Determination of IgG Response Profile in SARS-CoV-2 Patients Using a Multiplex Serological Assay

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Background

Since its first detection in Wuhan (China) in December 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has rapidly spread to reach other countries worldwide as the coronavirus 2019 disease (COVID-19) became pandemic [1]. SARS-CoV-2 is spreading through human-to-human contact and can cause respiratory infections among others illness. The clinical picture is very diverse, from asymptomatic infections of healthy carriers, which will increase the disease spreading, to fever, dry cough, breathing difficulties, headache, or pneumonia which make it difficult to differentiate from other respiratory infections such as flus or human Coronavirus (hCoVs). Moreover, if most cases are classified as mild (no or moderate signs) in the first stage of the disease, it can rapidly evolve to more severe and critical states and even cause death.

The virions has a nucleocapsid composed by genomic RNA and phosphorylated Nucleocapsid (N) protein, which is buried inside a phospholipid bilayer and covered by the Spike proteins trimmers (S) that gives the CoVs their crown-like appearance on which their names are based. The S protein has two subunits, the Spike 1 (S1) which contains the Receptor-Binding Domain (RBD) and N-Terminal Domain (NTD) and the Spike 2 (S2) [2]. The choice of the antigenic domain is important, as it must be specific to the SARS-CoV-2 for discrimination against other hCoVs for example, and sensitive enough so infection would not be missed [3]. Most commercial serological assays have demonstrated satisfying performances of the commercial multiplex immunoassay CoViDiag®, based on an array of five different antigens of the virus (the Nucleocapsid, the Spike 1 and Spike 2 subunits, and the RBD and NTD domains of the Spike), to investigate the profile of the IgG humoral response for patients with recent SARS-CoV-2 infection depending on the disease severity outcome, or the time post-PCR.

Objectives

No cross-reaction was observed with the four other seasonal coronaviruses (100% specificity, 0/28), 100% (20/20) of the hospitalized patients PCR-positive to SARS-CoV-2 presented detectable levels of IgGs. 14 days post-PCR diagnosis, 92.3% of the patients, PCR-positive, that did not required hospitalization are presenting IgG (36/39). Interestingly for CoViDiag-positive samples, detectable levels of anti-RBD were found mainly in hospitalized patients (85%, 17/20), while the presence of anti-S1 (60.9%, 28/46) combined with the absence of anti-RBD (6.5%, 3/46) was more characteristic of non-hospitalized patients. Screening campaign group lacked both anti-S1 (18.2%, 4/22) and anti-RBD (4.5%, 1/22).

Conclusion: The CoViDiag® IgG assay could be used to evaluate patients’ immunization and improve their management.

Keywords: SARS-CoV-2; COVID-19; Serological assays; Multiplexing; IgG profile

Study design and cohort

The study was conducted at Amiens University medical Center
Samples were derived from de-identified excess serum specimens as described in a previous study (Brochot et al., 2020b). The demographic information of the 167 patients are available in Supplementary Table 1. The study was approved by the institutional review board of the Amiens University Medical Center (number PI2020_843_0046, 21 April 2020).

Briefly, n=167 sera samples from patients PCR-positive to SARS-CoV-2 and hospitalized (n=20), non-hospitalized patients but PCR-positive to SARS-CoV-2 (n=57), patients participating in screening campaigns (n=62), and a control group of patients with a history of other seasonal coronavirus infection (n=28) before 2020. Sera from patients PCR-positive to SARS-CoV-2 were collected between 0 to 80 days post-PCR.

All samples have been tested on the CoViDiag® serological assay and compared to the results obtained with three other IgG assays widely used worldwide (Euroimmun®, Abbott® and Diasorin®) [4].

CoViDiag® assay and analysis

The assays have been performed according to the manufacturer instructions. The results have been automatically delivered using the SciReader® plate reader (Scenion GmbH) and associated analysis software, and an algorithm combining different cut-offs for the different antigens according to the manufacturer instructions (Supplementary Table 1).

Data and statistical analysis

The demographic information of the 167 patients has previously been described [4].

Diagnostic specificity was evaluated on samples PCR-negative to SARS-CoV-2 but PCR-positive to other hCoVs. Diagnostic sensitivity was evaluated on samples PCR-positive to SARS-CoV-2 collected between 0 to 80 days post-PCR from hospitalized or non-hospitalized patients.

For the statistical analysis, Generalized Additive Models (GAM) were used to calculate Odds Ratios (OR) and 95% Confidence Interval (CI) considering positivity/negativity for CoViDiag, N, S1, S2, NTD and RBD as the main outcomes (borderline results have been filtered for group to group comparison) and controlling for personal background effects (sex and age). No influence of the delay between PCR and serology has been observed. The general significance level was set at a p-value below 0.05. All analyses were performed using packages stats and odds ratio from the R statistical computing program v. 3.6.1 (Date of release 07/05/2019). Specifically, we compare the antibody response profile between patients group and depending on the time post-PCR to test whether a significant difference is present among different group variables.

Results

Diagnostic performances of the multiplex CoViDiag® IgG assay

All patients hospitalized for COVID-19 with a positive nasopharyngeal SARS-CoV-2 PCR were positive to the CoViDiag® IgG assay (n=20/20, 100%) (Table 1). We observed than only 80.7% of the patients with a positive SARS-CoV-2 PCR that did not require hospitalization were positive to the CoViDiag® IgG assay (n=46/57). The part of patients presenting IgG increases to 92.3% for samples collected at least 14 days after a positive PCR (n=36/39). We found 35.5% of the patients participating in the screening campaigns positives to the CoViDiag® IgG assay (n=22/62). Using the CoViDiag® assay, we observed that 25.8% (n=16/62) of the patients from the screening campaign were lacking either the anti-N, anti-S1 or anti-S2 antibodies. Similar incomplete response was observed for 31.6% (n=18/57) of the non-hospitalized patients, and 10% of the
Among the 57 non-hospitalized patients, five presented only anti-S2 IgG (see supplementary Table 1). Among the 62 screening campaign patients, four presented only anti-S, two only anti-N, and one only anti-NTD antibodies, highlighting the interest of targeting a wide scope of antibodies especially in the light form of the disease. There was no cross reactivity with the samples from patients PCR positive to other seasonal coronaviruses (OC43, HKU1, NL63, 229E), collected between day 7 and day 1153 post PCR (100 % diagnostic specificity, n=0/28).

Profile of the IgG antibody responses depending on the disease severity

For the patients presenting a positive IgG response to CoViDiag®, we find different profile of the immune response between the different patient groups (Figure 2). 95% (n=19/20) of the patients hospitalized presented anti-S1 IgG against 60.9% (n=28/46) of the patients non-hospitalized and 18.2% (n=4/22) of the patients from the screening campaign. Furthermore 85% (n=17/20) of the patients hospitalized presented anti-RBD IgG against 6.5% (n=3/46) of the patients non-hospitalized. The comparison of odds ratio for each antigen (Supplementary Table 2) confirmed that the presence of anti-RBD antibodies is the best marker for the chance of being in the hospitalized group versus non-hospitalized group (OR: 4.508, CI: 4.332-4.693, p-value: 7.34e-13) or screening campaign group (OR: 4.665, CI: 4.739-4.592, p-value: 2.48e-12). The presence of anti-S1 antibodies is the best marker for the chance of being in the non-hospitalized group versus screening campaign group (OR: 1.901, CI: 2.044-1.767, p-value: 0.002).

Profile of the IgG antibody responses depending on the time post-PCR

For the two groups of patients PCR positive to SARS-CoV-2, we investigated the profile of the IgG antibody responses depending on the delay between PCR and serology. Independently of the period of collection between 0 and 80 days post-PCR the majority of hospitalized patients presented detectable levels of anti-N, anti-S1, anti-RBD, and anti-S2 antibodies, but no anti-NTD antibodies (Figure 3A). However, for the non-hospitalized patients, the immune response appeared weaker, allowing to follow the IgG antibody different kinetics (Figure 3B). The number of patients with anti-N, anti-S1, anti-RBD and anti-S2 IgG antibodies, increased until 45 days post-PCR, before starting to drop, especially for anti-N IgG antibodies (∆= -27.5% between 31-45 and >45 days post-PCR). Furthermore in the 14 days following the PCR, the anti-N and anti-S are the main detected IgG antibodies (44.4% anti-N positives and 61.1% anti-S positives) while the anti-S1 IgG antibodies are generally detected latter (50% anti-S1 positives between 15-30 days).

Discussion

For routine diagnosis use, commercial serological assays must be evaluated in regard to their ability to detect early and weak infections. Several commercial assays have shown good performances focusing on the detection of total antibodies (IgG, IgM and IgA). However, as early diagnosis results are already delivered by PCR assays, serological assays detecting IgG seem more appropriate for the evaluation of an efficient and long lasting protection of the patients. Interestingly, the detection of antibodies against larger specter of antigens can also increase the diagnostic sensitivity, especially for generally weaker immune response of asymptomatic and mild forms. With diagnostic performances equivalent to other IgG commercial serological assays, the CoViDiag® multiplex assay gives a more comprehensive picture of
the IgG humoral response. This study investigates the profile of anti-
SARS-CoV-2 different antibodies. We observed different pattern of
IgG profiles between severe (hospitalized patients and PCR positives),
mild (non-hospitalized patients and PCR positives), or asymptomatic
(patients from the screening campaigns) form of the disease. On
samples more than 45 days post-PCR, the percentage of different
IgG positive results tends to decrease or remain constant for the mild
and more severe form of the diseases, respectively. Furthermore, a
lot of interrogations have been raised lately regarding the vaccination
protocol for previously infected patients. As most vaccines are based
on the RBD part of the S1 protein, multiplex serology has the potential
to differentiate between infection and vaccination, and between
variants, with a single assay. Future epidemiological study on a larger
panel of samples (especially extended to the population with mild or
asymptomatic form of the disease), combining the multiplex assay
with machine learning can be a convenient tool to investigate the
kinetics and mechanisms of the immune response and contribute to
the development of long lasting and efficient strategy of vaccination.

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Laboratory’s own resources

Declaration of Competing Interest

Authors Rémi Malbec, Gaël Even and Christophe Audebert
are employees of GD Biotech, while Pauline Ponthieu, Pauline
Follet, Vianney Souplet and Christophe Olivier are employees of
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