




# Determinants of IgG antibodies kinetics after severe and critical COVID-19

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## Abstract

The kinetics of IgG antibodies after coronavirus disease 2019 (COVID-19) remain poorly understood. We investigated factors influencing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG antibody levels and time to seronegativation during the follow-up of severe and critically ill patients. We retrospectively reviewed serological evaluations drawn during the follow-up of severe or critical laboratory-proven COVID-19 patients hospitalized at a large academic hospital. Specific IgG titers were measured using a chemiluminescent assay targeting anti-spike and anti-nucleocapsid protein IgG. The influence of time, demographic factors, clinical and paraclinical characteristics, and COVID-19 therapeutics on IgG levels were assessed through linear regression using a mixed-effect model, and delay until IgG negatvation through a Weibull regression model. The cohort included 116 patients with a total of 154 IgG measurements drawn at a median of 79 days after diagnosis. IgG antibodies were increased with age ( $p = 0.005$ ) and decreased significantly over time ( $p = 0.0002$ ). Using elapsed time and age as covariates, we demonstrated higher IgG levels in patients with a higher body mass index (BMI) ( $p = 0.0026$ ) and lower IgG levels in immunocompromised patients ( $p = 0.032$ ). A high BMI was further found to delay and immunodeficiency to hasten significantly seronegativation, whereas no significant effect was observed with corticosteroids. These data highlight the waning over time of IgG antibodies after severe or critical COVID-19. Age, BMI, and immunosuppression also appear to influence the IgG kinetics, while short-term corticotherapy does not. Those data improve the understanding of SARS-CoV-2 serology while further research should determine the determinants of long-term seroprotection.

## KEYWORDS

corticosteroids, COVID-19, IgG, kinetics, SARS-CoV-2, serology

## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a major human pathogen. Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 is primarily diagnosed through real-time reverse-transcription polymerase chain reaction (RT-PCR) testing.<sup>1</sup> Numerous serological tests have been developed and commercialized, relying mostly on enzyme-linked immunosorbent assay (ELISA) and chemiluminescence assays (CLIA) to detect IgA, IgM, or IgG against SARS-CoV-2 antigens (spike and nucleocapsid glycoproteins). The detection of antibodies can be used as a diagnostic tool at later timepoints in the disease course, when the sensitivity of RT-PCR decreases.<sup>2</sup> As IgG antibodies are expected to remain detectable after infection resolution,<sup>3</sup> serological testing is also used in seroprevalence studies, to diagnose past exposure to SARS-CoV-2, including in pauci or asymptomatic patients<sup>4</sup> although it has been stressed that the magnitude of the antibody response appears to be associated with disease severity.<sup>5</sup> Serological testing also carries the theoretical possibility to inform individuals about their immune protection against reinfections. Indeed, the presence of detectable antibodies after COVID-19 has been shown to correlate with immune protection for at least 6 months in a large cohort of healthcare workers.<sup>6</sup>

However, antibody kinetics remains incompletely understood. While most patients elicit a robust humoral response with detectable anti-SARS-CoV-2 antibodies, those antibodies will quickly become undetectable in a significant subset of patients.<sup>7,8</sup> Factors influencing the persistence of high antibody titers following COVID-19 remain poorly identified. In addition, whether treatment of COVID-19 with immunosuppressive agents such as dexamethasone influences antibody levels and kinetic has not been assessed.

This study aims (i) to describe the kinetics of serum IgG antibodies and (ii) to investigate the determinants underlying kinetics and IgG negativation at the convalescent phase of COVID-19 in a cohort of hospitalized patients with severe and/or critical disease.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and patients

Patients with RT-PCR-proven COVID-19 and who reached at least four on the WHO ordinal scale (i.e., patients requiring oxygen supplementation) were identified within our database of COVID-19 patients hospitalized in Cliniques universitaires Saint-Luc, an academic hospital of 1000 beds in Brussels, Belgium. We further selected for analysis the patients with at least one serological assessment drawn after the 14th day of diagnosis and before November 5, the date of analysis. We excluded patients who received convalescent plasma therapy.

All patients were treated according to Belgian and local guidelines. Besides best supportive care, and in accordance with ongoing Belgian guidelines at that time,<sup>9</sup> most patients were treated with

hydroxychloroquine until the end of May 2020. Although not recommended before the publication of Recovery trial results,<sup>10</sup> corticosteroids had been used earlier by some clinicians in case of progressing respiratory failure.

### 2.2 | Data collection

The following data were studied: demographics, clinical characteristics (signs and symptoms, comorbidities, and usual treatment), biological and imaging results, treatments administered, and outcome.

The local ethics committee approved our database (registration number 2020/06AVR/201) and waived the requirement for informed consent based on the retrospective observational design of the study.

### 2.3 | Definitions

With the aim of providing the most objective assessment of disease onset, the date of diagnosis was defined as the date of the first positive SARS-CoV-2 RT-PCR.

Immunosuppression was defined as any condition or treatment known to interfere with humoral or cellular immunity. Before running statistical analysis, we decided not to consider as immunosuppressed two HIV-positive patients with high CD4 cells count (650 and 1120 cells/mm<sup>3</sup>, respectively) and long-term viral replication control.

### 2.4 | SARS-CoV-2 serological testing

The detection of SARS-CoV-2 antibodies in human serum was performed using the MAGLUMI-800 CLIA system (Snibe Diagnostic). This system allows the detection of both IgM and IgG binding SARS-CoV-2 spike and nucleocapsid proteins. MAGLUMI assay has received CE marking and Food and Drug Administration's Emergency Use Authorization.

According to the manufacturer, the calculated sensitivities and specificities of IgG were respectively 91.2% and 97.3%. This high sensitivity was recently confirmed by Soleimani et al.<sup>11</sup> who reported a sensitivity of more than 95% by the 18th day after symptom onset. A result was considered positive if the index is more than or equal to 1.00 AU/ml, according to the manufacturer's recommendations.

### 2.5 | Statistical analysis

Results are presented as means  $\pm$  SD or median (interquartile range [IQR]) for continuous variables and as numbers and proportions for categorical variables.

Mixed effect model linear regressions were performed to assess the effect of independent variables on IgG titers. After evaluation of their effect on IgG titers, the time lapse from diagnosis to IgG

measurements (Model 1) and the time lapse in combination to the subject's age (Model 2) were added as model covariates. To account for non-normal IgG titer distribution, IgG titers were normalized by Box-Cox transformation. As we cannot a priori assume a linear relationship between IgG titers and time, time was expressed as a categorical variable by dividing the sample into five successive categories with equivalent numbers of patients: Day 14–64 (Period 1), Day 65–75 (Period 2), Day 76–85 (Period 3), Day 86–105 (Period 4), and Day 106–221 (Period 5).

For therapeutic interventions (corticosteroids, high-flow nasal cannula [HFNC], and mechanical ventilation [MV]), we completed the analysis with a propensity score: probability of receiving the intervention was calculated by logistic regression as a function of date of hospitalization, sex, age, BMI, renal function (inverse of serum creatinine), presence of fever, cough, dyspnea, hypertension, diabetes and immunodeficiency; the relationship between IgG titer and therapeutic intervention was studied and regressions on therapeutic interventions were weighted by the inverse of the probability of receiving the intervention.

We studied the delay until IgG negatization using a Weibull regression model; when the last available IgG measurement remained positive, this test date was considered as a censor; when the first available test was already negative, we assumed that the last positive date was Day 21. Hazard ratios (HR) were calculated with age as a covariate.

To minimize the bias related to the asymmetry of the independent variables, we used a sandwich-variance for regressions and calculated degrees of freedom according to Bell and McCaffrey.<sup>12</sup> All  $p$  values were two-sided and  $p$  values  $\leq 0.05$  were considered significant. Statistical analyses were performed using SPSS v27 (IBM).

### 3 | RESULTS

We included a total of 154 IgG measurements performed on 116 patients (Figure 1). The main baseline characteristics of our cohort are shown in Table 1. Briefly, the median age (IQR) was 58 (51–65)

years, with a majority (70/116, 60%) of males. About 88 patients (76%) had at least one comorbidity, with 55 (47%) suffering from cardiovascular disease, 39 (34%) from obesity, and 22 (19%) being immunocompromised.

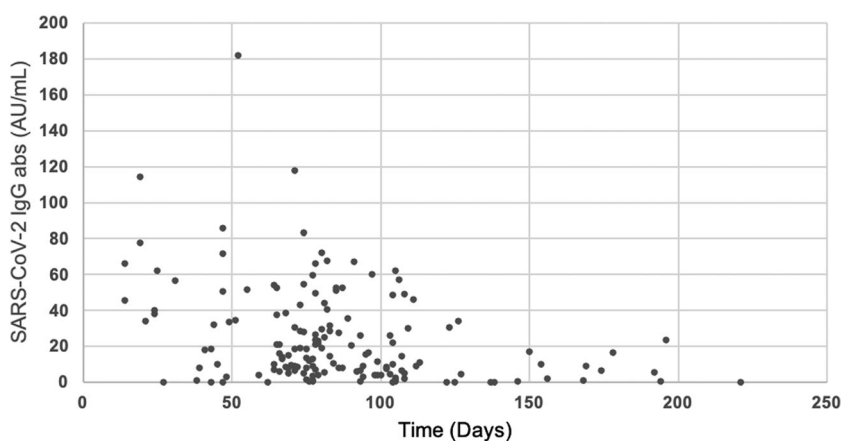
Of the 116 patients, 30 patients had 2 measurements while four patients had 3. IgG measurements were performed at a median of 79 days after COVID diagnosis (IQR 67–103), 63 measurements were performed more than 12 weeks and 8 more than 24 weeks after diagnosis. Ten patients had a negative IgG measurement, drawn at a median of 77 days (IQR 51–98), of which three patients had had a previous positive IgG serology.

#### 3.1 | Influence of time-lapse on IgG titer

We found a significant decrease of specific IgG titers over time ( $p = 0.0002$ ). Mean IgG titers decreased when comparing every time period to the next one, with the exception of the second to third period: mean IgG titers of 29.82 (95% confidence interval [CI], 19.31–44.39) for Period 1, 17.15 (95% CI, 13.60–21.39) for Period 2, 18.22 (95% CI, 13.50–24.13) for Period 3, 10.04 (95% CI, 6.98–19.99) for Period 4, and 6.85 (95% CI, 4.56–9.86) for Period 5. The distribution of IgG titers over time is further described with medians and CI in Figure S1.

#### 3.2 | Influence of demographics and comorbidities on IgG antibody levels

We found a trend towards an increase of IgG with increased age in univariate analysis ( $p = 0.077$ ), which became significant ( $p = 0.005$ ) when considering time elapsed between diagnosis and IgG measurements as a covariate. We found increasing IgG titers with higher BMI ( $p = 0.012$  and  $0.0026$ , respectively for Model 1 and 2) and lower IgG levels in the group of immunocompromised patients (two patients with hypogammaglobulinemia excluded) ( $p = 0.063$  and  $0.032$ , respectively for Model 1 and 2). We found



**FIGURE 1** Distribution of IgG antibodies over time after COVID-19. abs, antibodies; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

**TABLE 1** Characteristics of the 116 patients with serological assessment included in the cohort

Demographics and comorbidities	Whole cohort (n = 116)
Age, mean (SD), years	58.5 (11.9)
Male gender – no. (%)	70 (60.3)
Ethnicity – no. (%)	
Caucasian	68 (58.6)
Sub-Saharan African	21 (18.1)
Other	23 (19.8)
Unknown	4 (3.4)
BMI, mean (SD), kg/m <sup>2</sup>	28.6 (5.0)
Obesity (BMI ≥ 30) – no. (%)	39 (33.6)
Cardiovascular disease – no. (%)	55 (47.4)
Chronic pulmonary disease – no. (%)	18 (15.5)
Hypertension – no. (%)	56 (48.3)
Diabetes – no. (%)	19 (16.4)
Chronic kidney disease, Stage 4 or 5 – no. (%)	7 (6.0)
Immunosuppression – no. (%)	22 (18.9)
Solid organ transplant recipient	8 (6.9)
Auto-immune disease <sup>a</sup>	7 (5.2)
Ongoing treatment for cancer <sup>b</sup>	6 (6.0)
Hypogammaglobulinemia <sup>c</sup>	2 (1.7)
COVID-19 severity	
Highest grade of respiratory support	
HFNC	11 (9.5)
Mechanical ventilation	9 (7.8)
ECMO	5 (4.3)
COVID-19 treatment	
Chloroquine – no. (%)	2 (1.7)
Hydroxychloroquine – no. (%)	101 (87.1)
Corticosteroids – no. (%)	27 (23.3)

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; HFNC, high flow nasal cannula.

<sup>a</sup>1 receiving high-dose corticosteroids, 1 tocilizumab, 1 ocrelizumab, 1 corticosteroids and tocilizumab, 1 corticosteroids and mycophenolate mofetil, 1 dimethyl fumarate, and 1 etanercept.

<sup>b</sup>3 under chemotherapy, 1 chemoradiotherapy, 1 high-dose corticosteroids, and 1 durvalumab.

<sup>c</sup>One transplant patient had hypogammaglobulinemia.

no significant effect for other studied variables, including gender, ethnicity, and main co-morbidities (cardio-vascular disease, hypertension, pulmonary disease, diabetes, and chronic kidney disease) (Table 2).

### 3.3 | Association between clinical course, paraclinical findings, and IgG antibody levels

We did not find association between symptoms presented by the patients at admission and the IgG titers, with the exception of cough ( $p$  value respectively 0.078 and 0.035 for Model 1 and 2). Laboratory tests on admission known as associated with unfavorable outcome such as C-reactive protein, lactate dehydrogenase, creatinine, or low lymphocyte count did not show any influence on IgG levels (Model 1 and 2), with the exception of liver function test upon admission: high aspartate aminotransferase (ASAT) levels were significantly associated with higher IgG titers ( $p = 0.047$  and  $0.036$ ) while the association was not significant for alanine aminotransferase (Table S1). Concerning the severity of infection, the extent of lung involvement on the admission computed tomography scan showed no significant association with IgG levels. Moreover, we did not find an association between the severity of infection and IgG titers (Model 1 ad 2), as assessed by the need for HFNC ( $p = 0.92$  and  $0.66$ ), MV ( $p = 0.32$  and  $0.44$ ), or either respiratory support ( $p = 0.52$  and  $0.85$ ). Results were confirmed after weighting with a propensity score for the use of respiratory support (Table 2).

### 3.4 | Influence of corticosteroid treatment on IgG antibody levels

We did not find the influence of the administration of corticosteroids as COVID-19 treatment on IgG titers ( $p = 0.12$  and  $0.072$ ) using both models. Those results were confirmed after weighting with a propensity score for the use of corticosteroids (Table 2).

### 3.5 | Variables influencing time to seronegativation

We further assessed the association between the different variables and the occurrence of IgG seronegativity during follow-up. Age was not shown to affect the time to seronegativation ( $p = 0.34$ ). A higher BMI (HR = 0.843;  $p = 0.020$ ) and the presence of cough (HR = 0.203;  $p = 0.026$ ) were associated with delayed seronegativation. On the opposite, immunodeficiency was found significantly associated with shorter time to seronegativation (HR = 6.170;  $p = 0.0042$ ). Other comorbidities and severity of infection (assessed by the need for HFNC, MV, or either respiratory support) showed no significant effect. No patient treated with corticosteroids was found seronegative at follow-up. All results were confirmed in a bivariate analysis using age as a covariate (Table 3).

## 4 | DISCUSSION

In this study, we retrospectively analyzed IgG titers drawn during the follow-up of severe and critical COVID-19 patients. We found a significant decrease of IgG titers over time. We showed a negative

TABLE 2 Influence of demographic parameters, comorbidities, and clinical course on SARS-CoV-2 IgG titers<sup>a</sup>

	p value, considering time-lapse as covariate (Model 1) <sup>b</sup>	Standardized beta coefficient (95% CI)	p value, considering time lapse and age as covariates (Model 2) <sup>b</sup>	Standardized beta coefficient (95% CI)
Age	0.0050 **	+0.228 (+0.071 to +0.385)	/	+0.233 (+0.084 to +0.382)
BMI	0.012*	+0.212 (+0.049 to +0.375)	0.0026**	
<b>Comorbidities</b>				
Diabetes	0.074	+0.175 (-0.021 to +0.37)	0.079	+0.167 (-0.024 to +0.358)
Chronic kidney disease	0.73	+0.040 (-0.211 to +0.290)	0.96	+0.008 (-0.263 to +0.280)
Immunodeficiency	0.063	-0.178 (-0.374 to +0.018)	0.032*	-0.200 (-0.388 to -0.013)
<b>Clinical course</b>				
Need for HFNC	0.92	-0.010 (-0.197 to +0.177)	0.66	+0.041 (-0.147 to +0.229)
Need for HFNC after propensity score weighting <sup>c</sup>	0.93	+0.012 (-0.228 to +0.252)	/	
Need for MV	0.32	-0.090 (-0.271 to +0.091)	0.44	-0.073 (-0.264 to +0.119)
Need for MV after propensity score weighting <sup>c</sup>	0.62	-0.058 (-0.175 to +0.058)	/	
Need for either HFNC or MV	0.52	-0.062 (-0.254 to +0.129)	0.85	-0.019 (-0.215 to +0.177)
Need for either HFNC or MV after propensity score weighting <sup>c</sup>	0.83	-0.092 (-0.302 to +0.119)	/	
Corticoid treatment	0.12	+0.120 (-0.034 to +0.275)	0.072	+0.137 (-0.014 to +0.288)
Corticoid treatment after propensity score weighting <sup>c</sup>	0.13	+0.140 (-0.042 to +0.322)	/	

Abbreviations: BMI, body mass index; CI, confidence interval; HFNC, high flow nasal cannula; MV, mechanical ventilation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>Effect was evaluated on transformed values of IgG.

<sup>b</sup>Time lapse between diagnosis and IgG measurement is analyzed as a categorical covariable, measurements being divided in five consecutive time periods.

<sup>c</sup>The probability of receiving the intervention was calculated by logistic regression as a function of date of hospitalization, sex, age, BMI, renal function (inverse of creatinine), presence of fever, cough, dyspnea, hypertension, diabetes, and immunodeficiency.

association between immunosuppression and IgG levels at follow-up while the use of corticosteroids to treat COVID-19 did not show an influence within this timeframe. In contrast, we found that age, a high BMI and high ASAT at admission were associated with raised follow-up IgG antibody titers. Lastly, reinforcing our previous findings, immunosuppression was associated with reduced time to seronegativity while it appeared delayed by high BMI.

Antibodies waning after SARS-CoV-2 infection has been repeatedly reported, especially in mild/moderate COVID-19,<sup>13,14</sup> a feature raising concerns about similarities with immunity against other coronaviruses, where declining antibodies at 1 year do not fully protect against reinfection.<sup>15</sup> Long et al.<sup>7</sup> found that the IgG levels in 93% (28/30) of the asymptomatic patients and 97% (30/31) of the symptomatic patients declined during the early convalescent phase. Contrarily, a recent study of humoral response to SARS-CoV-2 in Iceland by Gudbjartsson et al.<sup>4</sup> using two pan-immunoglobulin assays found that among 1215 persons who had recovered from SARS-CoV-2 infection, 1107 (91%) were seropositive and antiviral antibody titers increased during 2 months after diagnosis by RT-PCR and remained on a plateau for 4 months.

Limited follow-up data is available after severe infection. Wang et al.<sup>16</sup> showed in a cohort of hospitalized patients that antibody levels peaked between day 31-40, followed by a slight decrease. In a small subset of 8 patients, they found diverse kinetics of neutralizing antibody, with some patients seeing increased and others decreased titers. Zhang et al.<sup>17</sup> showed in 112 COVID-19 hospitalized patients that IgG persisted for over 194 days after symptom onset, although patients showed a 46% reduction in antibodies titers against SARS-CoV-2 nucleocapsid protein compared with the acute phase.

A striking finding of our analysis is the association of age and obesity with higher IgG titers. This may appear counterintuitive, as both factors are usually thought to associate with poor adaptive immune response,<sup>18,19</sup> but confirms similar findings for age<sup>4,16,20-23</sup> and obesity<sup>4,21,22</sup> after COVID-19. Although absolute IgG levels have been shown to positively correlate with age, a decreased capacity of humoral response to novel antigens has been shown in elderlies, linked to a decreased naïve B cell repertoire.<sup>24</sup> Illustrating this reduced B-cell responsiveness, overall lower IgG levels after

vaccination have been demonstrated in elderlies.<sup>25</sup> Moreover, a decreased antibody quality in old age has also been shown.<sup>26</sup> The impact of obesity on humoral immunity appears more complex. A reduced level of immunoglobulin levels after hepatitis B and tetanus vaccination has been shown in this population,<sup>27</sup> but Sheridan et al.<sup>28</sup> found a significant correlation between BMI and IgG levels one month after influenza vaccination, but a greater decline at 12 months in obese patients. Further follow-up of the obese COVID-19 patient population is warranted to draw a better picture of long-term immunoglobulin kinetics.

In their seroprevalence study of the Icelandic population, Gudbjartsson et al.<sup>4</sup> found an association in recovered persons between higher antibody levels and older age, a higher BMI, but also disease severity. The latter finding corroborates other results showing that levels of anti-SARS-CoV-2 antibodies correlate with disease severity.<sup>23,29</sup> As age and obesity constitute major risk factors for severe COVID-19 and its dysregulated immune response,<sup>30</sup> higher antibody levels at follow-up might mirror the intensity of the immune response during infection. Interestingly, we also showed higher IgG titers in patients with elevated ASAT at admission, a feature that has been associated to COVID-19 severity.<sup>31</sup> One might argue that our analysis did not show any correlation between IgG levels and infection severity. However, one should keep in mind that our analysis focused on hospitalized patient with severe COVID-19, thus limiting its ability to detect an effect when considering severity as a spectrum ranging from asymptomatic to critical disease. This ability was further decreased by the fact that we could not include the sickest patients in our analysis, as death at the acute stage of infection precluded the drawing of follow-up blood samples.

The usefulness and yield of serological evaluation in immunocompromised patients remain unknown. Small case series<sup>32,33</sup> have found seroconversion of transplant recipients but reduced seroconversion has been shown in hematological malignancy patients.<sup>34</sup> Inflammatory bowel disease patients treated with infliximab showed reduced seropositivity and reduced antibody reactivity, compared with controls treated with the non-immunosuppressive vedolizumab.<sup>35</sup>

**TABLE 3** Influence of BMI and comorbidities on time to seronegativity of SARS-CoV-2 IgG

	Univariate analysis		Bivariate analysis considering age as a covariate	
	p value	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)
BMI	<b>0.020*</b>	0.843 (0.730–0.973)	<b>0.018*</b>	0.844 (0.733–0.791)
Cardio-vascular disease	0.23	2.328 (0.601–9.017)	0.13	2.990 (0.729–12.262)
Hypertension	0.23	2.301 (0.594–8.906)	0.15	2.836 (0.695–11.573)
Pulmonary disease	0.62	1.490 (0.316–7.018)	0.50	1.724 (0.355–8.365)
Diabetes	0.58	0.553 (0.070–4.364)	0.56	0.537 (0.068–4.248)
Immunodeficiency	<b>0.0042**</b>	6.170 (1.777–21.430)	<b>0.0030**</b>	6.703 (1.911–23.512)

Abbreviations: BMI, body mass index; CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



We found no correlation between the administration of corticosteroids as COVID-19 treatment and IgG titers. In the context of SARS-CoV-1, Woo et al.<sup>36</sup> described a biphasic IgG antibody response after infection and speculated this was the result of high-dose corticosteroid treatment. Glucocorticoids are potent immunosuppressive drugs with pleiotropic effects, including blocking the synthesis of numerous cytokines, inhibiting the expression of cellular adhesion molecules, and promoting lymphocyte apoptosis.<sup>37</sup> Short-course corticosteroid treatments have been shown to induce IgG as well as IgA level depression that may persist for several weeks.<sup>38</sup> More worryingly, a decreased neutralizing antibody response to naturally acquired herpes virus infection has been shown on volunteers exposed to short-term corticosteroids. However, in line with our findings, antibody response to antigens appeared unaffected.<sup>39</sup> Although limited by its sample size, our data provide some reassurance on the indirect effects of COVID-19 corticosteroid treatment on humoral response.

The strengths of this study include the rigorous selection of severe and critical patients with proven infection, the length of follow-up and serial serological assessments enabling evaluation of the effect of time on IgG levels. Moreover, the originality of this study is the in-depth characterization of patients and of COVID-19 clinical and paraclinical phenotype, and the study of their impact on IgG titers. Most published studies so far focused on overall antibody kinetics without taking into account individual factors to explain the variability in serological course.

The first limitation of this study is its retrospective design. Data were retrieved from electronic health records, and incomplete data reporting cannot be excluded, particularly regarding the reported symptoms, as other collected data are by nature less prone to errors.

The second limitation is that we did not assess the neutralizing activity of included serums. Valvidia et al.<sup>40</sup> studied the correlation between spike-binding neutralizing antibody and different commercial serology assays, including the MAGLUMI assay. Overall, all tested assays correlated with neutralizing activity, albeit at varying levels.<sup>40</sup> For the MAGLUMI test used in our study, they found an overall Rho value for correlation of 0.48 ( $p < 0.001$ ). Differences between assays regarding recognized epitopes may play an important role, as antibodies against immunodominant epitopes elicit the highest neutralizing activity. Interestingly, after the fourteenth day of symptoms, they found only one over 49 samples being discordant with negative MAGLUMI and positive neutralizing assay. As such, our results only provide a partial overview of humoral response to SARS-CoV-2. However, as stated, IgG values measured with MAGLUMI correlate with neutralizing activity. Moreover, we provided concordant results when studying determinants of IgG kinetics and seronegativity. Eventually, the fact that our main results corroborate results of other groups offers reassurance regarding their robustness. In any case, our results can help clinicians in their interpretation of serological tests.

Another limitation is that our work only assessed humoral immunity. Seronegativity should not be interpreted as the absence

of protecting immunity, as cellular immunity is a key component of response and protection against COVID-19.<sup>41</sup> Indeed, using interferon- $\gamma$  ELISPOT, Schwarzkopf et al.<sup>42</sup> observed that 78% of RT-PCR-positive volunteers with undetectable antibodies showed T-cell immunity against SARS-CoV-2. However, cellular immunity assessment is not routinely available, and serology is often used to determine past exposure. Different compartments of memory immunity have been shown to follow independent kinetics after COVID-19,<sup>43</sup> and all warrant specific interest. Determining predictors of IgG decay after severe COVID-19 is highly relevant in the context of the selection of plasma therapy donors, as hospitalized patients have been suggested as an interesting donor population.<sup>44</sup>

Lastly, the possibility of reexposure to SARS-CoV-2 during follow-up cannot be excluded. However, all but four long-term serological assessments had been drawn before October, when the second COVID-19 wave started in Belgium.

In summary, we measured specific IgG antibodies after severe or critical COVID-19 and found that time and immunodeficiency are associated with reduced IgG levels at follow-up, while age and BMI were associated to increased levels. Corticosteroids given as short-term treatment for COVID-19 did not show influence, and BMI and immunodepression were respectively associated with decreased and increased rates of seronegativity.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

Julien De Greef and Jean Cyr Yombi conceptualized the article. Julien De Greef and Leïla Belkhir undertook the data collection. Jean Cyr Yombi and Leïla Belkhir contributed equally to the article. Anaïs Scohy conducted the serological testing. Julien De Greef, Leïla Belkhir, Jean Cyr Yombi, Frank Aboubakar, Charles Pilette, Ludovic Gerard, Lucie Pothen, and Halil Yildiz took care of the patients during acute infection and follow-up. Francis Zech realized the statistical analysis. Julien De Greef wrote the initial draft with all authors providing critical feedback and edits to subsequent revisions. All authors approved the final draft of the manuscript.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/jmv.27059>

## DATA AVAILABILITY STATEMENT

The anonymized data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** De Greef J, Scohy A, Zech F, et al. Determinants of IgG antibodies kinetics after severe and critical COVID-19. *J Med Virol*. 2021;93:5416-5424.  
<https://doi.org/10.1002/jmv.27059>