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

Supplemental Data

S1. Methods

Characteristics of the cohorts

1) Convalescent cohorts. We selected three independent cohorts of convalescent unvaccinated COVID-19 individuals. In all cases, SARS-CoV-2 infection was microbiologically confirmed through real-time polymerase chain reaction (RT-PCR) from nasopharyngeal swab samples (NPS), taken at the time of hospital admission in the case of inpatients, and at their visit at their Primary Care Center in the case of outpatients with symptoms or close contact.

The characteristics and composition of the cohorts were as follows:

1.1) Patients needing hospital admission with severe COVID-19 and short-term (3-month) follow-up (**Inpatients, 3-months FU**), including consecutive subjects admitted to hospital between January 28th and February 8th, 2021 and reaching the 3-month FU visit unvaccinated.

1.2) Patients needing hospital admission with severe COVID-19 and long-term (12-month) FU (**Inpatients, 12-months FU**), including consecutive individuals admitted to hospital for COVID-19 between March 10th and June 30th, 2020, reaching the 12-month FU visit unvaccinated.

1.3) Patients with mild COVID-19 cared for in the primary care setting with long-term (12-month) FU (**Outpatients, 12-months FU**), including individuals with RT-PCR positive in

NPS between March 20th and July 30th, 2020, managed as outpatients, and reaching the 12-month FU visit unvaccinated.

2) Vaccination cohorts. We recruited two cohorts of vaccinated donors:

2.1) **SARS-CoV-2 naïve subjects**, comprising healthcare workers donors recruited from Hospital General Universitario de Elche among those with no history of COVID-19 symptoms or positive SARS-CoV-2 test who had been fully vaccinated with two mRNA vaccine doses (BNT162b2; Pfizer-BioNTech) 12 weeks before evaluation (those undergoing treatment with immunomodulatory drugs and pregnant women were not included).

2.2) SARS-CoV-2- infected subjects, comprising patients, with previous history of severe COVID-19, reaching the 12-month follow-up FU visit vaccinated with one **(SARS-CoV-2 infected-1 dose**) dose (BNT162b2; Pfizer-BioNTech) or two (**SARS-CoV-2 infected-2 doses**) mRNA vaccine doses (specifically 14 and 3 individuals received BNT162b2-Pfizer-BioNTech or mRNA-1273-Moderna & NIH, respectively).

3) Healthy unvaccinated cohorts. Two groups of SARS-CoV-2 -naïve unvaccinated subjects with negative serology for IgG/M antibodies (semi-quantitative anti-spike and anti-nucleocapside IgG-S/N antibodies, COVID-19 VIRCLIA® IgG MONOTEST, Vircell SL, Granada, Spain; and IgG/M rapid test, 2019-nCoV IgG/IgM Antibody Test, Wondfo Biotech Co., Guangzhou, China) were tested as controls:

3.1) **Household uninfected contacts of COVID-19** patients, comprising persons at high risk for infection because of household exposure to a person with SARS-CoV-2 infection; this group was selected among participants in a previous cohort study of close contacts of an index patient who had not acquired SARS-COV-2 infection, as measured by RT-PCR (1st, 8th and 15th day after confirmed close contact) in NPS and negative serology (at 15th day and 30th day).

3.2) **Healthy controls**, comprising SARS-CoV-2-naïve healthy donors with no history of SARS-CoV-2 infection or close contact with a confirmed infected individual, selected among subjects visiting the Travel Clinic of our institution for pre-travel counselling.

Experimental section

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. Blood was collected in serum tube, lithium heparin tube and K2-EDTA tube, consecutively. Serum tube was centrifuged, and serum used to perform the anti-spike antibody ELISA. Whole blood from lithium heparin tube was used for IGRA incubation within 4 hours. Manufacturer recommends processing the sample as soon as possible after collection, up to a maximum of 16 hours at room temperature for blood stability. K2-EDTA tube was centrifuged, and plasma was then aliquoted and stored at -80°C prior to performing the neutralizing antibodies ELISA.

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 21 h at 37ºC in the three tubes supplied: (1) blank tube for the individual IFN-γ background, (2) mitogen tube for unspecific IFN-γ secretion as control to verify whether the sample contains immune cells in a sufficient quantity and with a sufficient ability to be activated, and (3) stimulation tube coated with components of the S1 domain of the original sequence of SARS-CoV-2 spike protein for specific IFN-γ secretion. The IFN-γ concentration released in the plasma fraction obtained after centrifugation of the three tubes was then measured by an enzyme-linked

immunosorbent assay (Human interferon-gamma ELISA, Euroimmun, Lübeck, Germany) with an automated instrument (Dynex DS2® ELISA system) in international units per milliliter (IU/mL). 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 was considered borderline, and >200 was considered positive. Upper limit of quantification achieved was 5000 mIU/mL. Concentrations of IFN-γ above the calibration curve were defined as >5000 mIU/mL.

Detection of SARS-CoV-2–specific antibodies

IgG antibody serum levels against the trimeric spike protein (TrimericS-IgG) were quantified using commercial quantitative immunoassay kits (LIAISON® SARS-CoV-2 TrimericS IgG assay, DiaSorin, Saluggia, Italy) in an automated platform (LIAISON® XL Analyzer) following the manufacturer's instructions. Results were expressed as Binding Antibody Units (BAU) and interpreted according to the manufacturer criteria: ≥33.8 BAU/mL was considered positive with a numeric value for quantitative measurement.

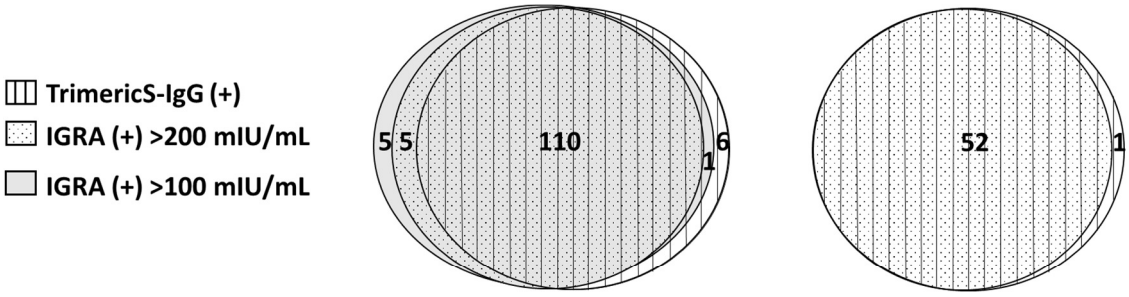
Detection of neutralizing antibodies

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), that determines the inhibitory effect of antibodies that can compete with the biotinylated host-cell receptor (ACE2) for the binding to the receptor-binding domain (RBD) of the S1 subunit of SARS-CoV-2 spike protein (inhibition percentage, %IH). Results were interpreted as inhibition percentage (%IH) as follows: %IH <20 was considered negative, %IH ≥20 to <35 was considered borderline, and %IH ≥35 was considered positive.

Statistical analyses

Statistical analyses were performed with R version 4.0.3 (2020-10-10) software. Percent positive or negative agreement and kappa coefficients (K) were calculated using the package caret [Kuhn M. 2020. Caret: classification and regression training. R package version 6.0-86. https://CRAN.R-project.org/package=caret]. For the graphical analysis, the ggplot2 package [Wickham H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag New York.] was used.

Figure S1. Positivity for SARS-CoV-2 IGRA and IgG anti trimeric spike protein in the convalescent and vaccinated cohorts.



Positivity	Convalescent cohorts (n=152)	Vaccinated cohorts (n=54)
TrimericS-IgG (+)	117 (77%)	53 (98.1%)
TrimericS-IgG (+) + IGRA (>200)	7+110+5 = 122 (80.3%)	53 (98.1%)
TrimericS-IgG (+) + IGRA (>100)	6+111+10 = 127 (83.6%)	53 (98.1%)