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FULL PAPER

Kinetics of Anti-SARS-CoV-2 IgG among healthcare workers in a General Hospital

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SUMMARY

SARS-CoV-2 is a newly-discovered positive-sense RNA virus, the cause of coronavirus disease 2019 (COVID-19) currently spreading worldwide. The SARS-CoV-2 S glycoprotein is considered a main target for neutralizing antibodies. In order to better understand the kinetics of the antibody response, we evaluated the relation between two consecutive antibody titers determined over an average period of four months. A total of 628 subjects were included in the study. A significant reduction of the antibody titers over time was found: Ab Titer 1: 8.1 Arbitrary Units (AU)/mL (IQR: 4.8-29.3); Ab Titer 2: 6.2 AU/mL (IQR: 0-28.5); p<0.0001. A Receiver Operator Characteristic curve analysis showed an Area Under the Curve (AUC) of 0.973 (95% CI: 0.962-0.984; p<0.0001) with an Ab titer 1 threshold of 110 AU/mL to predict an Ab Titer 2 ≥50 AU/mL with 100% specificity. Likewise, an AUC of 0.952 (95% CI: 0.930-0.974; p<0.0001) with an Ab titer 1 threshold of 185 AU/mL was found to predict an Ab Titer 2 ≥100 AU/mL. This study showed that the SARS-CoV-2 S1/S2 IgG median titer declined over an average period of four months and that the first IgG determination thresholds found can help predict IgG values after the same interval.

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INTRODUCTION

Between December 2019 and January 2020 in Wuhan, Hubei province, China, an outbreak of viral pneumonia caused by a new type of Coronavirus (CoV) was identified (Li *et al*., 2020, Wu *et al*., 2020, Zhou *et al*., 2020). Subsequently, the virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as cause of the coronavirus disease 2019 (COVID-19) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). In a few months, COVID-19 spread around the world, causing a global pandemic (Cucinotta & Vanelli, 2020).

SARS-CoV-2 is a single-stranded positive-sense RNA virus and has four main structural proteins: spike (S), envelope, membrane protein and nucleocapsid protein (Naqvi *et al*., 2020). The S glycoprotein facilitates angiotensin-converting enzyme-2 receptor binding on target cells, subsequent membrane fusion and virus entry (Ou *et al*., 2020), and is therefore considered a main target for neutralizing antibodies.

Key words:
COVID-19, SARS-CoV-2, COVID-19 Serological Testing.

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SARS-CoV-2 is a member of the human coronavirus family (HCoV), of which six were already known to cause disease (Yin & Wunderink, 2018). Among these, four are known as human endemic coronaviruses: HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 causing acute self-limiting common cold symptoms (Yin & Wunderink, 2018), while the other two, SARS-CoV and MERS-CoV, cause outbreaks of severe lower respiratory tract infection (Drosten *et al*., 2003, Zaki *et al*., 2012).

The symptoms of COVID-19 patients are quite variable, ranging from asymptomatic cases to flu-like symptoms (fever, dry cough, dyspnea, fatigue) up to cases of acute respiratory distress syndrome caused by bilateral interstitial pneumonia requiring admission to an intensive care unit (Hassan *et al*., 2020, Sheleme *et al*., 2020). In parallel, the most common laboratory alterations found in COVID-19 patients are an increase in C-reactive protein, erythrocyte sedimentation rate, interleukin-6, lactate dehydrogenase, and a decrease of albumin, eosinophils, and lymphocytes (Zhang *et al*., 2020).

In light of the foregoing, laboratory support is pivotal for a correct diagnosis of COVID-19, and the gold standard is RNA detection of SARS-CoV-2 by means of Real-Time PCR (RT-PCR) (Cheng *et al*., 2020, Loeffelholz & Tang, 2020). Nevertheless, serological tests are becoming more widespread (Deeks *et al*., 2020,

Mekonnen *et al*., 2020), showing the highest sensitivity at >14 days after symptom onset (Wang *et al*., 2020), and the combination of serum antibody testing against SARS-CoV-2 with RT-PCR has been described to improve diagnosis (Zhao *et al*., 2020). The kinetics of anti-SARS-CoV-2 antibodies is still little known and under debate (Huang *et al*., 2020, Yang & Ibarrondo, 2020); therefore, from the perspective of better understanding how long antibodies against SARS-CoV-2 persist over time, possibly protecting from disease and reinfection, we evaluated the kinetics of antibodies against SARS-CoV-2 S glycoprotein by comparing two measurements over an average period of four months.

MATERIALS AND METHODS

Design of the study and clinical samples

We performed a retrospective analysis of serum samples drawn from hospital care workers from May 2020 to October 2020, as requested by the occupational health service of the hospital for epidemiological purposes. The second sampling was scheduled after around four months, but in some cases it was performed earlier or a bit later, upon request of the worker. Serum samples were collected, stored at 4°C, and processed within 24 hours after sampling. Each sample was processed in duplicate.

Antibody detection

SARS-CoV-2 S1/S2 IgG were measured by means of the indirect chemiluminescent immunoassay LIAISON® SARS-CoV-2 S1/S2 IgG (Diasorin, Saluggia, Italy) according to the manufacturer's instructions. Briefly, the solid phase, represented by magnetic particles, is coated by specific recombinant S1 and S2 antigens and mouse monoclonal antibodies to human IgG are conjugated with an isoluminol derivative. During the first incubation, the SARS-CoV-2 IgG antibodies present in the serum samples bind to the solid phase coated by the recombinant S1 and S2 antigens. The second incubation involves the reaction between the antibody conjugate and the SARS-CoV-2 IgG antibodies linked to the solid phase. Finally, starter reagents are added, a flash chemiluminescence reaction is thus induced and the amount of isoluminol-antibody conjugate is measured by a photomultiplier. The analyzer automatically calculates SARS-CoV-2 S1/S2 IgG antibody concentrations expressed as arbitrary units (AU/mL) and grades the results from <3.80 AU/mL (lower limit of detection) to 400 AU/mL (upper limit of detection). A concentration ≥15 AU/mL is considered a positive result.

Statistical Analysis

Values are expressed as median and interquartile range (IQR) or absolute numbers and percentages. Median values were compared by means of the Mann–Whitney U test. A correlation analysis was performed using Spearman's rank correlation coefficient. A Receiver Operator Characteristic (ROC) curve analysis was performed to look for possible thresholds of IgG values at first sampling to predict IgG values measured at second sampling. SPSS statistical package, release 17.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. The significance level was set at $p \leq 0.05$.

Ethical considerations

The present study was designed a secondary analysis of data collected as part of standard care and subjects included in the database were deidentified before access. No personal information was stored in the study database. No patient intervention occurred with the obtained results.

RESULTS

A total of 4882 serum samples were drawn during the interval considered. Of these, only the subjects in whom the first antibody titer was above the lower limit of detection and for whom at least two antibody determinations were available were included in the study. A total of 628 subjects were therefore evaluated. The median age was 47 years [interquartile range (IQR): 36-54] and 450/628 (71.7%) were females. No significant difference was found in the comparison of median age between males and females: males: 45 years (IQR: 34-54); females: 47 years (IQR: 36-54); p=0.437. On the contrary, significantly greater values of Ab titers were found for males both at first and second sampling (*Figure 1A* and *1B*): first sampling: males: 11.5 AU/mL (IQR: 5-54.8); females: 7.2 AU/mL (IQR: 4.6-24.7); p=0.002: second sampling: males: 9.2 AU/mL (IQR: 0-51.7); females: 5.5 AU/mL (IQR: 0-24); p=0.005.

The median interval between the two samplings was 138 days (IQR: 137-139), and the comparison of median values of Ab titers (*Figure 2*) showed a significant reduction of the antibody titers over time: Ab Titer 1: 8.1 AU/mL (IQR: 4.8-29.3); Ab Titer 2: 6.2 AU/mL (IQR: 0-28.5); p<0.0001. A significant positive correlation was found between the two measurements (*Figure 3*). Nevertheless, the scatterplot comparing the first and second Ab titer showed that the population seemed to be composed of two groups of subjects: one group that had an increase in the Ab titer over time (upper left quadrant) and the other that experienced a decline of Ab titer (lower right quadrant). The subjects with values of 400 AU/mL were difficult to define, since this is the upper limit of detection of the assay; thus, their real Ab titer is unknown. Therefore, 482/628 (76.8%) of the patients experienced a decline of the Ab titer, conversely 146/628 (23.2%) had an increase. We then repeated the correlation analysis in these two populations; both the positive

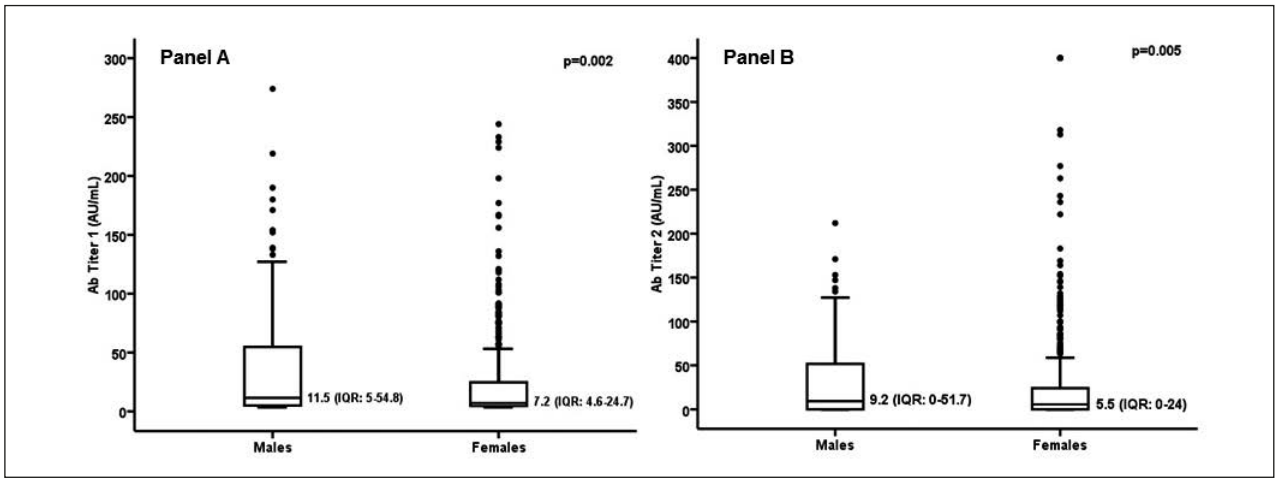

Figure 1 - *Comparison of median values of Ab titers between males and females at first (Panel A) and second (Panel B) sampling (n=628).*

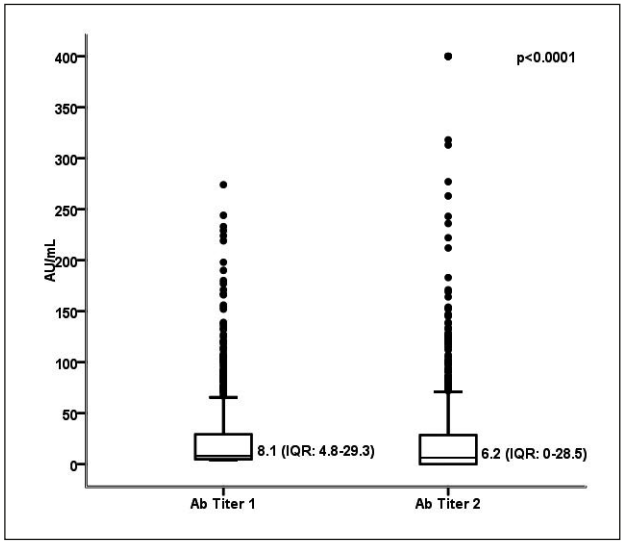

Figure 2 - *Comparison of median values of Ab titers between first and second sampling (n=628).*

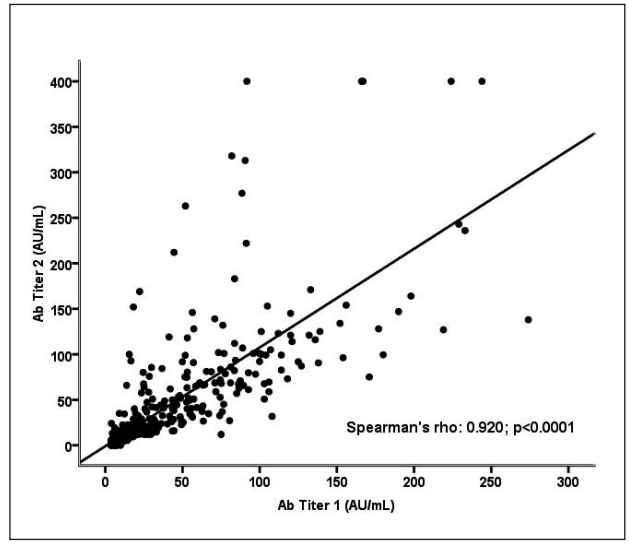

Figure 3 - *Correlation analysis between Ab titers 1 and 2 in the whole population (n=628).*

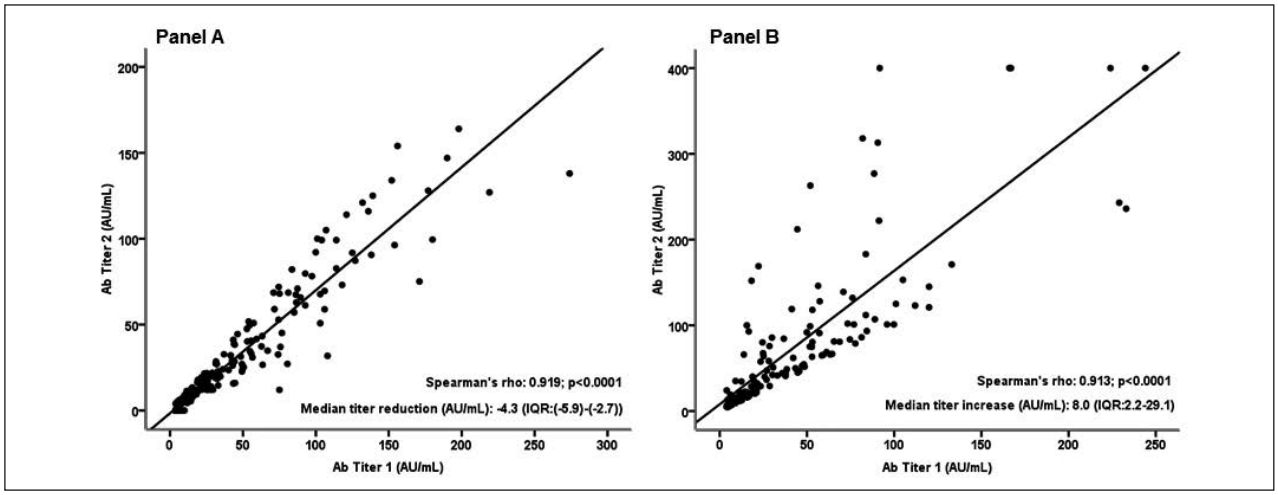

Figure 4 - *Correlation analysis between Ab titers 1 and 2 in the subpopulations of patients that had a reduction of the antibody titer (Panel A; n=482) and of those that had an increase (Panel B; n=146).*

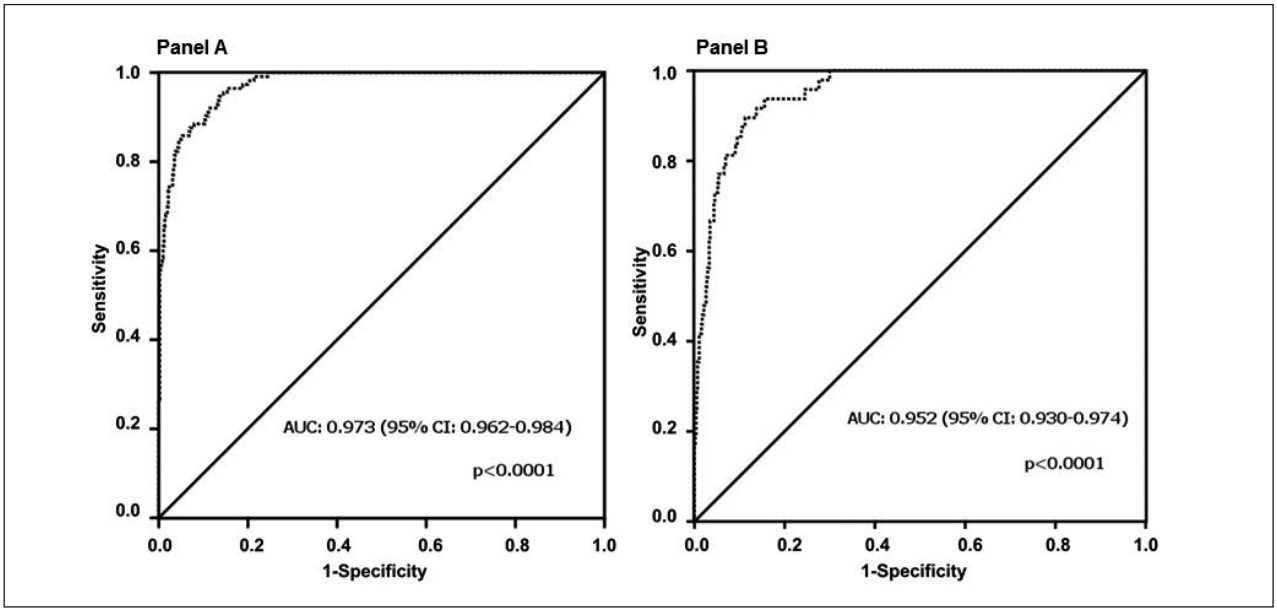

Figure 5 - *Receiver Operating Characteristic curve of Ab Titer 1 values in predicting Ab Titer 2 values ≥50 AU/mL (Panel A) and Ab Titer 2 values ≥100 AU/mL (Panel B).*

correlation and the median variations of the Ab titers are described in *Figure 4A* and *4B*.

We then considered as dependent variables Ab titers 2 with arbitrary values of 50 AU/mL and 100 AU/mL as examples, and performed a ROC analysis to check for possible thresholds of Ab Titer 1 values with 100% specificity to predict Ab titer 2 greater than or equal to the chosen values. *Figure 5A* shows the ROC curve of Ab Titer 1 values in predicting Ab Titer 2 values ≥50 AU/mL; an area under the curve (AUC) of 0.973 [95% confidence interval (CI): 0.962-0.984; p<0.0001)] was found, along with an Ab titer 1 threshold of 110 AU/mL. Likewise, *Figure 5B* shows the ROC curve of Ab Titer 1 values in predicting Ab Titer 2 values ≥100 AU/mL; the AUC was of 0.952 (95% CI: 0.930-0.974; p<0.0001) and the Ab titer 1 threshold found was 185 AU/mL. Unfortunately, no threshold of Ab titers at first sampling was found to predict an Ab value ≥150 AU/mL with 100% specificity at second sampling.

DISCUSSION

One of the main results of this study is the finding of a decline in the median anti-SARS-CoV-2 IgG titer during the period considered. The correlation analysis showed that, in most cases, the second Ab titer was proportional to the first one among the patients of this population that experienced a decline in the Ab titer over time. This finding is in line with other reports: in an evaluation of sequential serum samples up to 94 days after onset of symptoms both in 65 individuals with real-time quantitative PCR-confirmed SARS-CoV-2 infection and 31 seropositive healthcare workers, a decline in neutralizing antibody titers was found (Seow *et al*., 2020). Similarly, in a cohort of 32 recovered individuals, a decline in neutralizing antibody titers averaging about four-fold from one to four months post symptom onset was found (Crawford et al., 2020). In an 8-week follow-up of 37 asymptomatic individuals and 37 symptomatic who were diagnosed with RT-PCR-confirmed SARS-CoV-2 infection, Long et al. found a median percentage of decrease in IgG levels of more than 70% in both groups (Long *et al*., 2020). Likewise, in 343 North American patients infected with SARS-CoV-2, followed up to 122 days after symptom onset, a slow decay of serum antibody responses was observed (Iyer *et al*., 2020). Beaudoin-Bussières et al. analyzed serological samples from 31 convalescent donors that were collected at 6 and 10 weeks after the onset of symptoms and found a significant decrease in all receptor-binding-domain-specific IgG, IgM, and IgA titers (Beaudoin-Bussières *et al*., 2020). In a study evaluating 173 blood samples collected from 30 COVID-19 patients, a median decrease of 34.8% of the specific neutralizing antibodies titers over a 3-month study period was reported (Wang *et al*., 2020). Huang et al., in a population of 366 COVID patients, estimated a half-life of IgG of around 53 days and the diminish time at about 211 days post symptom onset (Huang *et al*., 2020). In a seroprevalence study conducted in Spain (Moncunill *et al*., 2021) that involved a cohort of 578 health care workers, an antibody decay rate of 0.66 was found for IgG after 3 months. In another seroprevalence study conducted in Italy (Stefanelli *et al*., 2020) that evaluated the longevity of IgG positive for anti-SARS-CoV-2 nucleocapsid antibodies, more than 40% of the patients became seronegative after 4

months. Likewise, in a study conducted in Germany, a decline in anti-receptor-binding domain IgG antibodies was observed (Glück *et al*., 2021).

The other main result is the finding of Ab titer thresholds useful for predicting Ab levels after a median interval of four months. In fact, in our series, all the subjects with an Ab titer 1 ≥110 AU/mL after a median time of 138 days had an Ab titer 2 ≥50 AU/mL and, after the same interval, all the subjects with an Ab titer 1 ≥185 AU/mL had an Ab titer 2 ≥100 AU/mL. This is feasible because, as described by Seow *et al*., patients who develop a strong neutralizing antibody response maintain high titers even though experiencing a decay over time (Seow *et al*., 2020). Likewise, in the Italian serosurvey (Stefanelli *et al*., 2020), individuals with high anti-NC IgG levels in the first serosurvey had the highest probability of being seropositive after four months.

This study has limitations. We evaluated the titers of IgG anti-S1 and IgG anti-S2 specific antibodies to SARS-CoV-2, not neutralizing antibody titers, although IgG anti-S correlate with neutralizing antibody titers. Concurrent nasopharyngeal swabs were not obtained and clinical data of the subjects included were not available. Therefore, we could not determine ongoing infection or evaluate symptoms onset.

CONCLUSIONS

In conclusion, this study showed that the median titer of SARS-CoV-2 S1/S2 IgG declined over a period of four months and that the first IgG determination thresholds found can be of help in predicting IgG values after the same interval.

Declaration of interest statement

The authors have no conflicts of interest to be declared.

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