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CLINICAL

Seroconversion in septic ICU patients presenting with COVID-19:  
necessary but not sufficient

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**Background.** As COVID-19 pandemic and vaccination effects progress, research now focuses on adaptive immunological response to SARS-CoV-2. Few studies specifically investigated intensive care unit (ICU) patients, and little is known about kinetics of humoral response in such critically ill patients. In this context, the main objective of the present work was to perform a longitudinal analysis of the humoral response in critically ill COVID-19 patients with prolonged ICU stays in regard with initial inflammatory response, disease severity and mortality.

**Methods.** Over a 3 week period, circulating immunoglobulins (Ig) against SARS-CoV-2 along with several immunological and clinical parameters were measured in 64 ICU COVID-19 patients.

**Results.** Critically ill COVID-19 patients mounted a dynamic and sustained antibody response of both IgM and IgG as soon as the first day of ICU hospitalization. This serological response was not associated with any of the classical immunological parameters measured at ICU admission or with initial severity clinical scores. IgM and IgG levels and seroconversion trajectories were not associated with unfavourable outcome.

**Conclusion.** Despite rapid seroconversion and elevated humoral response, COVID-19 patients are still characterized by elevated mortality. Additional studies, including cytotoxic T cell functions, are mandatory to understand the immunological mechanisms contributing to long stay of COVID-19 patients in ICU. © 2021 Instituto Mexicano del Seguro Social (IMSS). Published by Elsevier Inc. All rights reserved.

**Key words:** COVID-19, immunosuppression, seroconversion, sepsis, IFN- $\gamma$ , lymphocyte.

**Introduction**

The appearance of severe acute respiratory coronavirus-2 (SARS-CoV-2) has led to a rapidly spreading pandemic. Since the first cases of coronavirus disease-19 (COVID-19), more than 180 million cases and 3.9 million deaths have been reported worldwide (by June 25 — Johns

Hopkins University). COVID-19 mostly associates with asymptomatic and mild presentations but may progress in worst cases to severe pneumonia leading to intensive care unit (ICU) admission and acute respiratory distress syndrome (ARDS) requiring respiratory support (1). In such cases, COVID19 is, by international definition, a viral sepsis (i.e., infection + organ failure)(2). COVID-19 mortality, although currently decreasing, has remained dramatically high especially in patients requiring invasive mechanical ventilation. As of to date, most research focused on the description and understanding of early

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immune response to SARS-CoV-2 infection leading to COVID-19. In contrast, fewer studies focused on longitudinal immune monitoring in severe COVID-19 patients, whereas their hospital stay may last for several weeks. This is especially true in critically ill COVID-19 patients with ARDS who also present with the highest mortality. In such immunocompromised patients (i.e., elderly patients with immunosenescence, profound lymphopenia, comorbidities known to chronically alter immune surveillance), the question of mounting an effective immune response against SARS-CoV-2 appears thus of utmost importance.

In most cases of mild or moderate COVID-19, SARS-CoV-2 is adequately controlled by host immune system through the rapid development of a sustained humoral immune response and seroconversion (3,4). The role of such humoral immune response in prognosis of COVID-19 patients is still under debate as several studies showed that different disease severities of COVID-19 may generate similar antibody response while other groups raised the hypothesis that neutralization potency or unbalanced seroconversion might play a major role in patients favourable outcome (5–9). Thus, despite the unprecedented scientific research effort to shed light on humoral response in COVID-19, some parts of uncertainty remain. This is especially true regarding the most severe cases, as only few clinical studies focused on longitudinal serological status of ICU septic patients throughout their hospital stay, which may last for several weeks. This knowledge gap is important to be filled up since antibodies-based therapeutic options exist (convalescent plasma, anti-SARS-CoV2 monoclonal antibodies).

In this context, over a 3 week period, we longitudinally assessed circulating immunoglobulins (Ig) IgG and IgM against SARS-CoV-2 along with specific immunological and clinical parameters in a cohort of 64 COVID-19 patients admitted to ICU. We focused on initial severity and mortality to better delineate putative association between seroconversion and disease severity and progression.

## Material and Methods

### Patients

The present work is an ancillary study of a previous report based on RICO cohort (during COVID-19 first wave in France, spring 2020) (10). Critically ill patients admitted to three ICUs from academic hospital (Hospices Civils de Lyon, Lyon, France) who presented with pulmonary infection with SARS-CoV-2 confirmed by RT-PCR testing were prospectively included in the study. This project

was part of an ongoing prospective observational clinical study (RICO, REA-IMMUNO-COVID). It was approved by ethics committee (Comité de Protection des Personnes Ile de France 1-N°IRB / IORG #: IORG0009918) under agreement number 2020-A01079-30. This clinical study was registered at ClinicalTrials.gov (NCT04392401). Inclusion criteria were patients aged >18 years, diagnosis of COVID-19 confirmed by RT-PCR testing in one respiratory sample. Exclusion criteria were pregnancy, institutionalized patients, inability to obtain informed consent.

For each patient, demographics, comorbidities, time from onset of COVID-19 symptoms to ICU admission, initial presentation of the disease in ICU including the ratio of the arterial partial pressure of oxygen to the fractional inspired oxygen (PaO<sub>2</sub>/FiO<sub>2</sub> ratio) at admission, antiviral therapy targeting SARS-CoV-2 and organ support, were documented. Organ dysfunctions according to Sequential Organ Failure Assessment (SOFA) score (range 0–24, with higher scores indicating more severe organ failures), and Simplified Acute Physiology Score II (SAPS II; range, 0–164, with higher scores indicating greater severity of illness) were documented. Follow-up included ICU length of stay, in-hospital mortality, day 28 (D28) mortality, as well as occurrence secondary infection. Blood samples were drawn within the first 48 h after admission (Day 0: D0), between 72 and 96 h after admission (D3), between D7 and D9 (D7), between D12 and D15 (D12) and between D20 and D25 (D20).

### Serology

Immunoglobulin measurements were performed using Vidas® SARS-CoV-2 IgM and Vidas® SARS-CoV-2 IgG (bioMérieux, France) in vitro diagnostic (IVD) assays (11). Briefly, a solid-phase repository coated with the antigen (recombinant SARS-CoV-2 receptor-binding domain [RBD] of the viral spike [S] protein) served as both solid-phase and pipetting device. After a dilution step, SARS-CoV-2-specific IgM and IgG were captured on the coated antigen, and unbound components are washed out. In the second step, human IgM (Vidas® SARS-CoV-2 IgM) or IgG (Vidas® SARS-CoV-2 IgG) were specifically detected by mouse monoclonal antibodies conjugated to alkaline phosphatase and directed against human IgM or IgG, respectively. A relative fluorescence value (RFV) was generated (background reading subtracted from the final fluorescence reading). The assay was conducted with a standard solution (S1) as well as positive and negative controls. The results were automatically calculated by the in-

strument, according to standard (S1) and an index value (i) was obtained (where  $i = \text{RFV}_{\text{sample}}/\text{RFV}_{\text{S1}}$ ). The test is interpreted as negative when  $i < 1.00$  and positive when  $i \geq 1.00$ .

### Immunological Parameters

Cytokines were measured in serum using Simpleplex<sup>®</sup> technology using ELLA instrument (ProteinSimple<sup>®</sup>, San Jose, CA), following manufacturer's instructions. Plasma IFN $\alpha$ 2 concentrations were determined by single-molecule Array (SIMOA<sup>®</sup>) on a HD-1 Analyzer (Quanterix, Lexington, Massachusetts) using a commercial kit for IFN- $\alpha$ 2 quantification (Quanterix). Lymphocyte subsets was performed on an automated volumetric flow cytometer from Beckman Coulter (Aquios CL)(12). For IFN-stimulated genes (ISG) score (IFN score) calculation, whole blood was collected on PAXgene blood RNA tubes (BD, Grenoble, France) and frozen at  $-80^{\circ}\text{C}$  until RNA extraction. IFN score was obtained using nCounter<sup>®</sup> analysis technology (NanoString Technologies, Seattle, WA), as previously described (10).

### Statistical Analysis

All the statistical analyses were performed using R software (version 4.0.5). In comparative assays nonparametric Wilcoxon signed-rank test was performed. In all experiments p values  $< 0.05$  were considered significant.

### Results

Sixty-four patients enrolled between March 16 and May 15, 2020, were included. The main clinical and biologic parameters on admission are depicted in Table 1. Overall, this cohort presented with usual characteristics of COVID-19 patients admitted in ICU: mean age was 65 years, median SOFA score was 4 and 63% of patients required invasive mechanical ventilation. Upon admission, patients presented with elevated concentrations of cytokines (IL-6, IFN- $\alpha$ , IFN- $\gamma$ , IL-10, TNF) and IFN signature, marked lymphopenia and decreased mHLA-DR (Table 1). At day 28, mortality in this cohort was 22%.

IgM and IgG to SARS Cov-2 Spike protein were longitudinally assessed over a 3 week period after ICU admission. Overtime results are depicted in Figure 1. Upon admission, median IgM index was 2 (IQR: 0.4–5.2) (54% patients above positive threshold) and median IgG index was 2 (IQR: 0.2–6.9) (54% patients above positive threshold). The difference between patients (i.e., with or without seroconversion upon admission) was mainly explained by the delay between first symptoms and ICU arrival (Figure 2). Most Ig negative patients were admitted to ICU  $< 8$  d post first symptoms and were still in early step of disease as evidenced by high levels of IFN- $\alpha$  (Figure 2) while

most Ig positive patients developed symptoms  $> 8$  d before ICU admission and presented with low IFN- $\alpha$  level. Similar results were obtained with IFN signature (data not shown). Then, as elapsed time effect since first symptoms increased, most ICU patients exhibited similar serological profile. Regarding anti SARS-CoV-2 IgM, we observed a rise that peaked at day 7. In parallel, the IgG index sharply increased during first 10 d and then seemed to reach a plateau during following weeks (96% patients above positive threshold at D20).

When exploring the relationship between immunological data (cytokine levels, cell counts and phenotype) upon ICU admission and Ig index at D0 and D20, we did not observe any significant association (data not shown). Similarly, we investigated whether initial clinical characteristics of COVID-19 patients may affect seroconversion. As depicted in Figure 3A, SOFA score on ICU admission (categorized by quartiles) had no association with seroconversion trajectory overtime. We observed similar lack of association between SAPS II score and IgG levels (data not shown).

Lastly, as previous studies (6,13) suggested that delayed or unbalanced seroconversion kinetics may contribute to impaired viral response and thus worsens patients' outcome, we compared temporal 3 week dynamics of IgG values in regard with D28 mortality (Figure 3B). Again, this analysis did not show any significant difference regarding overall trajectories between D28 survivors and non-survivors. As non-survivors tended to exhibit a delayed onset of seroconversion (visible at day 3), we also compared IgG D3/D0 ratios between survivors and non-survivors to highlight difference in slope of seroconversion, but values largely overlapped and differences between groups were non-significant (data not shown).

### Discussion

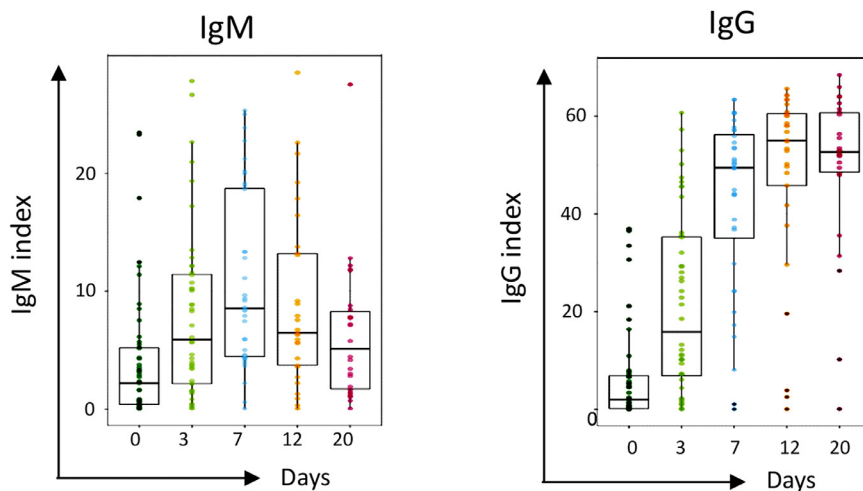
Antibody production and timely seroconversion kinetics are critical steps for a successful control of infectious diseases. In the present study, we reported on a longitudinal analysis of the humoral response in critically ill COVID-19 patients with prolonged ICU stays and we explored the antibody response in regard with initial inflammatory response, disease severity and mortality.

The main result revealed that, in a minutely detailed longitudinal study including a significant ICU cohort, severe COVID-19 patients mounted a dynamic and sustained antibody response as soon as the first day of ICU hospitalization. This Ig response was not associated with any of the classical immunological parameters monitored at admission or initial severity clinical scores SAPSII and SOFA. Most importantly, it was not predictive of favourable outcome. These results are in line with those of Ren et al. (14) showing that in the most severe patients, Ig response, although higher than that in mild disease,

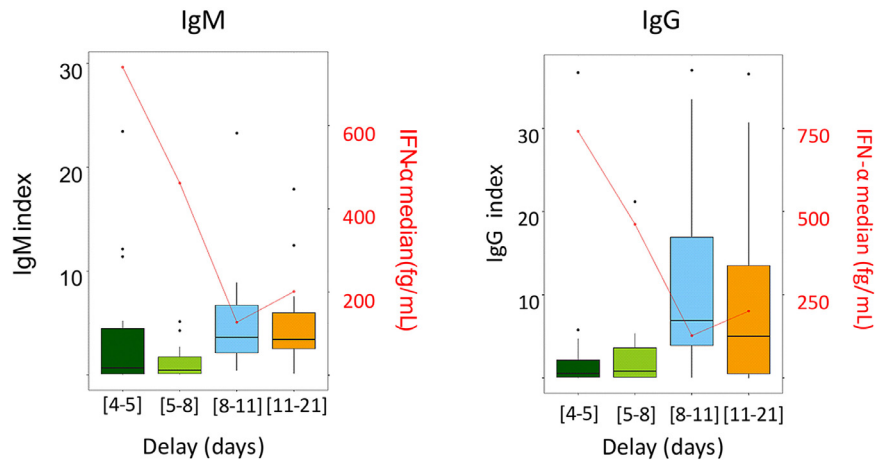
**Table 1.** Demographic and main clinical characteristics

Clinical characteristics		
Demographics		
Age	65 (52–72)	
Gender	51 (80%)	
Body mass index (kg/m <sup>2</sup> )	28 (26–32)	
Body mass index > 30 kg/m <sup>2</sup>	23 (36%)	
Severity scores		
SOFA score	4 (2–8)	
SAPS II score	34 (26–45)	
Organ support		
Mechanical ventilation	63 (98%)	
Noninvasive ventilation	23 (36%)	
Invasive ventilation	40 (63%)	
Follow-up		
Days in ICU	10 (4–30)	
Days in hospital	21 (11–56)	
Day 28 mortality	14 (22%)	
<b>Immunological parameters</b>		<b>Reference values</b>
Monocyte HLA-DR (AB/C)	11.125 (7.667–15.419)	(13.500–45.000)
Leucocytes (G/L)	7.9 (5.48–9.11)	(4–10)
Neutrophils (G/L)	6.3 (3.89–7.81)	(2–7)
Monocytes (G/L)	0.3 (0.2–0.5)	(0.2–1)
Lymphocytes (cells/ $\mu$ L)	0.9 (0.6–1.2)	(1 000–2 800)
T Lymphocytes (cells/ $\mu$ L)	425 (318.8–600.5)	(595–1861)
CD4+ T Lymphocytes (cells/ $\mu$ L)	297.5 (189.2–360.5)	(336–1.126)
CD8+ T Lymphocytes (cells/ $\mu$ L)	141 (86–189)	(125–780)
B Lymphocytes (% total Ly)	14 (10–17.9)	(4–19)
NK Lymphocytes (% total Ly)	13.5 (7.9–18)	(5–33)
Interleukine 6 (pg/mL)	107.5 (45–199.2)	<7
Interleukine 10 (pg/mL)	20 (14–30.1)	<8
TNF- $\alpha$ (pg/mL)	17.6 (13.18–20.25)	<10
IFN- $\gamma$ (pg/mL)	8.45 (5.25–15.45)	<3
IFN- $\alpha$ (fg/mL)	385 (67.5–2354.5)	<20
ISG score (ratio)	39.7 (12.79–64.21)	<2.3

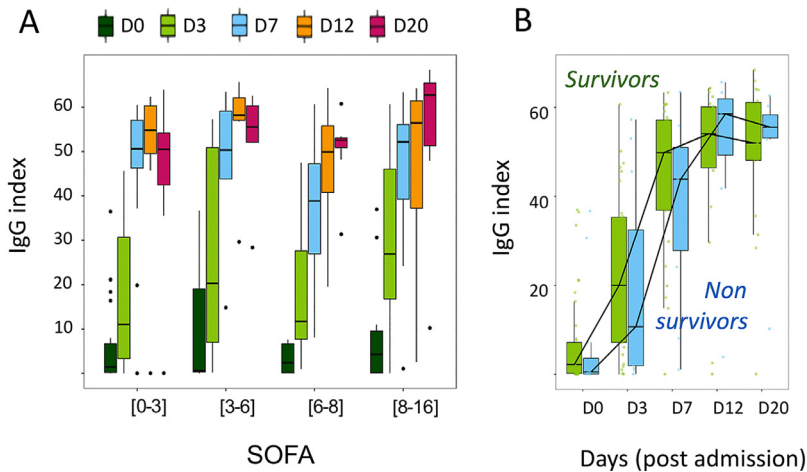
Results are shown as medians and interquartile ranges (Q1–Q3) for continuous variables or numbers and percentages for categorical variables. Sepsis related organ failure assessment (SOFA) and simplified acute physiology score II (SAPS II) scores were calculated during the first 24 h after intensive care unit (ICU) admission. Reference values are from routine laboratory. ISG: Interferon stimulated genes.



**Figure 1.** Longitudinal characterization of humoral response in 64 critically ill COVID-19 patients. Over time evolution of IgM (left panel) and IgG (right panel) levels (Vidas® index) are depicted during the first month after admission. Results are presented as individual data and boxplots.



**Figure 2.** ICU admission Ig titres according to elapsed time from first symptoms in 64 critically ill COVID-19 patients. IgM (left panel) and IgG (right panel) levels (Vidas® index) are presented as box plots according to delay between first clinical symptoms and ICU admission (days presented as quartiles). IFN- $\alpha$  concentrations at ICU admission (median values, red line) are similarly plotted according to delay between first clinical symptoms and ICU admission (red scale on right part of both histograms).



**Figure 3.** IgG titres association with SOFA score and mortality at day 28. A. SOFA. IgG titre kinetics (Vidas® index) are presented as box plots depending on admission SOFA score (stratified by quartiles). Colour boxes represent the different sample timing from 0–20 d after admission. B. Mortality. IgG titre (Vidas® index) are presented as box plots during the first month after admission according to mortality assessed at day 28.

did not correlate with clinical outcomes. This also agrees with the observation that elevated antibody levels did not systematically associate with clearance of virus since patients show prolonged viral shedding despite the presence of humoral response (15–17). On the other hand, some authors hypothesized that it was not the Ig level *per se*, but rather, the time of appearance of seroconversion that may play a role in patients prognosis as they observed delayed seroconversion in critical ill patients in comparison with less severe patients (18). Another explanation may lie in neutralizing capacity of secreted antibodies since not all anti-SARS-CoV-2 antibodies are believed to be neutralising whereas this property may be an important

protective factor (5,19). Finally, it was also reported that elevated titres of anti-SARS-CoV-2 may dampen immune responses by blocking cells able to respond to type I IFN (20). These latter aspects deserve further explorations.

Overall, the presence of high titres of specific antibodies in ICU patients clearly suggests that humoral immune response works properly in such severe patients. Whereas it is well accepted that severe COVID-19 patients present with features of immunosuppression (i.e., elderly patients with comorbidities known to chronically weaken immune surveillance), the present results indicate that antigens are correctly processed and presented by dendritic cells; that follicular helper CD4+ T cells are functional, and that



B cells are primed to produce related antibodies. Thus, the lack of association between humoral response and clinical outcomes suggests that the pathophysiological role of the immune response in COVID-19 severity and mortality may be complex and could be reminiscent of that seen in bacterial sepsis which is characterized by profound lymphopenia and altered cellular cytotoxic T cell functions (21–23). Indeed, the long-term immune response to the most severe forms of SARS-CoV-2 infections shares many characteristics with sepsis-induced immunosuppression: severe lymphopenia, low mHLA-DR, high rates of nosocomial pneumonia, altered IFN- $\gamma$  release by CD8+ T cells that over express PD-1 molecules (24–27). Considering the current results, it is tempting to focus on specific CD8+ T cell alterations that could be induced by viral proteins from SARS-CoV-2 (28). We may thus hypothesize that the lack of early control of SARS-CoV-2 infection by an innate immune response (i.e. altered type I response) along with subsequent persistence of viral load / proteins may amplify a progressive anergy of cytotoxic cells whereas those cells are the key players in all responses against viruses (29–31). The vicious circle between high viral load, lymphopenia and CD8+ T cell anergy may finally lead to viral persistence over weeks despite the presence of high number of antibodies. This hypothesis is partially supported by recent case reports linking immunosuppression and emergence of virus variants that can bypass a previously established humoral response (32,33) and by observations that high-titre convalescent plasma did not systematically improve survival or other clinical outcomes (34).

Overall, the present results shed light on putative treatments in ICU COVID-19 patients. So far, only dexamethasone showed beneficial effects to limit progression toward more severe form of the disease (35). In critically ill patients, in the absence of effective antivirals and efficient antibodies, an alternative would lie in boosting immune responses as presently assessed in sepsis (36–38). Preliminary results in COVID-19 with IFN- $\gamma$  (39) or IL-7 (40,41) are already published and would deserve additional trials.

Our study presents some limitations. As conducted in the first COVID-19 surge in France (spring 2020), dexamethasone was not part of standard care for ICU patients. Similarly, at this time, SARS-CoV2 viral load could not be routinely assessed. The present results need thus to be extended under current optimized practice (dexamethasone use, longitudinal viral load monitoring). Lastly, functional testing of T lymphocytes appears of utmost importance in next studies to reinforce the hypothesis of persisting altered cytotoxicity against SARS-CoV-2.

## Conclusion

The present study shows that critically ill patients with COVID-19 developed a rapid seroconversion leading to

high IgG and IgM levels. Nevertheless, this response did not correlate with initial immune response and severity or with patients' outcome. Additional studies are mandatory to understand the immunological mechanisms contributing to long ICU stays of COVID-19 patients still characterized by elevated mortality.

## RICO Study Group

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## Conflict of Interest

VC, GO, CT, EC and KBP are employees of bioMérieux SA. FC, VC, GO, CT, EC, KBP, ACL, TR and GM work in a joint research unit, co-funded by the Hospices Civils de Lyon, bioMérieux SA and Lyon-1 University (UR7426). Authors declare no other competing interests.

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## Ethical Statement

This project was part of an ongoing prospective observational clinical study (RICO, REA-IMMUNO-COVID).

It was approved by ethics committee (Comité de Protection des Personnes Ile de France 1-N°IRB/IORG #: IORG0009918) under agreement number 2020-A01079-30. This clinical study was registered at ClinicalTrials.gov (NCT04392401).

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