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Two SARS-CoV-2 IgG immunoassays comparison and time-course profile of antibodies response

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ABSTRACT

Introduction: The persistence of circulating antibodies to SARS-CoV-2 infection is not yet well known. We compare the results of 2 automated systems for the determination of IgG against SARS CoV-2 and assess the time-course of the IgG response. *Methods:* IgG were measured in 103 specimens of 55 patients with COVID-19 (time from the symptoms' onset: 3–187 days) using the automated tests "Abbott SARS-COV-2 IgG" and "MAGLUMI 2019-nCoV IgG". *Results:* The 2 methods had a concordance of 90.3%, but the quantitative correlation, although significant, showed dispersed results. All the specimens resulted positive after 17 days. However, the median concentrations of IgG rapidly increased up to 20 days and decreased for Maglumi IgG while Abbott IgG showed a constant trend up to 85 days, and then slowly declined. *Conclusions:* The titer of IgG against SARS-CoV-2 may significantly and rapidly decrease, but with a very different time-course depending on the method used for the determination.

1. Introduction

A new coronavirus (2019-nCoV) has emerged in December 2019 in the region of Wuhan, China, and the related disease (COVID-19) to date represents a major health concern in the world. The serological tests for CoV-2 antibodies determination could be useful for supporting the assessment of cases of uncertain identification or with moderate illness, as well as for contact tracing and for epidemiological studies (Tang et al., 2020). The latter could in turn be helpful for the correct identification of asymptomatic subjects and for correctly estimating the illness and death rate. To date there are several available SARS-CoV-2 serological tests. Different papers have already compared some of these methods, and found acceptable classification concordance although very dispersed results when quantitative data were evaluated. (Jaaskelainen et al., 2020; Kohmer et al., 2020; Lippi et al., 2020; Montesinos et al., 2020; Nicol et al., 2020; Wolff et al., 2020).

However, up to the present time it is uncertain whether the recovery from COVID-19 provides immunity (Kirkcaldy et al., 2020). A rapid seroconversion was demonstrated, but the persistence of IgG is undefined (Kontou et al., 2020). To date, a limited number of studies evaluated the time-course of the antibodies profile for a time of no more than 3 to 4 months (Bölke et al., 2020; Gudbjartsson et at., 2020; Kutsuna et al., 2020; Ibarrondo et al., 2020; Terpos et al., 2020.

The aim of this study is to compare 2 different automated methods for anti SARS-CoV-2 antibodies determination and the evaluation of the antibodies kinetic with both the methods up to about 6 months.

2. Materials and methods

2.1. Samples

We collected samples from patients who presented at the Ospedale dell'Angelo (Mestre, Italy) in March 2020 and were diagnosed as COVID-19 affected according to both clinical and laboratory criteria. Only patients with known date of symptoms' onset were considered. Fifty-five patients were then included in the study (46 males, 9 females, median age 63 years: range 28–89). The median time from the onset of symptoms to the date of the different withdrawals was 23 days (minimum 3, maximum 187). Other patients' characteristics (disease severity, symptoms, and number of withdrawals per patient) were reported at Table 1.

Serum was collected from residual blood samples taken for routine biochemical testing and stored at −80°C, with a maximum of only one freeze-thawing cycle.

2.2. Methods

IgG were measured with 2 two-step chemiluminescence microparticle immunoassays, the Abbott SARS-COV-2 IgG (nucleoprotein-based

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Table 1
Characteristics of the studied patients.

Symptoms at the onset of disease	Frequency (%)
Fever	75.5
Cough	56.6
Dyspnea	26.4
Asthenia	13.2
Nausea	7.5
Others	11.3
Disease severity	n of patients
Mild	9
Moderate	19
Severe	15
Critical	12
n of withdrawals	n of patients
1	31
2	12
3	4
4	5
5	2
6	1

The disease severity was classified according the WHO guidance Laboratory testing for coronavirus disease (COVID-19) in suspected human cases.

antigen) and the MAGLUMI 2019-nCoV IgG (S1, S2 and N proteins based). The good analytical characteristics of the two assays were previously evaluated and confirmed (Bryan et al., 2020; Dittadi et al., 2020; Padoan at al., 2020a) The Abbott SARS-COV-2 IgG assay is calibrated against an internal standard and the results are expressed as Index (ratio between the sample result and the calibrator result). The assay is reported as qualitative and the samples are considered reactive with an index ≥1.4. The MAGLUMI 2019-nCoV IgG is calibrated against an internal standard, is reported as qualitative, although it uses a 6-point standard curve and the results are expressed as Arbitrary Units/mL (AU/mL). The samples are considered reactive with a concentration >1.0 AU/mL.

The assays were carried out on the analyzers Architect I2000sr (Abbott; IL) and the Maglumi 800 (Snibe; Shenzen, China) according to the manufacturer's instructions.

2.3. Statistics and ethics

Sensitivity was calculated as the number of positive patients divided by all the patients for each group. The percentage of concordance between methods was calculated as the number of cases classified in the same way (both positive and both negative) with respect to the overall samples, and was evaluated by the kappa statistic. Quantitative differences between groups of patients were evaluated by the Kruskall-Wallis test. The statistical analysis was performed with MedCalc © Software, Version 19.2.1 (MedCalc Software, Mariakerke, Belgium). All investigations have been conducted by following the tenets of the Declaration of Helsinki and are compliant with institutional policies (Ethical Committee approval n 149/A CESC).

3. Results

3.1. Methods comparison

The qualitative overall concordance between method was 90.3% (kappa statistics, 0.49; 95% CI, 0.22–0.76). The quantitative relationship showed a statistically significant linear correlation (Architect= 0.054 Maglumi + 4.5) with, however, a very disperse distribution of cases. Indeed, the Passing-Bablock correlation showed a significant deviation from linearity (Fig. 1).

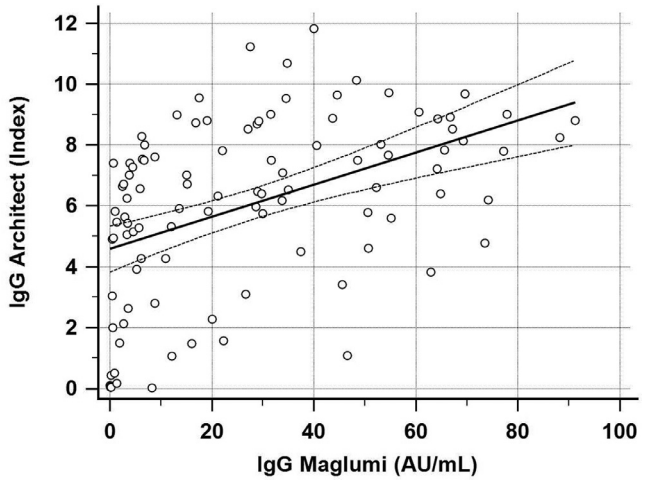

Fig. 1. Correlations between Maglumi and Architect SARS-CoV-2 IgG levels. The trend line represents the Passing-Bablock correlation [Architect = 0.071 (0.050/0.098) Maglumi + 4.3 (3.5/4.9)]

3.2. Sensitivity and time-course profile

The case study was subdivided into 6 groups, for which both the qualitative (Table 2) and the quantitative performance were evaluated.

Considering the quantitative results in the samples, the differences between groups were statistically significant for both methods (Kruskall-Wallis test P = 0.00007 for Maglumi and P = 0.00004 for Architect). However, after a rapid increase up to about 20 days, we can see a subsequent reduction of the concentrations for the levels measured by Maglumi (Fig. 2). A statistically significant decrease could be detected from the group 44 days after the symptoms' onset.

The same specimens measured by Architect showed a similar increase but a more stable behavior, with a modest although significant decrease only after about 85 days (Fig. 3).

There were no significant differences between specimens from patients with different disease severity (data not shown).

The results of the determination with the 2 methods in the 7 patients with at least 3 samples collected at least up to 50 days after the onset of symptoms are shown in Fig. 4.

4. Discussion

The persistence of antibodies against SARS-CoV-2 is not known. Studies on the immune response to other coronaviruses could aid in predicting a possible trend. Concentrations of IgG were found to decline a few months after the onset of symptoms, although the positivity rate remained relatively stable over a longer period (Cao et al., 2007; Wu et al., 2007). A model of antibodies kinetics (Rosado et al., 2020) mainly based on previous experience from other coronaviruses predicted a peak around 2 to 4 weeks and a subsequent slow

Table 2.
Sensitivity of the two methods for IgG determination in the different specimens subdivided in time frames according to the day from the onset of symptoms.

		Positivity rate	
Days from symptoms' onset	n of specimens	Maglumi	Architect
≤11	18	66.7%	66.7%
12–15	16	87.5%	81.2%
16–21	15	100.0%	93.3%
22–43	17	100.0%	100.0%
44–84	17	100.0%	100.0%
85–187	20	80.0%	100.0%

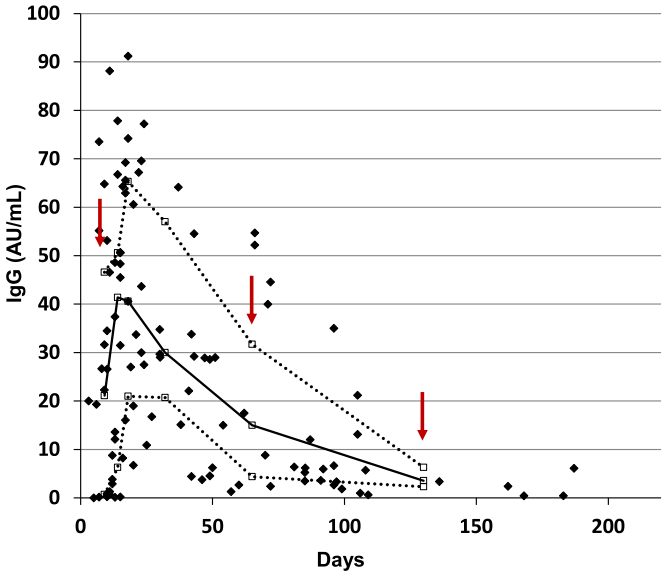

Fig. 2. Distribution of IgG levels of the single specimens measured by Maglumi in relation to the days since the onset of symptoms. In abscissa are reported the days from the onset of symptoms, in ordinate the concentrations of IgG Maglumi. The solid line connect the median concentrations of IgG for each class, the dotted lines connect the 25° to 75° percentile. Arrows represent the classes of cases significantly different from that with higher concentrations.

decrease of antibody titer, with the hypothesis that about 50% of cases will be negative 1 year after the infection.

Recent papers have evaluated the time course of IgG anti-SARS-CoV-2 for a time similar to our study. However, only the study of Gudbjartsson et al. (2020) used more than one method. This wide epidemiological study reported only a slight reduction of the antibodies titer up to 3 to 4 months after the diagnosis. Similar results were found in the smaller study of Bolke et al. (2020). On the other hand, Ibarrondo et al. (2020), Terpos et al. (2020), Kutsuma et al. (2020) and Long et al. (2020) found significant decrease of IgG titer within 3 to 4 months from the symptoms' onset, in accord with the present study.

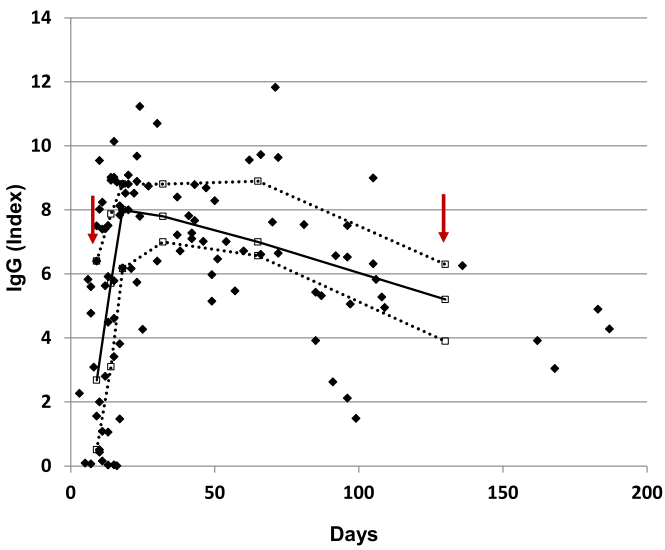

Fig. 3. Distribution of IgG levels of the single specimens measured by Architect in relation to the days since the onset of symptoms. In abscissa are reported the days from the onset of symptoms, in ordinate the concentrations of IgG Architect. The solid line connect the median concentrations of IgG for each class, the dotted lines connect the 25° to 75° percentile. Arrows represent the classes of cases significantly different from that with higher concentrations.

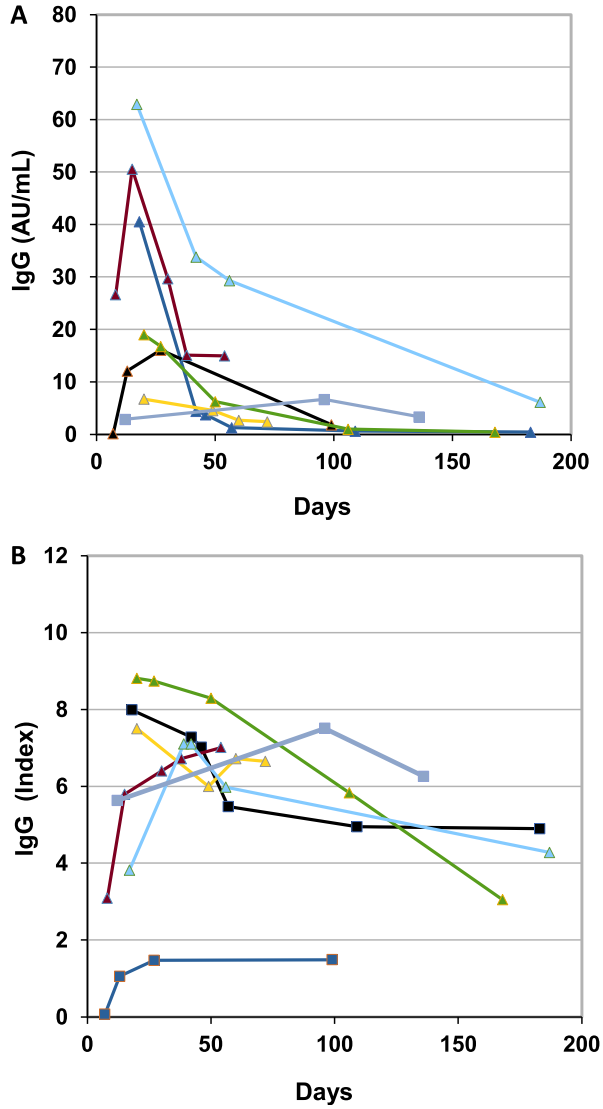

Fig. 4. Spaghetti plot of the 7 patients with at least 3 withdrawal in more than 50 days from the onset of symptoms, measured by Maglumi (A) and Architect (B).

The differences of the methods used and of the antigen targeted could partially explain these different performances. Our study is a clear example of this phenomenon, considering the different antibodies kinetics of the IgG found in the same patients with 2 different methods.

It is also interesting to note that Terpos et al. (2020) and Gudbjartsson et al. (2020) found different antibodies kinetics, also from a qualitative point of view, although the method used by Terpos (Euroimmun IgG anti-S1) was also used by Gudbjartsson. Then, the differences in the number and characteristics of the patients studied and the endpoint of the study should also be considered in the evaluation of the discrepancies in the antibodies time course.

In the present study, we evaluated the correlation between 2 different automated high throughput methods for the IgG determination. In particular, the Maglumi test was previously evaluated only in comparison with an ELISA test (Lippi et al., 2020; Montesinos et al., 2020). The correlation between Maglumi and Architect methods, although statistically significant, showed a much dispersed distribution of cases. These results could be expected, since the 2 methods measured antibodies against different virus proteins, although the Maglumi detects antibodies directed against both spike and nucleocapside proteins. Moreover, a different expression of the results was used in the different methods. In fact, statistically satisfactory but

conflicting quantitative correlations between methods were already previously reported (Jaaskelainen et al., 2020).

Anyway, when measured by Maglumi, the antibody concentrations showed a rapid decline, already significant after 45 days. In the class with specimens from 85 to 187 days after the onset of symptoms the sensitivity dropped from 100% to 80% and the median concentrations of IgG were less than 15% compared to the levels found after about 20 days. On the other hand, the same samples measured by Architect showed a quite constant trend up to 85 days, and then a moderate decline, with positivity rates that did not fall below 100%.

A limitation of this study is that more than a half of the patients were represented by only one sample. To extrapolate the trend, we included specimens at different times obtained from different patients. However, the parallel determination with the two methods in patients with at least 3 samples collected at least up to 50 days after the onset of symptoms confirms an evident decrease over time for Maglumi, and a constant trend or a limited decline for Architect (Fig. 4).

Another limitation could be the lack of the direct comparison with the neutralization test. However, both these types of antibodies seem to correlate with neutralizing antibodies responses (Jaaskelainen et al., 2020; Okba et al., 2020).

A methodological criticism that could be raised is that the methods used, as the majority of the methods worldwide, were reported as qualitative. It is worth noting that in our study the differences over time of the 2 methods were also qualitative. Anyway, the issue of the actual quantitative performance of the different methods is not trivial, since all the considerations about both the antibodies kinetics and the correlations of antibodies titers to disease severity could be regarded as questionable. However, although these methods are often declared as qualitative, they were built with quantitative characteristics. Both the methods used showed characteristics of linearity and imprecision at different levels consistent with a quantitative method (Dittadi et al., 2020; Padoan at al., 2020a; Padoan, 2020b). In particular, the Maglumi method is bases on an antibody standard curve for each assay. Anyway, it cannot be excluded that some of the differences in behavior may be due to the different quantization capacity of the 2 assays.

In conclusion, in this study we determined IgG anti SARS-CoV-2 with 2 methods that measure a different mix of antibodies against the main immunogenic proteins of the virus. The differences between methods should be taken into careful consideration, in particular regarding the possible discordant results in the medium-long term with respect to the onset of the symptoms.

Moreover, our data could suggest an unstable immune response to SARS-CoV-2 infection, more or less evident depending on the type of the antibodies measured and the methods used for the determination. All this should be considered by the laboratories, and could have consequences in carrying out epidemiological studies, in the evaluation of the time of collection of hyperimmune plasma for convalescent plasma therapy, as well as in the prediction of postinfection immunity.

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Declaration of competing interest

No potential competing interest was reported by the authors.

Author contributions

Conceptualization, R.D. and P.C.; sample collection and methodology, R.D. and H.A.; formal analysis, R.D.; writing-original draft preparation, R.D.; writing-review and editing, R.D., H.A. and P.C.; supervision, P.C. All authors have read and agreed to the published version of the manuscript.

Author statement

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