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Letter to the Editor

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To the Editor,

The quite recent emergence of the SARS-CoV-2 pandemic precludes long-term investigations of the immunologic response towards this new pathogen. Depending on the pathogen, serological persistence has been shown to last for months to years, as for SARS-CoV or other human coronaviruses (HCoV) [1]. Antibody responses to SARS-CoV-2 can be detected in most infected individuals 14 days after the symptom onset [2–4]. Recent reports are inconsistent regarding the persistence of antibodies directed against SARS-CoV-2 [5, 6]. These differences may be explained by multiple reasons but are more probably related to methodological issues than real different immunogenic effects. The aim of this study was to evaluate the long-term kinetics of anti-SARS-CoV-2 antibodies in a population of RT-PCR confirmed positive SARS-CoV-2 subjects and to describe the kinetics of antibodies in hospitalized patients compared to the one of non-hospitalized patients, including asymptomatic individuals.

A total of 197 patients with a confirmed SARS-CoV-2 RT-PCR were retrospectively included from March 21 to October 27, 2020. Demographic of patient participants are present in Supplementary Table 1. A total of 314 serum samples was analyzed for the detection of anti-SARS-CoV-2 antibodies. The World Health Organization (WHO) clinical progression scale was use to categorize patients according to disease severity (score 1 = asymptomatic, non-hospitalized; score 2–3 = mild disease, non-hospitalized; score >3 = moderate-severe disease, hospitalized) [7]. Information of symptom onset was gathered in clinical files of patients and/or by contacting the medical practitioners. Blood samples were collected into serum-gel or in lithium-heparin plasma tubes (BD Vacutainer® tubes, Becton Dickinson, New Jersey, USA) according to standardized operating procedures. Samples were centrifuged for 10 min at 1,885 × *g* (ACU Modular® Pre-Analytics, Roche Diagnostics®). The Elecsys anti-SARS-CoV-2 nucleocapsid (NCP) electrochemiluminescent immunoassay (ECLIA) (Cobas e801, Roche Diagnostics®, Basel, Switzerland) for the *in vitro* qualitative detection of total antibodies (IgG, IgM and IgA) to SARS-CoV-2 was used. The test result is given as a cut-off index (COI). According to the manufacturer, a result <1.0 is considered negative while a result ≥1.0 is considered positive. An optimized cut-off of 0.165 was also considered based on our previous validation [8] which has been confirmed by a recent study performed by the National SARS-CoV-2 Serology Assay Evaluation Group (i.e. 0.128) [9]. The specificity of the test was excellent in several independent evaluations (99.8–100%) [8–11]. The RT-PCR for SARS-CoV-2 determination in respiratory samples (nasopharyngeal swab samples) was performed on the LightCycler® 480 Instrument II (Roche Diagnostics®) using the LightMix® Modular SARS-CoV E-gene set.

Samples were subdivided according to the following categories since symptom onset, 0–1 week: 44 sera; 1–2 weeks: 30 sera; 2–4 weeks: 60 sera; 4–6 weeks: 18 sera; 6–11 weeks days:

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47 sera; 11–17 weeks: 57 sera; 17–26 weeks: 34 sera and 26–32 weeks: 24 sera. The antibody kinetics was determined separately for hospitalized and non-hospitalized patients. In asymptomatic patients, the day of RT-PCR positivity was used instead of the day of symptom onset. A kinetics for patients that had at least 3 blood sampling with a last collection time at more than 7 weeks since symptom onset was also evaluated separately (10 non-hospitalized and 11 hospitalized patients).

Descriptive statistics were used to analyze the data. The mean COI results (and 95% CI) were plotted against the different timeframes. Sensitivity was defined as the proportion of correctly identified COVID-19 positive patients initially positive by RT-PCR SARS-CoV-2 determination. Smoothing splines with four knots were used to estimate the time kinetics curve in hospitalized (WHO score >3) and non-hospitalized patients (WHO score 2–3). Dunn's multiple comparisons test was used to assess potential differences between sampling timings. Data analysis was performed using GraphPad Prism® software (version 9.0.0, California, USA). p-value<0.05 was used as a significance level. The study fulfilled the Ethical principles of the Declaration of Helsinki.

In symptomatic patients, a gradual increase in antibody titers up to the last timepoint was observed (Figure 1). We confirm that sampling before 2 weeks does not permit to identify previous or ongoing infection due to insufficient sensitivity. However, within the first week, the positivity trend was higher in hospitalized patients (i.e. 50%) compared to non-hospitalized patients (i.e. 20%), an observation already made by Long et al. [12] and by Gillot et al. [13]. From 4 to 6 weeks, excellent sensitivities were observed (Table 1, Figure 1). Individual results for hospitalized patients were largely above the manufacturer's cut-off. In non-hospitalized patients, one asymptomatic subject did not developed antibodies against the NCP (Figure 1A).

A trend towards higher antibody titers in hospitalized patients was also observed from weeks 6 to 11. The difference was higher if considering weeks 17 to 32 (Figure 1B). Other studies also reported higher levels of antibodies in patients with more severe disease [2, 14–16]. Of the 21 patients for which at least three independent blood drawn were available for a minimal follow-up period of 7 weeks, a decrease in antibody titer was observed for 4 non-hospitalized patients out 10 (40%). In hospitalized patient, the titer gradually increased to reach a plateau without any decrease (n=11; 100%) (Supplementary Figure 1). Nevertheless, the association was not found to be significant in our study (p>0.05).

Importantly, the antibody kinetics may vary according to the type of assay considered. Recent studies

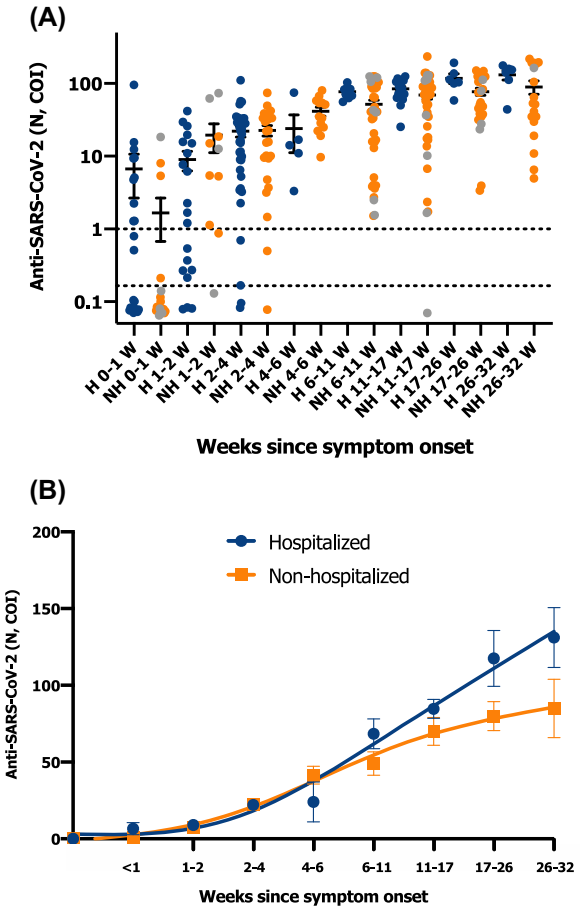

Figure 1: Anti-SARS-CoV-2 titers and long-term kinetics. (A) Anti-SARS-CoV-2 titers (mean COI and 95% CI) from symptom onset in hospitalized (blue points) and non-hospitalized (orange points) COVID-19 patients (timeframe in weeks). Grey points correspond to asymptomatic patients that had a positive RT-PCR. (B) Long-term kinetics of anti-SARS-CoV-2 in hospitalized (blue points) and non-hospitalized (orange points) COVID-19 patients (timeframe in weeks). Smoothing splines with four knots were used to estimate the time kinetics curve (mean standard ± error of the mean). Asymptomatic patients were excluded from the analysis.

are in line with the current results and also found a sustained antibody response against the NCP antigen using the Roche total antibody assay, on a lower follow-up period (i.e. 3–4 months) [15, 17]. A sustained antibody response against the receptor-binding domain (RBD) antigen, as assessed by the Wantai and the Siemens total antibody assays, was also observed up to 4 months [15, 17]. A decrease in anti-RDB IgG and anti-spike IgG levels was similarly observed over a period of up to 5 months in recent reports [6, 14, 15, 18, 19]. A significant decrease in sensitivity was also found using the Abbott assay (NCP IgG), in studies with up to 4 months of follow-up [15, 17].

Table 1: Anti-SARS-CoV-2 titers (mean COI and 95% CI) from symptom onset in hospitalized and non-hospitalized COVID-19 patients.

	Time intervals (weeks since symptom onset)	0–1	1–2	2–4	4–6	6–11	11–17	17–26	26–32
Non-hospitalized	n	14 (+6)	6 (+4)	23 (+2)	13 (+0)	31 (+7)	31 (+8)	24 (+4)	17 (+1)
WHO score 2–3 (+1)	Mean (95% CI)	1.0	7.7	22.4	41.4	49.1	70.0	80.0	84.8
		(−0.4 to 2.4)	(0 to 15.5)	(14.8 to 30.0)	(28.7 to 54.1)	(33.4 to 64.7)	(51.5 to 88.5)	(60.4 to 99.5)	(44.5 to 125.3)
	Sensitivity (%)	20.0	90.0	96.0	100	100	97.4	100	100
		(5.7–43.7)	(55.5–99.9)	(79.7–99.9)	(75.3–100)	(90.8–100)	(86.4–99.9)	(87.7–100)	(81.5–100)
Hospitalized	n	24	20	35	5	9	18	6	6
WHO score >3	Mean (95% CI)	6.7	9.0	22.1	24.0	68.5	84.7	117.6	131.2
		(−1.6 to 14.9)	(3.3 to 14.6)	(14.4 to 30.0)	(−11.8 to 59.9)	(46.0 to 90.9)	(71.7 to 97.7)	(70.5 to 164.7)	(80.9 to 181.4)
	Sensitivity (%)	50.0	85.0	94.3	100	100	100	100	100
	(95% CI)	(29.2–70.1)	(62.1–96.8)	(80.8–99.3)	(47.8–100)	(66.4–100)	(81.5–100)	(54.1–100)	(54.1–100)

Numbers between brackets correspond to asymptomatic patients (WHO score 1). The cut-off used to calculate sensitivity was 0.165.

The sustained antibody response as measured with total antibody assays (NCP and RBD) compared to IgG assays may be due to the additional response of non-IgG antibody isotypes. The reasons for the differences in assay performance over time for assays targeting the same antigen remain however unclear [17]. Whether the antibodies measured with commercial assays has a neutralizing capacity is paramount to indicate the potential level of protective immunity against SARS-CoV-2 infection. Antibody titers generated with available assays correlated differently with neutralizing antibody titers [17, 20–22]. The Roche assay was the weaker predictor of neutralizing capacity (r=0.56, p=0.0001) compared to the Abbott assay (NCP IgG) (r=0.69, p<0.0001), Siemens assay (RDB total antibodies) (r=0.74, p<0.001), and the S1/S2-based DiaSorin assay (S1/S2 IgG) (r=0.84, p<0.0001) [17]. Jahrsdörfer et al. and Padoan et al. confirm that the weaker correlation was observed using the Roche assay [11, 22], and McAndrews et al. found that 86% of individuals positive for RBD-directed antibodies exhibited neutralizing capacity, whereas only 76% of positive NCP-directed antibodies exhibited neutralizing capacity [23]. The fact that anti-NCP assays have the lowest neutralizing capacity could be expected, as neutralizing antibodies are directed to the spike protein responsible for enabling cell entry. Indeed, a strong correlation between levels of anti-RBD or anti-spike antibodies and neutralizing capacity has been found in independent evaluations [5, 6, 14, 19, 24]. Neutralizing capacity remained robust from 1 to 5 months in several studies [14, 19, 20], although modest declines at 3–5 months were observed by Wajnberg et al. and Isho et al. [5, 18]. Other studies however observed a significant decrease of 2 to 4-fold, in neutralizing capacity up to 3 months [16, 17, 25–27].

It is important to keep in mind that some patients may develop anti-spike or anti-RBD antibodies but may not have detectable neutralizing antibodies. These are only correlation studies which are not related to direct measures of neutralizing capacity [17]. The fact that neutralizing antibodies constitute a major protective mechanism against SARS-CoV-2 infection deserves that further investigation are done in this area to assess to long-term inhibition capacity of SARS-CoV-2 antibodies [5, 9, 17]. The contribution of B cells and T cells to immunity to SARS-CoV-2 should also be more explored and it seems important to remind that previous exposure to SARS-CoV-2 might not guarantee total immunity in all cases since reinfection with SARS-CoV-2 have been described [28, 29].

In conclusion, we found a gradual increase in anti-NCP total antibody titers for up to 32 weeks since symptom onset. Even if some non-hospitalized patients showed a slight tendency towards a decrease of their antibody titer, this study found that detection rates were similar in

hospitalized or non-hospitalized patients after one week from symptom onset and last at least 7.5 months. Although the majority of asymptomatic patients (95%) developed a sustained antibody response, one patient did not developed antibodies 11 weeks after the RT-PCR positivity supporting the claim that caution is advised when interpreting anti-SARS-CoV-2 antibodies in asymptomatic subjects.

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