

## SHORT PAPER

# Neutralizing antibody responses against SARS-CoV-2 spike receptor-binding domain 13 months after the recovery from the disease

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*Key words:* SARS-CoV-2, COVID-19, coronavirus, antibody responses, immunity

*Parole chiave:* SARS-CoV-2, COVID-19, coronavirus, risposta anticorpale, immunità

### Abstract

**Background.** Information regarding the kinetics and longevity of acquired immunity in recovered COVID-19 patients requires thorough analysis and documentation. This is an update to an ongoing monocentric pilot observational study, that longitudinally analyzed the presence of antibodies after SARS-CoV-2 infection.

**Study design.** Antibody titers against nucleocapsid protein (NCP) of SARS-CoV-2 analyzed at 8 months was followed by adoption of a more specific immunoassay, anti-Spike-Receptor binding domain IgG CLIA for analysis at 12 and 13 months post infection.

**Methods.** MAGLUMI® SARS-CoV-2 S-RBD IgG Chemiluminescence immunoassay (CLIA) was adopted for measurement of antibody titres at 12 and 13 months after SARS-CoV-2 infection.

**Results.** 97% (34 out of 35) patients resulted positive for anti-SARS-CoV-2 RBD IgG at 12 and 13 months.

**Discussion and Conclusions.** In areas with vaccine and resource scarcity, vaccination could be prioritized for those individuals who have never been infected or for the ones who have recovered but show the absence of protective antibodies.

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## Introduction

As the vaccination implementation programs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causing Coronavirus disease 2019 (COVID-19) are progressing worldwide in full swing, information regarding the kinetics and longevity of acquired immunity following the natural infection necessitates analysis as well as documentation. The SARS-CoV-2 shares approximately 79.5% genomic homology with SARS-CoV-1, with a similar receptor-binding domain (RBD) structure (1). Therefore, much understanding of the immunity offered post-SARS-CoV-2 infection is derived from previous experiences with SARS-CoV-1, where protective antibodies were found to persist for at least 2 years, as well as real-time emerging data (2-4).

We present an update to a previous monocentric pilot observational study, that longitudinally analyzed the presence of antibodies against SARS-CoV-2 nucleocapsid protein (NCP) in  $n=30$  patients at 8 months (5).

From late Feb 2021, an additional  $n=12$  patients (8 female and 4 male), who met the eligibility criteria for participation, were enrolled in the study. These patients ( $n=12$ ), similar to the original cohort, had a history of testing positive for SARS-CoV-2 by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) in March 2020. Since the legal provisions adopted by the Italian Ministry of Health advised mandatory vaccination for all Healthcare Workers, irrespective of previous disease status,  $n=7$  patients (4 female and 3 male) were gradually vaccinated from mid-March 2021 and hence excluded from the study, making the revised final sample size at 12 and 13 months to be  $n=35$  (figure 1). The study group was divided into two subgroups based on disease severity; Mild ( $n=21$ ) and Moderately-Severe ( $n=14$ ).

## Materials and Methods

This study aimed at analyzing the anti-Spike-Receptor-Binding Domain (anti-S-RBD) antibody levels (IgM and IgG) in 35 patients, using MAGLUMI® SARS-CoV-2 S-RBD IgG Chemiluminescence immunoassay (CLIA - New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China) at 12 and 13 months after SARS-CoV-2 infection. This immunoassay was granted under an Emergency Use Authorization by the US Food and Drug Administration. As per the Specifications, anti-SARS-CoV-2 S-RBD IgG assay had a sensitivity 100% with CI [99.9%-100.0%] at  $\geq 15$  days post symptom onset and specificity of 99.6%; CI [98.7%-100.0%]. High concentration samples were diluted automatically by analyzers and the recommended dilution was 1:9 with the diluent in kit. The sample, buffer and magnetic microbeads coated with S-RBD recombinant antigen were mixed thoroughly and incubated, forming immune-complexes. After precipitation, decanting of supernatant, and performing a wash cycle, N-(4-aminobutyl)-N-ethylisoluminol (ABEI) labeled with anti-human IgG antibody was added, and incubated to form complexes. Again after precipitation in a magnetic field, decanting of supernatant, and performing another wash cycle, the Starter 1+2 were added to initiate a chemiluminescent reaction. The light signal was measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of SARS-CoV-2 S-RBD IgG present in the sample. The measurements and interpretation of results were made according to the manufacturer's instructions. The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results were expressed in AU/m. A result less than 1.00 AU/mL ( $<1.00$  AU/mL) was

considered to be non-reactive, while a result greater than or equal to 1.00 AU/mL ( $\geq 1.00$  AU/mL) was considered to be reactive (6).

## Statistical Analysis

In the univariate analysis, absolute frequencies and percentage were used to describe categorical variables and median and 1st (q1) and 3rd (q3) quartile for quantitative variable. Shapiro Wilk tested the normal distribution of the data. For quantitative comparisons of antibody levels at 12 and at 13 months into severity groups,

the Wilcoxon test for paired data was used. All statistical tests were 2-sided, with a significance level set at  $p < 0.05$ . Analyses were performed using the R software environment for statistical computing and graphics (version 3.4.1; <http://www.r-project.org/>).

## Serologic status at 12 and 13 months post-infection

At both, 12 and 13 months post-infection, the percentages of anti-SARS-CoV-2 S-RBD, IgM and IgG positive subjects were analyzed. 97% (34 out of 35) patients were

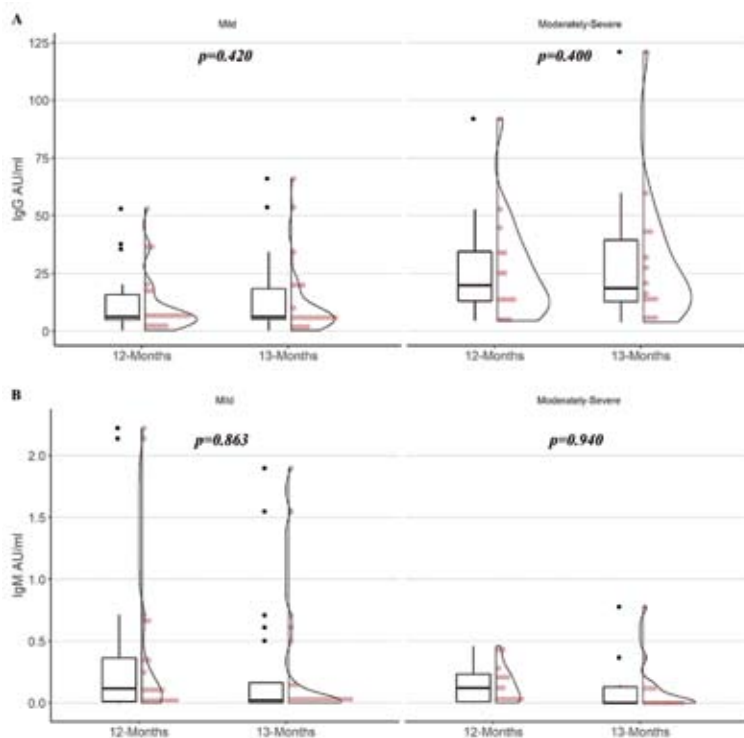


Figure 1 - Box plots describing the antibody titers IgM and IgG (anti-S-RBD) expressed as a median [q1-q3] for Mild (n=21) and Moderately-Severe group (n=14) at 12- and 13-months post-infection.

A: At 12 months, IgG median neutralizing titer (MNT) for the Mild group was 6.12 [4.9-15.8] AU/ml, and 19.8 [13.1-34.5] AU/ml for the Moderately-Severe group. At 13 months, IgG MNT for the Mild group was 6.04 [5.0-18.4] AU/ml, and 18.5 [12.8-39.4] AU/ml for the Moderately-Severe group.

B: At 12 Months, IgM MNT for the Mild group was 0.12 [0.01-0.4] AU/ml and 0.1 [0.01-0.2] AU/ml for the Moderately-Severe group. At 13 Months, IgM MNT for the Mild group was 0.02 [0.00-0.16] AU/ml and 0.00 [0.00-0.13] AU/ml for the Moderately-Severe group.

The p-value results from the Mann U Whitney test.

positive for anti-SARS-CoV-2 RBD IgG at 12 and 13 months. The patient non-reactive for Anti-S-RBD IgG at 12 and 13 months, is a known case of multiple myeloma, under treatment.

The patients of the Moderately-severe group (n=14) had a higher Median Neutralization titer (MNT) at 12 months (19.8 AU/ml) and at 13 months (18.5 AU/ml) as compared to the patients of the Mild group (n=21), with MNT of 6.1 AU/ml at 12 months and 6.0 at 13 months.

## Discussion and Conclusions

Neutralizing antibodies (nAbs) are capable of preventing an infectious agent from infecting a cell by neutralizing or inhibiting its biological effect. The most critical target for SARS-CoV-2 nAbs is the RBD within the SI subunit of S protein. Such nAbs can interrupt the interaction of RBD and its receptor ACE2. Thus, SARS-CoV-2 S-RBD IgG antibody level in human serum or plasma correlates with protective immune responses in individuals who have recovered from SARS-CoV-2 infection and could also reflect herd immunity at a population level, aiding in planning the clinical management of patients with past or ongoing COVID-19 infection.

The results of our study are consistent with the literature, where a correlation between antibody titers and disease severity was documented (7).

Patients who have developed more pronounced post-infectious symptoms tend to develop higher antibody titers than patients who have experienced mild symptoms. Furthermore, the antibody titer tends to vary also in relation to age and comorbidities. The results of this antibody persistence at 13 months document a good immunological response following SARS-CoV-2 infection and could also provide informations helpful for shaping future policies and adaptive

measures regarding the use of vaccines and related boosters. In areas experiencing vaccine and resource scarcity, prioritization of vaccination could be either for those individuals who have never been infected or for the ones who have recovered but are non producing protective antibodies.

## Riassunto

*Risposte anticorpali neutralizzanti contro lo Spike Receptor-Binding-Domain di SARS-CoV-2 a 13 mesi dalla guarigione dell'infezione*

**Contesto.** Le informazioni relative alla cinetica e alla durata dell'immunità acquisita nei pazienti guariti da COVID-19 richiedono un'analisi ed una documentazione approfondita. Questa comunicazione è un aggiornamento di uno studio osservazionale monocentrico attualmente in corso, che ha analizzato nel tempo la persistenza degli anticorpi dopo l'infezione da SARS-CoV-2.

**Disegno dello studio.** I titoli anticorpali contro la proteina del nucleocapside (NCP) del virus SARS-CoV-2, analizzati a 8 mesi, sono stati nuovamente misurati dopo l'adozione di un test immunologico più specifico, anti-Spike-Receptor binding domain IgG CLIA, per l'analisi a 12 e 13 mesi dall'infezione.

**Metodi.** MAGLUMI® SARS-CoV-2 S-RBD IgG immunodosaggio in chemiluminescenza (CLIA) è stato adottato per la misurazione dei titoli anticorpali a 12 e 13 mesi dopo l'infezione da SARS-CoV-2.

**Risultati.** Il 97% (34 su 35) dei pazienti era positivo per IgG anti-SARS-CoV-2 RBD a 12 e 13 mesi.

**Discussione e Conclusioni.** Nelle aree con scarsità di vaccini e risorse, la vaccinazione potrebbe essere prioritaria per quegli individui che non sono mai stati infettati o per quelli che si sono ripresi ma non dimostrano presenza di anticorpi protettivi.

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