



FULL LENGTH ARTICLE

A peptide-based assay discriminates individual antibody response to SARS-CoV-2

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Abstract SARS-CoV-2 virus is responsible for the current worldwide coronavirus disease 2019 (COVID-19) pandemic, infecting millions of people and causing hundreds of thousands of deaths. Understanding the antibody response to SARS-CoV-2 is crucial for the development of vaccines, therapeutics and public health interventions. However, lack of consistency in methods used to monitor antibody response to SARS-CoV-2 leaves some uncertainty in our fine understanding of the human antibody response mounted following SARS-CoV-2 infection. We developed a peptide-based enzyme-linked immunosorbent assay (ELISA) by selecting 7 synthetic peptides from the spike, membrane, and nucleocapsid protein sequences of SARS-CoV-2, which effectively detects the antibody response mounted by all COVID-19 convalescent tested. Strikingly, the assay shows a profound difference in antibody response among individual subjects, which may have a significant impact on disease severity. Together, our results define an efficient and specific serological assay to consistently measure the antibody response following SARS-CoV-2 infection, as well as help the design of vaccine and therapeutics for prevention and treatment of COVID-19.

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Introduction

The novel human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus disease 2019 (COVID-19) pandemic.^{1,2} Limited pre-existing immunity is supposed to account, at least in part, for the extraordinary escalation of cases worldwide. Understanding of the human antibody response to SARS-CoV-2 infection is relevant to vaccines development and planning of vaccination strategies, and to guide appropriate design, implementation and interpretation of serological assays for surveillance purposes. As of today, more than 200 studies have been published that analyze the human antibody response following SARS-CoV-2 infection. Indeed, the vast majority of infected individuals mount a SARS-CoV-2-specific antibody response, with many studies reporting 100% seroconversion.^{3–9} However, assays used to detect and quantify antibody response were diverse, with target antigens including spike (S), S1 and S2 subunits, receptor binding domain (RBD) and nucleocapsid (N). Indeed, the lack of consistency in methods used leaves some uncertainty in our complete understanding of the human antibody response mounted following SARS-CoV-2 infection. For example, mean or median time to seroconversion for IgG has been reported to range from 12 to 15 days post symptom onset^{3,10–17} with wide variation in first to last detection from 4 to 73 days post symptom onset. Time to seroconversion for IgA has also been measured in several studies, ranging from 4 to 24 days post symptom onset, although most were within 4–11 days,^{18–20} including two reports of 24 days to first detection.^{8,9} Therefore, a standardized assay based on synthetic reagents would greatly help the uniformity of measurement of serological responses. Here we present an ELISA for detection of anti-SARS-Cov-2 antibodies that has synthetic peptide antigens as the immunoabsorbent solid-phase. Strikingly, the assay finds significant differences in antibody specificity in the sera of COVID-19 convalescents, indicating that each antibody responses in SARS-CoV-2 infected patients has an individual repertoire feature.

Results and discussion

10 peptides predicted to be immunodominant by the BebiPred 2.0 algorithm⁶ were synthesized as candidate antigens from the S, M and N proteins of SARS-Cov-2 Wuhan-Hu-1 strain (GeneBank: MN908947) (Table 1 and Fig. S1). Candidate peptides were tested by ELISA procedure for serologic reactivity to a panel of serum samples from 24 COVID-19 convalescents (COVIDpos1–COVIDpos24) treated at San Pio Hospital in Benevento, Italy, in May 2020 (Table S1). For the peptide-based SARS-Cov-2 ELISA, wells of microtiter plates were coated with 2 µg/ml of a single or a mixture of the S, M, and N protein-derived peptides, and serologic reactivities were determined. Results were scored on the basis of the signal/cutoff (S/C) ratio, where cutoff absorbance was determined from the mean of four pre-2019 sera plus 3 standard deviations. No reactivity was observed towards peptides n.1, n. 4 and n. 9, which were therefore excluded from further analysis (data not shown).

The results shown Figure 1 and summarized in Table 2 indicate that all convalescent sera tested contain IgG that immuno-react with at least one of the 7 selected peptides, individually or mixed. Only the serum of one convalescent (n. 15) contained IgG for all 7 peptides. On the other hand, sera from 10 convalescents immuno-react only with one peptide: sera n. 3, n. 22 and n. 23 with peptide n.7; sera n. 9, n. 16 and n. 17 with peptide n. 10; sera n. 10, n. 14 and n. 21 with peptide n. 3 and serum n. 19 with peptide n.1. Peptide n. 3 (M1–24), n. 7 (N153–172) and n. 10 (S524–598) are the ones most frequently seen by the tested sera.

Competition experiments, conducted using in soluble form the coated peptide or irrelevant peptides confirmed the specificity of the sera response of sera to the coated peptides (Fig. S2).

The same type of assay was subsequently conducted on a cohort of 23 additional convalescent sera collected in October 2020 (COVIDpos101–COVIDpos123), with consistent results (Table S1 and Fig. S3).

The selected peptides were also tested for IgA immunoreactivity (Fig. 2 and Table 3). Similarly to the IgG response, the IgA response also appears varied among the

Table 1 List of peptides used for this study.

| Peptide | Sequence | Protein | Position |
|---------|--|---------|----------|
| 1 | NKHIDAYKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTLPAADM | N | 355–401 |
| 2 | DAVDCALDPLSETKCTLLKSFTVEKGIYQTSN | S | 287–317 |
| 3 | MADSNGTITVEELKLLLEQWNLVI | M | 1–24 |
| 4 | FGAGAALQIPFAMQMAYRFNGI | S | 888–909 |
| 5 | FSQILPDPSKPSKRSFIE | S | 802–819 |
| 6 | GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS | S | 601–640 |
| 7 | NNNAATVLQLPQGTTLPKGF | N | 153–172 |
| 8 | PLLESELVIGAVILRGHLRI | M | 132–151 |
| 9 | RPQGLPNNTASWFTALTQHGK | N | 42–62 |
| 10 | VCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSFQGVSVI | S | 524–598 |

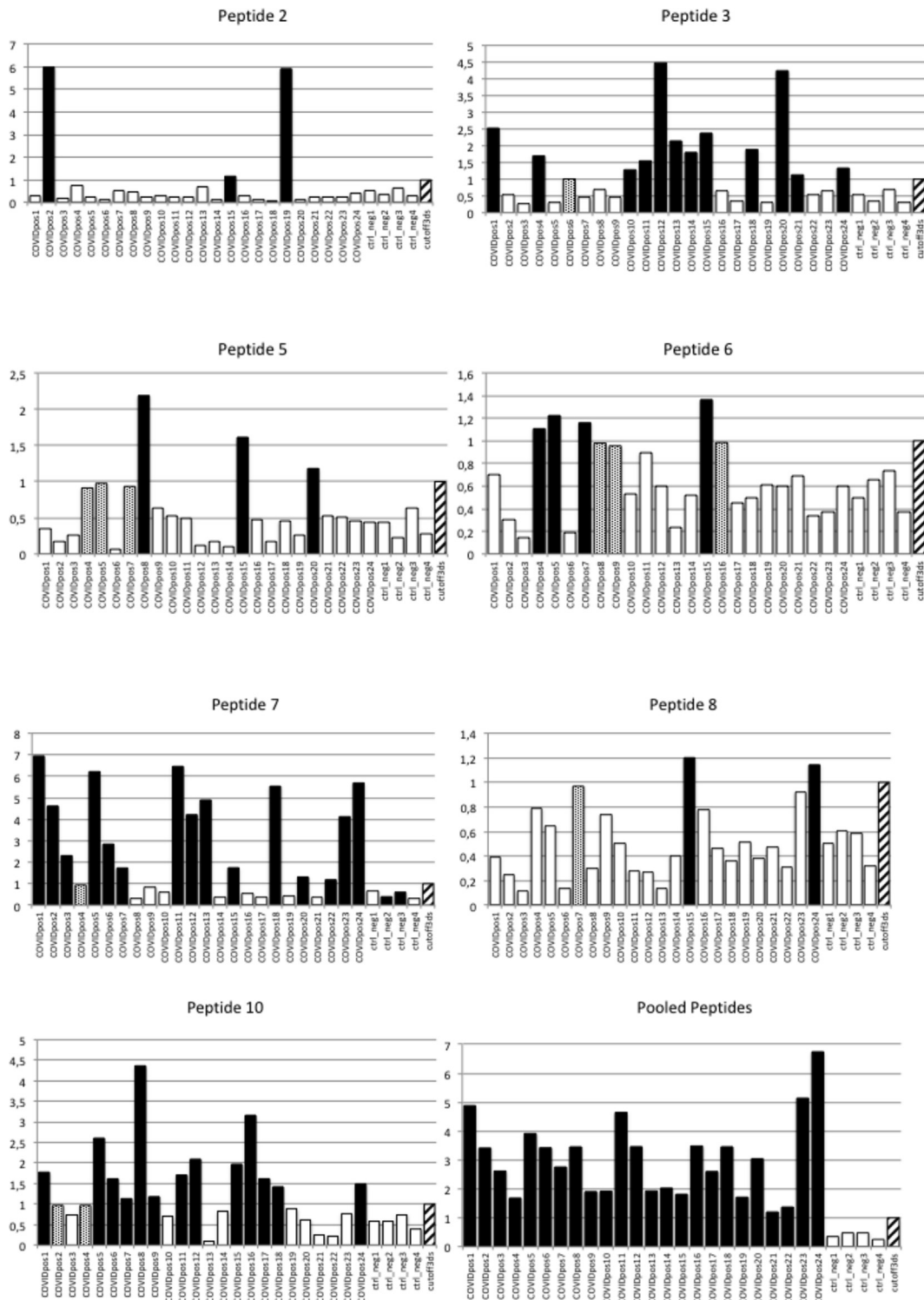


Figure 1 IgG immuno-reacting with the selected peptides at 15–30 days after the RT-qPCR positive detection in 24 COVID-19 convalescents. Results were scored on the basis of the signal/cutoff (S/C) ratio, where the cutoff (C) value (striped bar) was determined from the mean of four pre-2019 sera plus 3 standard deviations. Samples with absorbance values corresponding to $C \pm 10\%$ (dotted bars) were scored as uncertain. Data shown is representative of at least 50 independent experiments.

Table 2 Summary of IgG sero-reactivities shown in Figure 1.

| Patient | Peptide | | | | | | | Pool |
|---------|---------|---|---|---|---|---|----|------|
| | 2 | 3 | 5 | 6 | 7 | 8 | 10 | |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
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| 15 | | | | | | | | |
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| 18 | | | | | | | | |
| 19 | | | | | | | | |
| 20 | | | | | | | | |
| 21 | | | | | | | | |
| 22 | | | | | | | | |
| 23 | | | | | | | | |
| 24 | | | | | | | | |
| Neg 1 | | | | | | | | |
| Neg 2 | | | | | | | | |
| Neg 3 | | | | | | | | |
| Neg 4 | | | | | | | | |

different convalescents, five of which (n. 9, n. 15, n. 16, n. 17 and n. 22) do not recognize any peptide. Of all the peptides, peptide n. 3 is the most recognized by IgA (15 out of 19 reactive sera).

The COVID-19 pandemic is continuing to spread globally, and there is an urgent need to better understand the immune response to the virus so that effective immune-based treatments and vaccines can be developed. Here, we settled a peptide-based ELISA assay that measures the IgG and IgA responses in COVID-19 patients. Every SARS-CoV-2 infected subject we tested ($n = 47$) had detectable IgG antibodies reacting with at least one peptide used for the assay.

It is important to note that the assay does not ascertain the immunodominance of some peptides towards others, as the different composition and hydrophobicity of the peptides might influence the efficiency of plate coating. However, under the same coating condition, the assay reveals a profound difference in antibody response among subjects, ranging from reactivity toward only one peptide to all 7 peptides. This variety in antibody response was further confirmed in a serological screening done on a cohort of more than a thousand asymptomatic persons

(Polvere, unpublished data). Finally, the peptide-specific antibody response remained constant for at least two months in 5 patients of the cohort who subsequently underwent serological analysis (data not shown).

Our finding confirms the evidence that antibody responses in SARS-CoV-2 infected patients appears to have unique repertoire distribution patterns without preference for particular antibody families.²¹ Together, our results define an efficient and specific serological assay to measure antibody responses to SARS-CoV-2 infection.

Materials and methods

Peptides

Peptides were synthesized by Proteogenix and dissolved in DMSO.

Blood samples

This observational study used anonymized fresh or frozen residual samples collected at San Pio Hospital, Benevento, at 15–30 days from COVID-19 symptoms onset. After clotting, serum was separated using centrifugation for 10 min at 1000 rcf and aliquoted before storing at -80°C . Serum was heat-inactivated for 30 min at 56°C before usage for this study.

Elisa assay

96-well high-binding plates (NUNC Maxisorp, ThermoFisher) were coated overnight at 4°C with single or pooled peptides each at $2\ \mu\text{g}/\text{ml}$ in $100\ \mu\text{l}$ of Hank's balanced salt solution (HBSS). Unbound antigen was removed by washing once with $300\ \mu\text{l}/\text{well}$ with phosphate buffered saline (PBS) containing 0.05% Tween 20. Plates were blocked with 5% BSA (Sigma) dissolved in TBS containing 0.05% Tween-20 (TBST) for 1 h at room temperature with continuous gentle agitation. Sera samples were diluted in blocking buffer (1:500 for IgG and 1:300 for IgA) and incubated for 1 h at room temperature with continuous agitation. Plates were washed three times with $300\ \mu\text{l}/\text{well}$ of PBST-0.05% Tween-20. $90\ \mu\text{l}$ of HRP-conjugated goat anti-human immunoglobulins were added and incubated for 1 h at RT in 2.5% BSA dissolved in TBST for 50 min at room temperature with agitation. These secondary antibodies were diluted 1:60,000 for goat anti-Human IgG (MERK) and 1:50,000 for goat anti-human IgA (SIGMA). Unbound antibody was removed by washing a six times with $300\ \mu\text{l}/\text{well}$ PBS containing 0.05% Tween-20. After washing, $70\ \mu\text{l}$ of freshly prepared TMB (Thermo Fisher) substrate diluted 1:3 in PBS was added to every well and left for 15–30 min to allow colour to develop. $0.3\ \text{M}\ \text{H}_2\text{SO}_4$ ($70\ \mu\text{l}/\text{well}$) was used to stop the reaction. Absorbance readings at 450 nm were taken using a microplate reader Seac-Sirio-S.

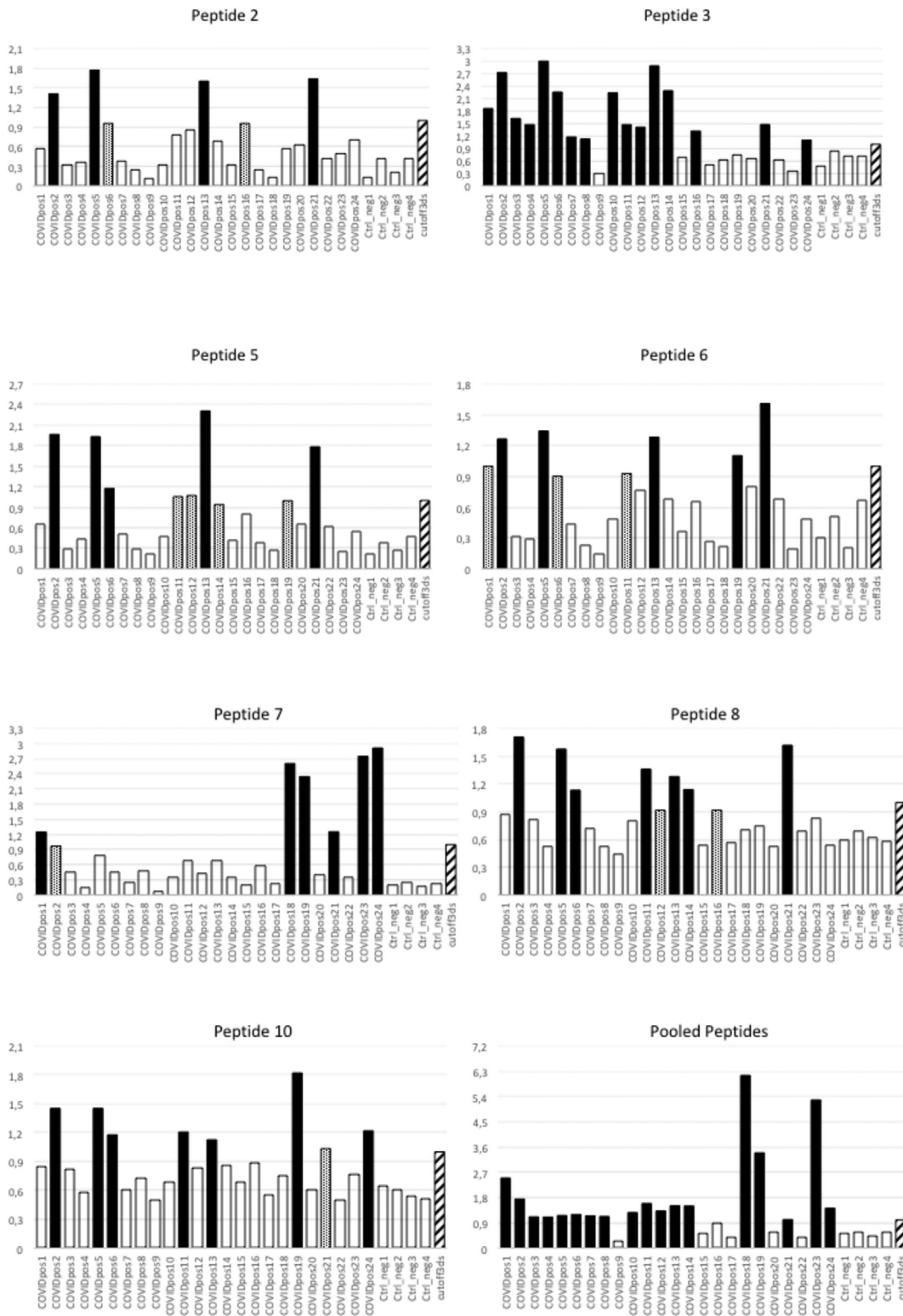


Figure 2 IgA immuno-reacting with the selected peptides at 15–30 days after the RT-qPCR positive detection in 20 COVID-19 convalescents. Results were scored as indicated in Figure 1. Data shown is representative of at least 50 independent experiments.

Table 3 Summary of IgA sero-reactivities shown in Figure 2.

| Patient | Peptide | | | | | | | |
|---------|---------|---|---|---|---|---|----|------|
| | 2 | 3 | 5 | 6 | 7 | 8 | 10 | Pool |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
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| 20 | | | | | | | | |
| 21 | | | | | | | | |
| 22 | | | | | | | | |
| 23 | | | | | | | | |
| 24 | | | | | | | | |
| Neg 1 | | | | | | | | |
| Neg 2 | | | | | | | | |
| Neg 3 | | | | | | | | |
| Neg 4 | | | | | | | | |

Author contributions

SV, GC, MF, FA, AP, PP and JRM performed the experiments; TZ, IP and PP analyzed the data; RS and PV wrote the manuscript.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgendis.2021.01.008>.

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