Clinical usefulness of fully automated chemiluminescent immunoassay for quantitative antibody measurements in COVID-19 patients

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RESEARCH ARTICLE

Abstract
Since December 2019, we have been in the battlefield with a new threat to the humanity, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), characterized by viral pneumonia. It may be asymptomatic or cause various symptoms, ranging from flu-like symptoms to acute respiratory distress syndrome and eventually death. At present, the only reliable test for COVID-19 diagnosis is quantitative reverse transcriptase-polymerase chain reaction. Assessing the immune response against SARS-CoV-2 could increase the detection sensitivity of infected population. Hereby, we report the performances of a fully automated chemiluminescent immunoassay (CLIA) on 276 serum samples. One hundred samples obtained from COVID-19 negative subjects (COVID-19 free) were analyzed to evaluate the diagnostic specificity of antibody (Ab) detection. Thereafter, 176 samples obtained from 125 patients with confirmed COVID-19 (COVID-19 patients) were selected to assess the diagnostic sensitivity of the CLIA. All samples were analyzed on MAGLUMI 800 platform. All COVID-19 free samples had Ab levels below the cutoff values. Hence, the diagnostic specificity was estimated at 100% (95% confidence interval [CI] = 96.3-100.0; positive predictive value = 100%). By the 18th day from the onset of symptoms, we reached an optimal diagnostic sensitivity (more than 95.0%) In fact, the diagnostic sensitivity increased over time and between 15 and 25 days after symptoms onset, reached 95.5% (95% CI = 84.9-99.2). The new automated CLIA analyzer appeared to be a robust and reliable method to measure specific Ab against COVID-19 at high throughput. Our data suggest that combining Ab and nucleic acid detection could increase diagnostic sensitivity.

KEYWORDS
COVID-19, Euroimmun, immunoassay, MAGLUMI, SARS-CoV-2, serology
1 | INTRODUCTION

Since December 2019 we have been on the battlefield with a new threat to humanity, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19), characterized by bilateral viral pneumonia. It may be asymptomatic or cause a variety of symptoms, ranging from anosmia and dysgeusia to acute respiratory distress syndrome and eventually death. There is no specific treatment and by 27 April 2020, SARS-CoV-2 has infected and killed more than 2 805 000 and 194 000 patients, respectively. These figures seem optimistic as we cannot yet detect all infected patients by the quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR).

At present, the only reliable test for SARS-CoV-2 detection and COVID-19 diagnosis is the RT-qPCR, which is an expensive, time-consuming, and laborious method to implement and requires some expertise and proficient clinical laboratories to perform the assay. Multiple factors such as suboptimal sampling, lower viral charges, sampling medium, and contamination could bias test results. One other way is to assess the immune response against SARS-CoV-2 by the automated analyzer, which is faster, less expensive, random access, and could be considered as a complementary diagnostic tool in suspected patients with a negative RT-qPCR result.

Measuring specific antibodies (Abs), therefore, will increase the sensitivity of the detection of the infected population. It also could be useful to assess how the human immune system responds over time. Hereby, we report the performances of a fully automated chemiluminescent immunoassay (CLIA).

2 | MATERIALS AND METHODS

2.1 | COVID-19 positive subjects (COVID-19 patients)

A total of 176 serum samples (samples) from 125 with confirmed COVID-19 (COVID-19 patients) were randomly collected into serum gel tubes from 25 February to 10 March 2020. The inclusion criteria were symptomatic and hospitalized patients with positive RT-qPCR tests on nasopharyngeal swab samples and characteristic radiological lung patterns such as ground-glass opacity and/or bilateral involvement. There were not any exclusion criteria besides the age (only patients ≥ 18 year old were included). Immunocompromised patients were not excluded from the study as we sought to know how their immune system will set in against COVID-19.

Among 125 selected COVID-19 patients, we longitudinally followed 45 patients (≥2 samples) up to 25 days after the onset of symptoms to observe Ab development. All data included demographic, clinical, radiographic, and laboratory findings and time between symptoms onset and hospital admission, or full recovery or eventual death were obtained from patients’ medical records. The two first authors checked all medical records at least three times until 27 April 2020.

The sample size was derived from Noordzij et al. paper using \( \alpha = .05 \) and \( \beta = .20 \), suggesting ≥100 subjects/group.

2.2 | Sampling and preanalytical procedure

All fresh samples were taken by venipuncture and centrifuged at 2054g for 10 minutes. The samples, at last, were extracted from whole blood, respecting all hygiene measures and stored at +4°C before analysis for a maximum of 3 days. After analysis, all residual samples were stored at −20°C.

2.3 | Immunoglobulin G and immunoglobulin M measurements by MAGLUMI 2019-novel coronavirus (CLIA)

We measured immunoglobulin G (IgG)/IgM Ab using Snibe 2019-Novel Coronavirus (nCoV) Kit, a CE-IVD marked assay on MAGLUMI 800 (Shenzhen New Industries Biomedical Engineering [Snibe] Co., Ltd., Shenzhen, China), a fully automated CLIA analyzer. The method uses nucleocapsid and spike proteins as antigens (Ags), as they were appeared to be promising targets for Ab detection against other coronaviruses. The produced light emission (relative light unit [RLU]) by indirect immunoassay (IA) reaction is proportional to the specific Ab quantity in the sample. RLU’s are then transformed to arbitrary units (AU/mL), using a computed 10-point calibration curve.

A level greater than 1.00 AU/mL is interpreted as positive for both Ab. The claimed diagnostic sensitivity for IgG, IgM, and combined IgG and IgM (IgG/IgM) were 91.21%, 78.65%, and 95.60%, respectively. The manufacturer did not report any cross reactivity with other Ab against bacterial and viral respiratory infectious agents (including other CoVs). The claimed diagnostic specificity for IgG, IgM, and IgG/IgM was 97.33%, 97.50%, and 96.00%, respectively. All claimed within- and between-run coefficients of variation (CVs) for positive quality controls were less than 10.0%. The manufacturer did not claim within nor between-run CVs for negative quality controls.

2.4 | Diagnostic specificity

For this purpose, we analyzed 100 residual samples (60 females; mean age = 37.2 years [95% confidence interval, 95% CI = 34.5-37.2]) obtained from COVID-19 negative subjects (COVID-19 free), and evaluated the method based on the guideline published by the “Haute Autorité de Santé in France” (reliable if diagnostic specifies are ≥95%). Samples (serum samples were separated and frozen at the time of sampling) were divided into three subgroups. Forty samples with no known confounding factors and 40 others with supposedly confounding factors (cf. Supporting Information Table) known to interfere with serological assays such as autoimmune Ab
(n = 17) and infectious diseases Ab (23). Samples were randomly selected among stored sera collected between October 2018 and February 2019. Another 20 samples were collected from asymptomatic subjects during the overlapping period of Flu epidemic and COVID-19 outbreak in March 2020. All samples were tested in batch to detect any potential cross reactivity. The specificity was defined as the percentage of true negative (TN) results correctly identified by the method and calculated by the formula $Sp = TN/(TN + \text{false positives} \ [FP])$. We also verified the cutoff of seropositivity for each Ab based on COVID-19 free sample levels (if <1 AU/mL).

### 2.5 | Diagnostic sensitivity

For this purpose, we performed the IA to measure Ab in all samples (n = 176) collected from COVID-19 patients (n = 125) at different stages of infection. COVID-19 was confirmed by positive RT-qPCR on nasopharyngeal swab and by radiographic criteria (bilateral chest involvement and/or ground-glass opacity [GGO] identified by X-ray or computed tomography [CT] scan). For each of these samples, the date symptoms onset were determined based on the clinical records. The diagnostic sensitivity was estimated based on the receiver operating characteristic (ROC) curves for four different time intervals depending on symptoms onset: 0 to 4 days after, 5 to 9 days after, 10 to 14 days after, and 15 to 25 days after.

We analyzed samples in batch and evaluated the method based on the guideline published by the “Haute Autorité de Santé in France” (reliable if diagnostic sensitivities are >95%). At last, positive and negative predictive values (PPV and NPV) and test accuracy were statistically estimated to assess the proportion of true positive (TP), TN, and accuracy of the CLIA method.

The sensitivity was defined as the percentage of TP results correctly identified by the method and calculated by the formula, $S = TP/(TP + \text{false negatives} \ [FN])$. The PPV was defined as the probability of a positive result among true patients and calculated by the formula $PPV = TP/(TP + FP)$. The NPV was defined as the probability of a negative result among healthy subjects and calculated by the formula $NPV = TN/(TN + FN)$. Accuracy was defined as the closeness of the measurements to an expected value and calculated by the formula $ACC = (TP + TN)/(TP + FP + TN + FN)$.

### 2.6 | Precision and trueness

To verify the method accuracy, we used precision and trueness according to ISO 5725-2:2019. Precision was assessed by comparing the locally calculated within-run and the between-run CVs to those claimed by the manufacturer in package inserts. For this purpose, two control levels per Ab types (IgG = 0.29 and 3.92 AU/mL; IgM = 0.29 and 3.92 AU/mL) were analyzed according to CLSI EP15-A3. During 5 consecutive days, each control level analyzed 3 times a day. We considered the verification satisfactory if calculated CVs for positive control levels were less than those claimed by the manufacturer. In the absence of standard material or proficiency testing programs for the moment, the positives control level (per Ab types) with known target value was analyzed 20 times during 20 days to estimate the trueness.

Trueness was calculated using the formula $bias \% = [(M - V) \times 100]/V$. With $M$ as the mean value obtained on 20 replicates and $V$ as known value for positive quality controls provided by the manufacturer’s package inserts.

### 2.7 | Limit of blank, limit of detection, limit of quantitation, linearity, and carry-over

(A) The limit of blank (LOB) was defined as the mean of blank replicates $+ (1.645 \times SD_{blank \ replicates})$. For this purpose, negative samples (NS) with no measured (NaCl 0.09% solution) analyzed 10 times in row (IgG mean = 0.02 AU/mL; IgM mean = 0.44 AU/mL). Limit of detection (LOD) was defined as $LOD = (1.645 \times SD_{blank \ plasma \ pool \ replicates})$. Limit of quantitation (LOQ) was verified by analyzing the results of the $3 \times 3$ serum pools with different concentrations of IgG (means at 191.15, 83.27, and 3.57 AU/mL), during 5 consecutive days. For IgM, as we did not have samples with high Ab concentration, only one pool (2.58 AU/mL) was analyzed in triplicate for 5 days, to evaluate the lower LOQ. The LOQ was verified if overall CV (including within- and between-run CVs) for each pool was less than 20%. LOB, LOD, and LOQ were verified according to the CLSI EP17-A2 guideline and the Armbruster and Pry 5,10 paper.

(B) The linearity assessments were carried out by analyzing five serum pools in triplicate. For this purpose, we used two initial pools/Ab types (IgG = 104.07 and 0.07 AU/mL; IgM = 15.00 and 0.51 AU/mL), to prepare three other pools/Ab types according to CLSE EP06-A.11 The linearity was verified by calculating the percentage of recovery using the formula $(%Rec) = [(M \times \text{dilution titer: } 1.00, 1.33, 2.00, 4.00, 1.00) \times 100]/V$. With $M$ as the mean value obtained on each sample pool and $V$ as known value, 104.07 AU/mL for IgG, and 15.00 AU/mL for IgM. To assess the matrix effect on the linearity, we used NaCl 0.09% solution to perform serial dilutions (dilution factor: 1, 2, 4, and 8) on two other serum pools (IgG mean = 197.63 AU/mL and IgM mean = 9.10 AU/mL). All samples were analyzed in triplicates.

(C) As the analyzer uses sample probe, we assessed the sample contamination (carry-over). For this purpose, a sample with a high Ab level (IgG mean = 91.92 AU/mL; IgM mean = 8.97 AU/mL) was analyzed in triplicate (namely Ab1, Ab2, and Ab3). Immediately, an NS were tested (IgG mean = 0.079 AU/mL, IgM mean = 0.079 AU/mL) in triplicates (namely Ab1, Ab2, and Ab3). The carry-over calculated based on the formula $(%C-O) = (NS1 - NS3/Ab) \times 100$ according to CLSI EP07 and Burner et al’s12 paper. We verified the method if calculated carry-over were less than 1% as an arbitrary criterion.
2.8 | IgG and IgA measurements by Euroimmun anti-SARS-CoV-2 (enzyme-linked immunosorbert assay)

To evaluate the CLIA method, we performed a brief comparison with a CE-IVD ELISA Kit. The comparison was carried out with the Euroimmun anti-SARS-CoV-2 enzyme-linked immunosorbert assay (ELISA) method (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany), on 77 samples (for which enough serum volumes were available) of which, 47 classified as COVID-19 patients and 30 as COVID-19 free subgroups. In brief, using a calibration curve, the measured optical density which is proportional to Ab concentration in samples was transformed to AU/mL. Considering the result interpretation, Ab levels ≥1.1 AU/mL were considered positive.

2.9 | RT-qPCR assay

The laboratory diagnosis of SARS-CoV-2 infection was confirmed by using the coronavirus COVID-19 genesig Real-Time PCR assay (Primerdesign Ltd, Chandler’s Ford, UK) on nasopharyngeal swab (Universal Transport Medium tubes; COPAN, Brescia, Italy) targeting the RNA-dependent RNA polymerase gene. The extraction of the viral RNA was carried out on the m2000sp platform (Abbott Molecular Inc, Illinois) and the amplification was performed on the LightCycler 480 instrument (Roche Diagnostics, Mannheim, Germany) according to the manufacturers’ recommendations. The amplification parameters were as follows: 10 minutes at 55°C for reverse transcription, 2 minutes at 95°C for activation, followed by 45 cycles of 10 seconds at 95°C and 60 seconds at 60°C. A cycle threshold value less than 40 was defined as a positive test.13

2.10 | Statistical analysis

Means, 95% CIs, medians, and interquartile ranges (IQR) were determined in COVID-19 free subjects and in COVID-19 patients. The diagnostic sensitivities and specificity, PPV and NPV, areas under the ROC curve (AUCs), and accuracies were estimated based on the ROC curve results. The degree of agreement between the two methods was determined by the kappa index. Absolute values rounded to two digits after the point and percentages rounded to one digit after the point. A Mann-Whitney test was performed on COVID-19 patients to find a possible correlation between patient’s characteristics (demographic, clinical, radiographic, and laboratory findings) and Ab development. A P < .05 was considered as statistically significant.

Data analysis was performed using GraphPad Prism software (Version 8.0; California, CA). All CVs, PPV, and NPV were computed using Analyse-it (Version 5.30; Analyse-it Software, Ltd., Leeds, United Kingdom). Dixon-Reed and Tukey’s tests were used to detect potential outliers.14

2.11 | Ethical aspects

Our study fulfilled the Ethical principles provided by the Declaration of Helsinki and was approved by the Local Medical Ethics Committee (Ref: 2020/06AVR/203).

3 | RESULTS

3.1 | COVID-19 positive subjects (COVID-19 patients)

All results related to baseline demographics, radiography, laboratory, and clinical findings including symptoms comorbidities and treatments of COVID-19 patients are summarized in Table 1. COVID-19 was confirmed in all patients based on RT-qPCR results through nasopharyngeal swab samples at admission. The thorax CT scans (109/n = 125) and chest X-ray (16/125), performed at admission, showed the specific profile compatible with COVID-19 (lung bilateral involvement and/or GGO) on radiographic images. None of these patients had pneumonia caused by Streptococcus pneumoniae and/or Legionella pneumophilia. There was only one case of concomitant infection of influenza (type B) and no case of respiratory syncytial virus was documented. The analysis of demographic data from COVID-19 patients who developed Ab (80.0%; n = 125) and those without any immune response, showed the mean age (63.7 vs 71.4 years), was the sole statistically significant difference (P < .03; Table 2). The analysis on clinical features showed that the number of deceased (8/100 vs 9/25 COVID-19 patients) cases was statistically different between two latter subgroups (P < .001; Table 2).

3.2 | Diagnostic specificity

The means of IgG and IgM levels and their 95% CI in the COVID-19 free group (100 samples; 100 subjects) were 0.12 (0.09-0.15) and 0.35 AU/mL (0.32-0.37), respectively. All COVID-19 free samples had Ab levels less than the seropositivity cutoff value claimed by the manufacturer (1 AU/mL). Hence, the diagnostic specificity was estimated at 100.0% (96.3-100.0). The PPV was also estimated at 100.0%. As none of these samples had Ab levels >1 AU/mL, the seropositivity cutoffs were also successfully verified at the same time (data are provided in).

3.3 | Diagnostic sensitivity

The diagnostic sensitivities for IgG and IgM and their 95% CI in COVID-19 patients’ group (176 samples; 125 patients) were 62.5% (55.2-69.3) and 60.2% (52.9-67.2), respectively. (Figure 1A,B). Combining IgG/IgM as a criterion for the diagnostic sensitivity, we would reach to overall 70.5% sensitivity (63.3-76.7). Overall, 80.0% of patients (n = 125) developed immune response over time.
TABLE 1  Associations and discrepancies according to the diagnosis criteria (positive RT-qPCR and suggestive chest CT scan and/or X-ray) used for including positive COVID-19 patients, their laboratory results, clinical information, and treatments

| Demographics | Deceased (n = 17; F = 9) | Remained at hospital by 30 April 2020 (n = 7; F = 0) | Recovered patients (n = 101; F = 49) |
|--------------|--------------------------|-------------------------------------------------|-----------------------------------|
| Mean age (95% CI) | Male = 79.0 (67.9-90.1); female = 84.9 (75.3-94.5); overall = 82.1 (75.5-88.7) | Male and overall = 62.6 | Male = 61.2 (57.5-64.9); female = 64.0 (58.9-69.0); overall = 62.5 (59.5-65.9) |
| Male = 63.5 (60.1-66.8) | | | |
| Female = 67.2 (62.4-72.1) | | | |
| Overall = 65.2 (62.3-68.1) | | | |
| 18-39 y (F) | 0 (0) | 0 (0) | 3 (5) |
| 40-59 y (F) | 1 (0) | 3 (0) | 22 (15) |
| 60-79 y (F) | 4 (3) | 4 (0) | 23 (17) |
| ≥80 y (F) | 3 (6) | 0 (0) | 4 (12) |
| Time from symptoms onset to hospital admission, d | Mean (95% CI) 3.8 (1.6-6.0) | 6.6 (5.7-7.5) | 6.6 (5.9-7.4) |
| Median (IQR) | 2.0 (1.0-7.0) | 7.0 (6.0-7.0) | 6.0 (3.0-8.0) |
| Time from hospital admission to final outcome, d | Mean (95% CI) 11.1 (7.8-14.5) | >29.4 (26.2-32.7) | 9.6 (8.5-10.6) |
| Median (IQR) | 11.0 (6.0-18.5) | 28.0 (27.0-31.0) | 8.0 (5.0-12.5) |
| Time from symptom onset to final outcome, d | Mean (95% CI) 14.9 (11.3-18.6) | >36.0 (32.9-39.1) | 16.0 (14.9-17.1) |
| Median (IQR) | 15.0 (8.5-20.0) | 35.0 (33.0-39.0) | 15.0 (12.0-20.0) |
| Serological profile (n = 176 serum samples) | | | |
| IgG levels (AU/mL) | | | |
| Mean (95% CI) | 24.1 (6.0-42.2) | 41.3 (2.8-85.4) | 23.9 (17.4-30.3) |
| Median (IQR) | 0.3 (0.04-29.4) | 15.3 (1.7-32.9) | 5.0 (0.3-32.6) |
| IgM levels (AU/mL) | | | |
| Mean (95% CI) | 1.4 (0.7-2.0) | 2.4 (1.4-3.5) | 4.7 (2.3-7.1) |
| Median (IQR) | 0.5 (0.26-2.1) | 2.4 (1.2-3.1) | 1.7 (0.6-4.6) |
| Time from symptom onset to immune response (days) | | | |
| Mean (95% CI) | 12.9 (6.9-18.9) | 12.0 (9.0-15.0) | 12.4 (11.4-13.4) |
| Median (IQR) | 13 (7.3-15.0) | 12.0 (8.0-16.0) | 12.0 (9.0-15.0) |
| Other laboratory findings at admission | | | |
| Hemoglobin (12-16 g/dL): n (%) | N = 9 (53.0); I = 0 (0.0); D = 8 (47.0) | N = 6 (85.7); I = 0 (0.0); D = 1 (14.3) | N = 64 (63.4); I = 0 (0.0); D = 37 (36.6) |
| White blood cells count (4-10×10⁹/L): n (%) | N = 12 (70.6); I = 3 (17.6); D = 2 (11.8) | N = 6 (85.7); I = 1 (14.3); D = 0 (0.0) | N = 66 (63.5); I = 21 (20.8); D = 14 (13.9) |
| Neutrophil count (1.6-7×10⁹/L): n (%) | N = 11 (64.7); I = 5 (29.4); D = 1 (5.9) | N = 5 (71.4); I = 2 (28.6); D = 0 (0.0) | N = 64 (63.4); I = 28 (27.7); D = 9 (8.9) |
| Lymphocyte count (0.8-5×10⁹/L): n (%) | N = 5 (29.4); I = 1 (5.9); D = 11 (64.7) | N = 4 (57.1); I = 0 (0.0); D = 3 (42.9) | N = 58 (57.4); I = 0 (0.0); D = 43 (42.6) |
| Platelet count (150-450×10⁹/L): n (%) | N = 10 (58.8); I = 3 (17.6); D = 4 (23.6) | N = 2 (28.6); I = 1 (14.3); D = 4 (57.1) | N = 88 (87.1); I = 4 (4.0); D = 9 (8.9) |
| CRP (<50 mg/L): n (%) | N = 0 (0.0); I = 17 (100.0) | N = 0 (0.0); I = 7 (100.0) | N = 1 (1.0); I = 100 (99.0) |
| LDH (<250 U/L): n (%) | N = 1 (5.9); I = 16 (94.1) | N = 0 (0.0); I = 7 (100.0) | N = 16 (15.8); I = 85 (84.2) |
| ALT (N < 36 U/L), and/or AST (N < 48 U/L) and/or GGT (N < 49 U/L), and/or T.Bili (N < 17 umol/L) and/or ALP (N = 40-145 U/L): n (%) | N = 4 (23.5); I = 13 (76.5) | N = 4 (57.1); I = 3 (42.9) | N = 41 (40.6); I = 60 (59.4) |
| Urea (N = 2.9 mmol/L) and/or SCr (N = 53-106 µmol/L): n (%) | N = 9 (52.9); I = 8 (47.1) | N = 5 (71.4); I = 2 (28.6) | N = 67 (66.3); I = 34 (33.7) |
| Hs-Tpn-T (N < 10 ng/L): n (%) | N = 4; I = 3 (42.9; NR in 10 patients) | N = 1; I = 2 (66.7; NR in 4 patients) | N = 14; I = 10 (41.7; NR in 76 patients) |
| Symptoms at admission | | | |
| Fever (>38-38.5°C; temporal temperature): n (%) | 17 (100.0) | 7 (100.0) | 101 (100.0) |

(Continues)
Interestingly, the diagnostic sensitivities increased for samples (n = 44) collected between 15 and 25 days (mean = 17.9 days [17.1–18.7]) after symptoms onset to 90.9 (78.8–96.4) for both IgG and IgM (Figure 1C,D). Using combined IgG/IgM, the diagnostic sensitivity reached to 95.5% (84.9–99.2). The statistical analysis showed the time from symptoms onset to hospital admission (mean days = 6.8 vs 3.9), and to sera sampling (mean days = 12.8 vs 7.7) were significantly different between COVID-19 patients who developed Ab.

| TABLE 1 (Continued) |
|----------------------|
| COVID-19 patients (n = 125; F = 58) | Deceased (n = 17; F = 9) | Remained at hospital by 30 April 2020 (n = 7; F = 0) | Recovered patients (n = 101; F = 49) |
| Cough: n (%) | 16 (94.1) | 7 (100.0) | 96 (95.0) |
| Dyspnea: n (%) | 16 (94.1) | 7 (100.0) | 97 (96.0) |
| Myalgia and or fatigue: n (%) | 17 (100.0) | 7 (100.0) | 24 (23.8) |
| Rhinorrhea and sore throat: n (%) | 2 (11.8) | 1 (14.3) | 13 (12.9) |
| Diarrhea: n (%) | 3 (17.6) | 1 (14.3) | 10 (9.9) |
| Nausea, vomiting and abdominal pain: n (%) | 0 (0.0) | 0 (0.0) | 14 (13.9) |
| Other symptoms (including headache, confusion, unconsciousness, anosmia and dysgeusia): n (%) | 6 (35.3) | 3 (42.9) | 46 (45.5) |

Complications during hospitalization

| Viral pneumonia and acute hypoxemia: n (%) | 17 (100.0) | 7 (100.0) | 101 (100.0) |
| Critical care procedures (including invasive ventilation and/or ECMO): n (%) | 6 (35.3) | 7/7 (100.0) | 20/101 (19.8) |
| ARDS: n (%) | 17 (100.0) | 7 (100.0) | 10 (9.9) |
| Cerebro-cardiovascular diseases: n (%) | 3 (17.6) | 2 (28.6) | 14 (13.9) |
| Septic choc: n (%) | 1 (5.9) | 0 (0.0) | 0 (0.0) |
| Acute kidney failure: n (%) | 2 (11.8) | 1 (14.3) | 6 (5.9) |
| Respiratory super-infection: n (%) | 2 (11.8) | 1 (14.3) | 11 (10.9) |
| Other concomitant infections: n (%) | 1 (5.9) | 1 (14.3) | 8 (7.9) |

Antiviral/bacterial/fungal treatments during hospitalization

| Hydroxychloroquine (other anti-retroviral): n (%) | 12 (70.1) | 7 (100.0) | 96 (95.0) |
| Azithromycin: n | 4 | 0 | 24 |
| Other antibiotics: n | 4 | 2 | 20 |
| Overall antibiotics: n (%) | 8 (47.1) | 2 (28.6) | 44 (43.6) |
| Anti-fungal treatments: n (%) | 0 (0.0) | 0 (0.0) | 2 (2.0) |
| Corticosteroids: n (%) | 4 (23.5) | 0 (0.0) | 6 (5.9) |

Comorbidities

| All comorbidities: n (%) | 14 (82.4) | 5 (71.4) | 88 (87.1) |
| Arterial hypertension: n (%) | 7 (41.2) | 3 (42.9) | 45 (44.6) |
| Diabetes: n (%) | 2 (11.8) | 3 (42.9) | 28 (27.7) |
| Cardiovascular diseases: n (%) | 8 (47.1) | 2 (28.6) | 15 (14.9) |
| Chronic kidney: n (%) | 5 (29.5) | 0 (0.0) | 28 (2) |
| Lung diseases (asthma, COPD): n (%) | 2 (11.8) | 0 (0.0) | 8 (7.9) |
| Leucopenia (immunosuppressive treatments (autoimmune diseases and organ transplantation), chemotherapies, active cancers): n (%) | 3 (17.6) | 2 (28.6) | 12 (11.9) |
| HIV condition: n (%) | 1 (5.9) | 0 (0.0) | 1 (1.0) |
| Pregnancy: n (%) | 0/9 (0.0) | NA | 1/49 (2.0) |

Note: Considering there were not enough subjects (<20 patients) in deceased and remained at hospital subgroups, we did not realize any statistical analysis.

Abbreviations: 95% CI, 95% confidence interval; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CT, computed tomography; D, decreased; ECMO, extracorporeal membrane oxygenation; Flu, influenza A and B; GGO, ground-glass opacity; GGT, gamma glutamyl transpeptidase; hs-TpTn-T, high-sensitive cardiac troponin T; I, increased; IQR, interquartile range; LDH, lactate dehydrogenase; N, normal; NA, not applicable; NR, not realized; pro-BNP, B-type natriuretic peptide; RSV, respiratory syncytial virus; RT-qPCR, quantitative reverse transcriptase polymerase chain reaction; SCr, serum creatinine; T.Bili, total bilirubin; Urea, blood urea nitrogen.
and those without any immune response. All data regarding diagnostic sensitivities, NPVs, AUCs, and test accuracies between 0 and 25 days after symptoms onset are summarized Table 3.

### 3.4 | Precision, trueness, LOB, LOD, LOQ, linearity, and carry-over

The bias (%) within and between-run CVs to assess the method precision are summarized in Table 4. Both negative control levels had target values less than estimated LODs. Hence, we did not take into account the highly elevated CV for negative control levels. Both positive control level CVs were less than 10%. The precision (<10%) and trueness (<±10%) assessments were satisfactory. The LOB and LOD of IgG and IgM were also shown in Table 4. The LOQ of IgG was verified as all overall CVs/serum pool were less than 20%. The carry-over assessments were satisfactory as they appeared less than 1%. The linearity assessments were not satisfactory. The higher the dilution titer, the greater the result. Dilution testing using NaCl 0.09% solution were also conclusive as the recovery after 1:8 dilution titer/Ab types (data not shown). Hence, we propose to avoid any sample dilution in samples with high Ab concentrations.

### 3.5 | Comparison of Ab detection between MAGLUMI and Euroimmun assays

The comparison showed that the Euroimmun Kit was less specific (100.0% vs 93.0%). Two FP were observed, the first one was a sample from 2018, and anti-alanyl-tRNA synthetase (anti-PL 12) was presumed to be the cause of the cross reactivity. The other was a fresh sample collected from a healthy volunteer in Mars 2020. All examinations, including clinical, radiological, and biological (RT-qPCR and serology) were negatives. However, MAGLUMI Kit appeared to be less sensitive as it detected about 64.0% of COVID-19 patients (30/n = 47 vs 79.0% detected by Euroimmun Kit), when combining IgG and IgM vs IgG and IgA couples. The degree of agreement estimated by the kappa was 0.77 (95% CI = 0.63-0.91) which could be considered as a substantial, to almost perfect agreement between the two methods.
DISCUSSION

Here, we have demonstrated that an immune response can be expected in almost all patients after more than 15 days from the onset of symptoms. The overall diagnostic sensitivity reached to 95.5%, about 18 days after symptoms onset. There were two patients who did not develop Ab in the subgroup of 15 to 25 days after symptoms onset: a 76-year-old man who showed an immune response at day 25 after the onset of symptoms and died shortly after; and a 32-year-old woman on chemotherapy because of an active invasive ductal carcinoma. The latter patient fully recovered 19 days after symptoms onset (date of the last sampling). As there was not any statistical difference (P = .15) between immunocompromised patients who did not develop Ab to those who developed (including

**FIGURE 1**  A, Receiver operating characteristic (ROC) curve for IgG levels obtained from 176 samples at different stages of infection. Area under the ROC curve (AUC) was estimated = 0.846 (CI = 0.801-0.891), P < .0001. The latter AUC suggests excellent discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups. B, ROC curve for IgM levels obtained from 176 samples at different stages of infection. AUC was estimated = 0.831 (CI = 0.782-0.880), P < .0001. The latter AUC suggests excellent discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups. C, ROC curve for IgG levels obtained from 44 samples between 15 and 25 days after symptoms onset. AUC was estimated = 0.963 (CI = 0.916-1.000), P < .0001. The latter AUC suggests outstanding discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups. D, ROC curve for IgM levels obtained from 44 samples between 15 and 25 days after symptoms onset. AUC was estimated = 0.970 (CI = 0.931-1.000), P < .0001. The latter AUC suggests outstanding discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups. CI, confidence interval; COVID-19, coronavirus disease 2019; IgG, immunoglobulin G
analyzed the MAGLUMI Kit on 70 serum samples. The discrepancies be-

opted for such. As patients

response (Table

who developed Ab (80.0%; n = 125) to those without any immune

deaths (n = 9). Hence, the elderly patients had less time to develop an

delayed 

immunocompromised patients) an immune response, we did not 
exclude these data from the statistical analysis. More studies should 
be carried out on immunocompromised patients suffering from
COVID-19 to fully understand how their immune system responds 
to SARS-CoV-2 infection. 

The statistical analysis showed age (P = .03) and time from 
symptoms onset to hospital admission (P = .001), and to sera sampling 
(P < .001) were significantly different between COVID-19 patients 
who developed Ab (80.0%; n = 125) to those without any immune 
response (Table 2). As the immune system takes several days (gen-

erally more than 4 days) to set in for developing Ab against a specific 
Ag, it was not odd to observe such differences.15 Compared to our 
younger patients (mean = 64 years old), the more advance was the 
age (mean = 71 years old), the earlier was the hospital admission 
(mean = 5 days earlier). Other explanations could be the functional 
decline of the immune system in elderly patients and premature 
deaths (n = 9). Hence, the elderly patients had less time to develop an 
immune response if considering the hospital admission as the starting 
point for Ab measurement. 

Padoan et al16 analyzed the MAGLUMI Kit on 70 serum samples 
(87 samples collected) obtained from 37 COVID-19 patients at dif-
ferent stages of infection. Their results show 100.0% diagnostic 
sensitivity, 11 days after symptoms onset. 

The discrepancies be-
tween the immunocompromised and immunocompetent patient in 
our subcohorts. We also did not consider the fever as a sole criterion 
for the symptom onset as Padoan et al16 opted for such. As patients 
could present various symptoms, we considered all symptoms (fever, 
cough, and dyspnea) to obtain the date of symptoms onset. 

### TABLE 3 Median (IQR) of subgroups Ab levels and overall/subgroups DSn, NPV, AUC, and test accuracy

| Antibody, AU/mL | Days after SO (n = samples) | 0-4 Days (n = 21) | 5-9 Days (n = 50) | 10-14 Days (n = 61) | 15-25 Days (n = 44) | 0-25 Days (n = 176) |
|----------------|-----------------------------|------------------|------------------|-------------------|------------------|-------------------|
| IgG Median IgG (IQR) | | 0.07 (0.039-1.07) | 0.32 (0.08-9.03) | 5.03 (0.87-18.89) | 47.37 (4.53-103.00) | 3.70 (0.18-32.43) |
| DSn% (95% CI) | | 23.81 (10.63-45.09) | 38.00 (25.86-51.85) | 75.41 (63.32-84.49) | 90.91 (78.84-96.41) | 62.50 (55.15-69.31) |
| NPV% (95% CI) | | 86.21 (83.11-88.81) | 76.92 (72.66-80.69) | 86.96 (81.12-91.19) | 90.76 (86.73-93.94) | 60.61 (55.92-65.11) |
| Accuracy % (95% CI) | | 87.60 (79.42-92.25) | 80.00 (72.70-86.08) | 90.68 (85.10-94.69) | 97.22 (90.04-99.24) | 76.45 (70.99-81.33) |
| AUC (95% CI) | | 0.55 (0.40-0.71) | 0.78 (0.69-0.87) | 0.92 (0.86-0.97) | 0.96 (0.92-1.00) | 0.85 (0.80-0.89) |
| IgM Median IgM (IQR) | | 0.30 (0.18-0.53) | 0.59 (0.32-1.42) | 1.81 (0.67-4.14) | 2.87 (1.62-4.50) | 1.30 (0.46-3.13) |
| DSn% (95% CI) | | 9.52 (1.69-28.91) | 40.00 (27.61-53.82) | 72.13 (59.83-81.81) | 90.91 (78.84-96.41) | 60.23 (52.85-67.17) |
| NPV% (95% CI) | | 84.03 (82.08-85.81) | 76.92 (72.66-80.69) | 85.47 (79.71-89.80) | 96.15 (90.76-98.45) | 58.82 (54.36-63.15) |
| Accuracy % (95% CI) | | 84.30 (76.57-90.27) | 80.00 (72.70-86.08) | 89.44 (83.63-93.73) | 97.22 (90.04-99.24) | 74.64 (69.08-79.66) |
| AUC (95% CI) | | 0.52 (0.340.70) | 0.74 (0.64-0.84) | 0.92 (0.87-0.98) | 0.97 (0.92-1.00) | 0.83 (0.78-0.88) |
| IgG/IgM | | 28.57 (13.81-49.96) | 56.00 (42.31-68.84) | 80.33 (68.69-88.37) | 95.45 (84.87-99.19) | 70.45 (63.34-76.70) |
| DSn% (95% CI) | | 86.96 (83.57-89.73) | 81.30 (76.30-85.45) | 89.29 (83.38-93.26) | 98.04 (92.81-99.49) | 65.79 (60.49-70.73) |
| NPV% (95% CI) | | 87.60 (80.38-92.89) | 84.67 (77.89-90.02) | 92.55 (87.34-96.09) | 98.81 (95.07-99.83) | 81.16 (76.04-85.60) |
| Accuracy % (95% CI) | | 0.55 (0.36-0.73) | 0.80 (0.71-0.89) | 0.94 (0.89-0.99) | 0.98 (0.94-1.00) | 0.73 (0.67-0.79) |

Note: All P values were < .0001, except for IgG (P = .43), IgM (P = .77), and IgG/IgM (P = .50) obtained by computing 0 to 4 days results after the onset of symptoms. 

Abbreviations: Ab, antibody; AU, arbitrary unit; AUC, area under the ROC curve; CI, confidence interval; DSn, diagnostic sensitivity; IgG, immunoglobulin G; IgG/IgM, both IgG and IgM at the same time; IQR, interquartile range; NPV, negative predictive value; ROC, receiver operating characteristic; SO, symptoms onset.
A study using 535 plasma samples obtained from 173 confirmed COVID-19 patients on an ELISA platform supplied by Beijing Wantai Biological Pharmacy Enterprise Co, Ltd, showed about 99.0% diagnostic specificity and 93.0% overall diagnostic sensitivity with a median of 11 days (IgG = 14 days and IgM = 12 days) after symptoms onset. The latter study also showed it may take more than a month to reach 100.0% seroconversion. Interestingly, the diagnostic sensitivity at all stages of infection increased and reached more than 99.0% (n = 173) using RT-qPCR (65.0%) and Ab measurement (94.0%) in combination.

Our results are in line with the latter report, we obtained an overall diagnostic sensitivity of 95.5%, 18 days after symptoms onset, and a diagnostic specificity of 100.0% regardless of the sampling period. These estimations should be nuanced as we included only hospitalized patients to assess the diagnostic sensitivities. Considering the current prevalence for seasonal coronaviruses, which is thought to be greater than 70.0%, and provided data by the manufacturer in package inserts (using 104 sera with Ab against other bacterial and viral respiratory infectious agents, including CoVs), one could suggest that there is no evidence for cross-reactivity in our cohorts. Another study with a home-brew-designed ELISA Kit using SARSr-CoV Rp3 N protein as an Ag showed an increase of IgM levels from 50.0% to 81.0% (n = 16) and of IgG levels from 81.0% to 100.0%, from 0 to 5 days after hospital admission. Assuming it takes between 7 and 10 days from the onset of symptoms to hospital admission, we can conclude optimal Ab sensitivities will be achieved after more than 12 to 15 days from symptoms onset.

On the basis of previous studies and our data, if the first test is negative but the clinical suspicion for COVID-19 remains, a second test should be carried out 2 weeks later to reach an optimal diagnostic sensitivity. Our data are preliminary, and we suggest to confirm the diagnostic specificity and PPV in the more general subset. The claimed seropositivity cutoffs by the manufacturer appeared to be accurate. Hence, it could be used in all clinical laboratories.

We have also verified the analytical performance of the CLIA method. All analytical verifications were successfully fulfilled beside the linearity assessments which did not confirm Padoan et al report. One explanation could be the latter report did not assess the linearity in higher IgG and/or IgM concentrations suggesting the lack of linearity in high concentrations. We assume that the lack of linearity/Ab types was not caused by the matrix effect as dilution tests using the NaCl 0.09% solution were not conclusive. We also

| Analysis                              | IgG          | IgM          |
|---------------------------------------|--------------|--------------|
| Precision                             |              |              |
| Mean of NCL, AU/mL                    | 0.02         | 0.36         |
| NCL within-run CV%                    | 19.2         | 12.6         |
| NCL between-run CV%                   | 40.0         | 43.5         |
| Mean of PCL, AU/mL                    | 3.56         | 3.57         |
| PCL within-run CV%                    | 1.2          | 2.2          |
| PCL between-run CV%                   | 6.3          | 4.8          |
| Trueness                              |              |              |
| Means of PCLs, AU/mL                  | 3.72         | 3.69         |
| Bias (%)                              | −5.0         | −6.0         |
| LOB, AU/mL                            | 0.02         | 0.51         |
| LOD, AU/mL                            | 0.03         | 0.58         |
| LOQ                                   |              |              |
| Means of pool 1’ Ab levels (AU/mL); overall CV% | 3.57; 6.5  | 2.58; 4.8  |
| Means of pool 2’ Ab levels (AU/mL); overall CV% | 83.27; 12.3 | NR          |
| Means of pool 3’ Ab levels (AU/mL); overall CV% | 191.15; 7.9 | NR          |
| % Carry-over                          | 0.01         | 0.90         |

Abbreviations: Ab, antibody; AU, arbitrary unit; CLIA, chemiluminescent immunoassay; CV, coefficients of variation; LOB, limit of blank; LOD, limit of detection; LOQ, limit of quantification; NCL, negative control level; PCL, positive control level.
successfully determined the LOB, LOD, and carry-over which were not reported by Padoan et al.\textsuperscript{16} In our experience, MAGLUMI 800 appeared to be user-friendly and reliable with a throughput of 80 tests per hour. All reagents were in the same cartridge and there was neither mixing nor preparation before analysis.

Considering the correlation between methods, we compared the overall diagnostic sensitivity and specificity of Snibe 2019-nCoV Kit (IgG/IgM), with Euroimmun anti-SARS-CoV-2 (IgG/IgA) using 77 samples (30 COVID-19 free and 47 COVID-19 patients). As the measured Abs, type of assays and limits of quantification were different for two methods, the kappa index was calculated on combined Ab results. Our results showed Snibe CLIA method was more specific (about 100.0% vs 93.0%) and less sensitive (about 64.0% vs 79.0%). These results were in line with those observed in a recent article from our research group, published by Montesinos et al.\textsuperscript{19} Results obtained from 200 samples (from patients and healthy subjects) showed that Snibe CLIA method (IgG/IgM) was more specific (about 100.0% vs 87.5%) but less sensitive (about 64.3% vs 84.4%) comparing to Euroimmun ELISA (IgG/IgA).\textsuperscript{17} On the other hand, a comparison between the two latter methods carried-out by Lippi et al\textsuperscript{20} using 48 samples collected at different stages of infections, showed better diagnostic sensitivity of the CLIA method comparing to the ELISA method. These discrepancies could be explained by the number of tested samples, inclusion/exclusion criteria, sampling period, infection stages, and age criteria in each study. More studies should be carried out to confirm these observations. On the basis of these studies, the overall agreement between the two methods was more than 82.0% (Kappa's Cohen = 0.65), which could be considered a substantial agreement.

We also gathered all patients' data to check whether they were in agreement with previous clinical/biological findings and if they were related to the seropositive status. We observed that the median time from symptoms onset to hospital admission, to hospital discharge, and to death were 6.0, 15.0, and 15.0 days, respectively. Overall, about 80.0% of our 125 COVID-19 patients developed Ab against COVID-19 with a median time of 12.0 days after symptoms onset. The median time between symptoms onset and Ab development in recovered remained at the hospital and deceased subgroups were 12.0, 12.0, and 13.0 days, respectively (Table 1). Our results also suggested that all patient subgroups developed Ab over the same period regardless of their outcome. Three studies with 453 (121 dead) patients reported the median time between symptoms onset and the hospital admission ranged from 7.0 to 10.0.\textsuperscript{21-23} The median times from symptoms onset to hospital discharge and death were 26.0 days (IQR = 22-29) and 16.0 days (12-20), respectively, comparing 113 deceased and 161 recovered cases.\textsuperscript{22-23} These results were in agreement with our findings. The statistical analysis showed a number of deceased cases was statistically different between patients who developed Ab and those without any immune response (Table 2).

In our cohort, the fever and viral pneumonia were observed in 100.0%, dyspnea in 96.0%, cough in 95.0%, fatigue and/or myalgia in 38.0%, gastrointestinal symptoms in 24.0%, and nonspecific types including headache, confusion, anosmia, or dysgeusia in 44.0% of COVID-19 patients (Table 1). The analysis of two other studies with 412 hospitalized patients (n = 412; 119 dead) showed patients had various symptoms such as fever (93.0%), cough (65.0%), fatigue (57.0%), dyspnea (40.0%), anorexia (29.0%), and myalgia (26.0%) in the majority of cases.\textsuperscript{22,23}

Our clinical findings on 125 patients were in agreement with these two studies besides dyspnea and cough which were more present in our cohort. On the basis of our data, we propose to clinically/serologically monitor suspected cases at least for 2 weeks and to look for more specific symptoms such as fever, cough, and dyspnea (Table 1). In our cohort, all patients were symptomatic and only 80.0% of them developed an immune response. In our cohort, some symptomatic patients did not develop Ab (25/125), and in addition, COVID-19 asymptomatic carriers were not included in our study. However, we could conclude that all symptomatic patients did not develop an immune response.

These latter studies show the majority of patients did not suffer from any comorbidities (52.0%; n = 412 vs our data = 14.0%) and the more common comorbidities were arterial hypertension (33.0% vs our data = 44.0%), followed by diabetes (15.0% vs our data = 26.0%) and cardiovascular diseases (12.0% vs our data = 20.0%).\textsuperscript{22,23} We documented more comorbidities in our cohort and confirmed the age factor (but not the comorbidity) impacted drastically the final outcome. The mean age of the deceased subgroup in our study was higher with a mean of 82.1 years old compared to the recovered subgroup, which had a mean of 62.5 years old (Table 1). It appeared there was not any correlation between comorbidities and Ab development in our cohort (Table 2).

Our data showed that other than radiology (100.0%), complete blood count (CBC) and blood biochemistry markers could be useful in COVID-19 patient’s diagnosis if they are available. Over 86.0% and 99.0% of our cohort experienced increased lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels, respectively. These results are in agreement with previous studies, suggesting pathological radiology profile (98.0%; n = 552), skewed biochemistry (>59.0%; n = 413), and CBC markers (>58.0%; n = 414).\textsuperscript{21-24} Hence, in addition to patient monitoring, CT scan, CBC (lymphocyte count), and blood biochemistry markers (LDH, CRP, and other hepatic markers) could also be useful as a triage tool if are available (Table 1). It appeared there was not any correlation between these biomarkers and Ab development (Table 2). Studies should be carried-out among pauciymytic, asymptomatic, and hospitalized subjects to assess the correlation with biological profiles.

Besides supportive care to monitor patient’s state and increase oxygen saturation of organism by the means of noninvasive oxygen therapy, intubation, extracorporeal membrane oxygenation, and looking for concomitants or superinfections (bacterial, fungal, or viral), there is no specific treatment, nor effective vaccine for the treatment of COVID-19.\textsuperscript{25} Two recent reviews, in which a considerable number of articles were methodically analyzed, do not suggest any specific COVID-19 treatment.\textsuperscript{26,27} In our cohort 92.0%
of patients received hydroxychloroquine, 43.0% antibiotics, and only 8.0% corticosteroids in addition to supportive treatments (Table 1). Our data did not suggest that treatments were correlated to Ab development (Table 2).

The overall case fatality rate (OCFR), in our cohort, was 13.6%. There appeared to be a positive correlation between age and the risk of death. On the basis of World Health Organization daily situation reports by 27 April 2020, the OCFR was 7.0% globally (n = 3,090,445). On the basis of daily situation report published on 27 April 2020, by Belgian center of Epidemiology of Infectious Diseases (Sciensano), the OCFR was 15.7% (n = 49,032) in Belgium. Discrepancies in OCFRs among countries could be explained by different testing politics, hygienic measures, lockdown rules, case definitions, and age subgroups of infected population.\(^5\)\(^-\)\(^2\)\(^9\)

Our study had limitations. First of all, all included patients were symptomatic and needed to be hospitalized. Hence, we can only suggest the same immune response in asymptomatic or pauci-symptomatic carriers. We did not perform any neutralizing Abs assessment and it is unknown whether the CLIA method detects such Abs. One could only suggest Abs are neutralizing based on previous studies on animal and humans.\(^5\)\(^-\)\(^2\)\(^9\)

Finally, we could not verify the upper LOQ of IgM as we did not have enough samples with sufficiently high concentrations.

5 | CONCLUSION

The serology of COVID-19 patients alone could not allow diagnosis without the clinical, biological, and radiographic data of the patients. Our results showed that we could expect a high number of clinically FNs, particularly in the early stages of infection. On the other hand, clinical FP results appeared to be rare. Hence, all negative tests should be interpreted cautiously. Our data on patients' characteristics showed the age and the number of deceased cases were negatively related to Ab development, while the time from symptoms onset to sampling, and to hospital admission, were positively related to Ab development. At present, RT-qPCR using nasopharyngeal swabs or lower respiratory tract samples is still the standard laboratory test for SARS-CoV-2 detection, despite the fact that the diagnostic sensitivity does not reach to 100.0%. Other methods which do not detect the virus but the disease (COVID-19), such as serology and radiological examination, could be useful in the patient management. In fact, the radiological examination could allow the early detection and triage of patients. Therefore, a combination of RT-qPCR, serology, and radiographic examination would increase COVID-19 detection. As a result of these findings, we propose to check the immune response of suspected patients up at least 15 days after symptoms onset. The CLIA method appeared to be a robust and reliable to measure Abs against COVID-19 at a high throughput.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors have approved the entire content of the submitted manuscript and any subsequent revised version and have accepted responsibility for the entire work. RS (clinical pathology resident, Medical Microbiology Service): Conceived the project (methodology, validation protocol, samples collections, and ethical committee approval), reviewed all patient’s medical records, collected the data, and wrote the manuscript in collaboration with BK-M, reviewed the literature, and responded to the reviewers. MK (clinical pathology resident, Medical Biochemistry Service): Reviewed all patient’s medical records, gathered all clinical and laboratory records with RS, and wrote the abstract. DG (clinical pathologist, head of Medical Biochemistry Service): Supervised the whole analytical aspects (preanalytic, analytical validation, automation, postanalytic, and laboratory technician training for using of the analyzer) of the project and revised the manuscript. HR-V (clinical pathologist, head of Virology and Molecular Biology Services, head of National Reference Centers of HIV, Hepatitis B and C Viruses, Borrelia burgdorferi, Bartonella henselae, and Clostridium difficile): Supervised the whole project; methodology, medical validation, manuscript preparation, responses to reviewers, and covered costs of the work/publication.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the main manuscript and the supplementary material of this article. The detailed clinical/biological/radiological data of COVID-19 confirmed patients are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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