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

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- 1- Reza Soleimanin, 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, wrote the manuscript in collaboration with Benoît Kabamba-Mukadi, reviewed the literature and responded to the reviewers.
- 2- Mehdi Khourssaji, Clinical pathology resident, Medical Biochemistry Service: Reviewed all patient's medical records, gathered all clinical and laboratory records with Reza Soleimani and wrote the abstract.

- 3- Damien Gruson, Clinical pathologist, head of Medical Biochemistry Service: Supervised the whole analytical aspects (pre-analytic, analytical validation, automation, post-analytic and laboratory technician training for using of the analyzer) of the project and revised the manuscript.
- 4- Hector Rodriguez-Villalobos, Clinical pathologist, head of medical microbiology service: Revised the manuscript.
- 5- Mathilde Berghmans, Leila Belkhir and Jean-Cyr Yombi, Specialists in infectious diseases: Patients' management (clinical and treatment).
- 6- Benoît Kabamba-Mukadi, Clinical pathologist, head of Virology and Molecular Biology Services, head of National Reference Centres of HIV, Hepatitis B & C Viruses, *Borrelia burgdorferi*, *Bartonella henselae* and *Clostridium difficile*: Supervised the whole project; methodology, medical validation, manuscript preparation, responses to reviewers, covered costs of the work/publication.

Abstract

Background: Since December 2019 we have been in the battlefield with a new threat to the humanity, known as SARS-CoV-2, which causes COVID-19, characterized by viral pneumonia. It may be asymptomatic or cause various symptoms, ranging from flu like symptoms to ARDS and eventually death. At present, the only reliable test for COVID-19 diagnosis is RT-qPCR. 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.

Methods: One hundred samples obtained from COVID-19 negative subjects (COVID-19 free) were analyzed to evaluate 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.

Results: All COVID-19 free samples had Ab levels below the cut-off values. Hence, the diagnostic specificity was estimated at 100% (95%CI= 96.3-100.0; PPV= 100%). By the 18th day from the onset of symptoms we reached to an optimal diagnostic sensitivity (more than 95.0 %) In fact, the diagnostic sensitivity increased over time and between 15 to 25 days after symptoms onset, reached to 95.5% (95% CI= 84.9-99.2).

Conclusion:

The new automated CLIA analyzer appeared to be a robust and reliable method to measure specific Ab against COVID-19 at a high throughput. Our data suggest that combining Ab and nucleic acid detection could increase the diagnostic sensitivity.

Introduction and objectives

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 the coronavirus disease-19 (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 (ARDS) and eventually death. There is no specific treatment and by April 27, 2020, SARS-CoV-2 has infected and killed more than 2,805,000 and 194,000 patients,

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respectively. These figures seem optimistic as we cannot yet detect all infected patients by the reverse transcriptase polymerase chain reaction (RT-qPCR) [1].

At present, the only reliable test for SARS-CoV-2 detection and COVID-19 diagnosis is the RT-qPCR, which is expensive, time consuming and laborious method to implement and requires some expertise and proficient clinical laboratories to perform the assay. Multiple factors such as sub-optimal 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 [2] by 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 (Ab) therefore, will increase the sensitivity of the detection of infected population. It also could be useful to assess how the human immune system respond over time. Hereby, we report the performances of a fully automated chemiluminescent immunoassay (CLIA).

Material and methods

COVID-19 positive subjects (COVID-19 patients)

One hundred and seventy-six serum samples (samples) from 125 with confirmed COVID-19 (COVID-19 patients) were randomly collected into serum-gel tubes from February 25, to March 10, 2020. The inclusion criteria were symptomatic and hospitalized patients with positive RT-qPCR test on nasopharyngeal swab samples and characteristic radiological lung patterns such as ground glass opacity and/or bilateral involvement. There were not any exclusion criteria beside the age (only patients ≥ 18 years 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 April 27, 2020.

The sample size was derived from Noordzij et al.' paper using $\alpha = 0.05$ and $\beta = 0.20$, suggesting ≥ 100 subjects/group [2].

Sampling and preanalytical procedure

All fresh samples were taken by venipuncture and centrifuged at $2,054 \times g$ for 10 minutes. The samples at last were extracted from whole blood, respecting all hygiene measures and stored at $+4$ Celsius (C) before analysis for maximum of three days. After analysis, all residual samples were stored at -20°C .

Immunoglobulin (Ig) G and IgM measurements by MAGLUMI™ 2019-nCoV (CLIA)

We measured IgG/IgM Ab using Snibe 2019-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 (Ag), as they were appeared to be promising targets for Ab detection against other coronaviruses [3]. The produced light emission (RLU) by indirect immunoassay (IA) reaction is proportional to the

specific Ab quantity in the sample. RLUs are then transformed to arbitrary units (AU/mL), using a computed 10-point calibration curve.

A level > 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 were 97.33%, 97.50% and 96.00%, respectively. All claimed within and between-run coefficients of variation (CVs) for positive quality controls were $< 10.0\%$. The manufacturer did not claim within nor between-run CVs for negative quality controls [4].

Diagnostic specificity

For this purpose, we analyzed 100 residual samples (60 females (F); 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 $> 95\%$) [5]. Samples (serum samples were separated and frozen at the time of sampling) were divided in 3 subgroups. Forty samples with no known confounding factors and 40 others with supposedly confounding factors (*cf.* the supplementary 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 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 results (TN) correctly identified by the method and calculated by the formula $Sp = \frac{TN}{TN + \text{False positives (FP)}}$. We also verified the cut-off of seropositivity for each Ab based on COVID-19 free sample levels (if < 1 AU/mL).

Diagnostic sensitivity

For this purpose, we performed the immunoassay 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 GGO identified by X-ray or CT-scan). For each of these samples, the date symptoms onset was determined based on the clinical records. The diagnostic sensitivity was estimated based on the receiver operating characteristic (ROC) curves for 4 different time intervals depending on symptoms onset; 0-4 days after, 5-9 days after, 10-14 days after and 15-25 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 $> 95\%$) [5]. At last, positive and negative predictive values (PPV and NPV) and test accuracy were statistically estimated to assess the proportion of true positive, true negative and accuracy of the CLIA method.

The sensitivity was defined as the percentage of true positive results (TP) correctly identified by the method, and calculated by the formula, $S = \frac{TP}{TP + \text{False Negatives results (FN)}}$. The positive predictive value (PPV) was defined as the probability of a positive result among true patients and calculated by the formula $PPV = \frac{TP}{TP + FP}$. The negative predictive value (NPV) was defined as the probability of a negative result among healthy subjects and calculated by the formula $NPV = \frac{TN}{TN + FN}$.

TN/(TN+FN). Accuracy was defined as the closeness of the measurements to an expected value and calculated by the formula $ACC = (TP+TN)/(TP+FP+TN+FN)$.

Precision and trueness

To verify the method accuracy, we used precision and trueness according to ISO 5725-2:2019 [6]. Precision was assessed by comparing the locally calculated within-run and the between-run coefficients of variation (CVs) to those claimed by the manufacturer in package inserts. For this purpose, 2 control levels per Ab types (IgG= 0.29 & 3.92 AU/mL; IgM= 0.29 & 3.92 AU/mL) were analyzed according to CLSI EP15-A3. During 5 consecutive days, each control level analyzed 3 x/day. We considered the verification satisfactory if calculated CVs for positive control levels were < 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 20x 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 PQC provided by the manufacturer's package inserts. [7, 8].

Limit of blanc (LOB), limit of detection (LOD), limit of quantitation (LOQ),

Linearity and carry-over

A) LOB was defined as the mean of blank replicates + $(1.645 \times SD_{\text{blank replicates}})$.

For this purpose, negative samples with no measurand (NaCl 0.09% solution) analyzed 10x in row (IgG mean= 0.02 AU/mL; IgM mean= 0.44 AU/mL).

LOD was defined as $LoB + (1.645 \times SD_{\text{blank plasma pool replicates}})$. LOQ were

verified by analyzing the results of the 3 x 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 1 pool (2.58 AU/mL) was analyzed in triplicate for 5 days, to evaluate the lower LOQ. The LOQ were verified if overall CV (including within & between-run CVs) for each pool was < 20 %. LOB, LOD and LOQ were verified according to the CLSI EP17-A2 guideline and the Armbruster & Pry's paper [9, 10].

- B) The linearity assessments were carried out by analysing 5 serum pools in triplicate. For this purpose, we used 2 initial pools/Ab types (IgG= 104.07 & 0.07 AU/mL; IgM= 15.00 & 0.51 AU/mL), to prepare 3 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, & 8) on 2 other serum pools (IgG mean= 197.63 AU/mL & 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, a negative sample (NS) were tested (IgG mean= 0.079 AU/mL, IgM mean= 0.22 AU/mL) in triplicates (namely NS1, NS2 and NS3). The carry-over calculated based on the formula (%C-O) =

(NS1 - NS3 / Ab3 - NS3) x 100 according to CLSI EP07 and Burner, *et al.*'s paper [3, 12]. We verified the method if calculated carry-over were < 1% as an arbitrary criterion.

IgG and IgA measurements by Euroimmun anti-SARS-CoV-2 (ELISA)

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 immunosorbent 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 (OD) which is proportional to Ab concentration in samples were transformed to AU/mL. Considering the result interpretation, Ab levels ≥ 1.1 AU/mL were considered positive.

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 (RdRp) gene. The extraction of the viral RNA was carried out on the m2000sp platform (Abbott Molecular Inc., Illinois, USA) 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

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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].

Statistical analysis

Means, 95% confidence intervals (CIs), medians, interquartile ranges (IQR) were determined in COVID-19 free subjects and in COVID-19 patients. The diagnostic sensitivities and specificity, positive and negative predictive values (PPV & NPV), areas under the receiver operating characteristic curve (AUCs) and accuracies were estimated based on the receiver operating characteristic (ROC) curve results. The degree of agreement between the 2 methods was determined by the kappa index. Absolute values rounded to 2 digits after the point and percentages rounded to 1 digit after the point. A Mann-Whitney test was performed on COVID-19 patients in order to find a possible correlation between patient's characteristics (demographic, clinical, radiographic and laboratory findings) and Ab development. A *p* value of < 0.05 was considered as statically significant.

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

Ethical aspect

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).

Results

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 pneumophila*. There was only 1 case of concomitant infection of influenza (type B) and no case of respiratory syncytial virus (RSV) 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 ($p < 0.03$; 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 2 latter subgroups ($p < 0.001$; Table 2)

Diagnostic specificity

The means of IgG and IgM levels and their 95% CI in COVID-19 free group (100 samples, 100 subjects) were 0.12 AU/mL (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 cut-off 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 cut-

offs were also successfully verified at the same time (data are provided in the supplementary table).

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 1-A & 1-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.

Interestingly, the diagnostic sensitivities increased for samples (n= 44) collected between 15 to 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 1-C & 1-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 (80.0%; n= 125) and those without any immune response. All data regarding diagnostic sensitivities, NPVs, AUCs and test accuracies between 0 to 25 days after symptoms onset are summarized Table 3.

Precision, trueness, limit of blanc (LOB), limit of detection (LOD), limit of quantification (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 ($< \pm 10\%$) assessments were satisfactory. The LOB and LOD of IgG and IgM were also showed 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 not also conclusive as we reached to more than 400% of 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.

Comparison of Ab detection between MAGLUMITM and Euroimmun[®] assays

The comparison showed that the Euroimmun kit was less specific (100.0% vs 93.0%). Two false positives (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 (HV) 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 to 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 18.0 days after symptoms onset. There were two

patients who did not develop Ab in the subgroup of 15-25 days after symptoms onset: a 76-year-old man who showed an immune response at day of 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 ($p= 0.15$) between immunocompromised patients who did not developed Ab to those who developed (including 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 ($p= 0.03$) and time from symptoms onset to hospital admission ($p= 0.001$), and to sera sampling ($p< 0.001$) 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 (generally more than 4 days) to set in for developing Ab against a specific Ag, it was not odd to observe such differences [16]. 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 al.* analyzed the MAGLUMI™ kit on 70 serum samples (87 samples collected) obtained from 37 COVID-19 patients at different stages of infection. Their results show 100.0% diagnostic sensitivity, 11 days after symptoms onset [15]. The discrepancies between the diagnostic sensitivities could be explained by the number

of included patients in each study. One advantage of our study was the number of the patients (125) included and the number of the samples (176) tested. We did not use the immunosuppression criterion (caused by various diseases/therapies) to exclude 14 patients' data, as it appeared there was not any significant difference ($p=0.15$) between immunocompromised and immunocompetent patient in our sub-cohorts. We also did not consider the fever as a sole criterion for the symptom onset as Padoan, *et al.* [15] opted such. As patients could present various symptoms, we considered all symptoms (fever, cough and dyspnea) to obtain the date of symptoms onset.

A study using 535 plasma samples obtained from 173 confirmed COVID-19 patients on a 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 & 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 [16].

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 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 $> 70.0\%$ [17], 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-reactivities 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 to 10 days from the onset of symptoms to hospital admission, we can conclude an optimal Ab sensitivities will be achieved after more than 12-15 days from symptoms onset [18]. Based on 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 more general subset. The claimed seropositivity cut-offs 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 [15]. 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 successfully determined the LOB, LOD and carry-over which were not reported by Padoan, *et al.* 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 prior to analysis.

Considering 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 & 47 COVID-19

patients). As the measured antibodies, 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* [19]. Results obtained from 200 samples (from patients & 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) [19]. On the other hand, a comparison between the 2 latter methods carried-out by Lippi, *et al.* using 48 samples collected at different stages of infections, showed a better diagnostic sensitivity of the CLIA method comparing to the ELISA method [20]. 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. Based on these studies the overall agreement between the 2 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 antibodies 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 [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 [23]. These results were in agreement with our findings. The statistical analysis showed 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 2 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%), myalgia (26.0%) in the majority of cases [22, 23].

Our clinical findings on 125 patients were in agreement with these 2 studies beside dyspnea and cough which were more presents in our cohort. Based on 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 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 (AHT; 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%) [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 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 patients 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) [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). We could not any correlation between these biomarkers and Ab development (Table 2). Studies should be carried-out among pauci-symptomatic, 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 non-invasive oxygen-therapy, intubation, extracorporeal membrane oxygenation (ECMO) and looking for concomitants or super-infections

(bacterial, fungal or viral), there is no specific treatment, nor effective vaccine for the treatment of COVID-19 [25]. Two recent reviews, in which a considerable number of articles were methodically analyzed, do not suggest any specific COVID-19 treatment [26, 27]. In our cohort 92.0% of patients received hydroxychlorquin, 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. Based on WHO daily situation reports by April 27, 2020 the OCFR was 7.0% globally (n= 3,090,445). Based on the daily situation report published on April 27, 2020, by Belgian center of Epidemiology of Infectious Diseases (Sciensano) the OFCR 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 [28, 29].

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 antibodies assessment and it is unknown whether the CLIA method detects such antibodies. One could only suggest antibodies are neutralizing based on previous studies on animal and humans [16, 30].

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

Conclusion

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The serology of COVID-19 patients alone could not allow a diagnosis without the clinical, biological and radiographic data of the patients. Our results showed we could expect a high number of clinically false negatives, particularly in the early stages of infection. On the other hand, clinical false positive 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 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 antibodies against COVID-19 at a high throughput.

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Availability of Data: The data that supports the findings of this study are available in the main manuscript and 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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Figure

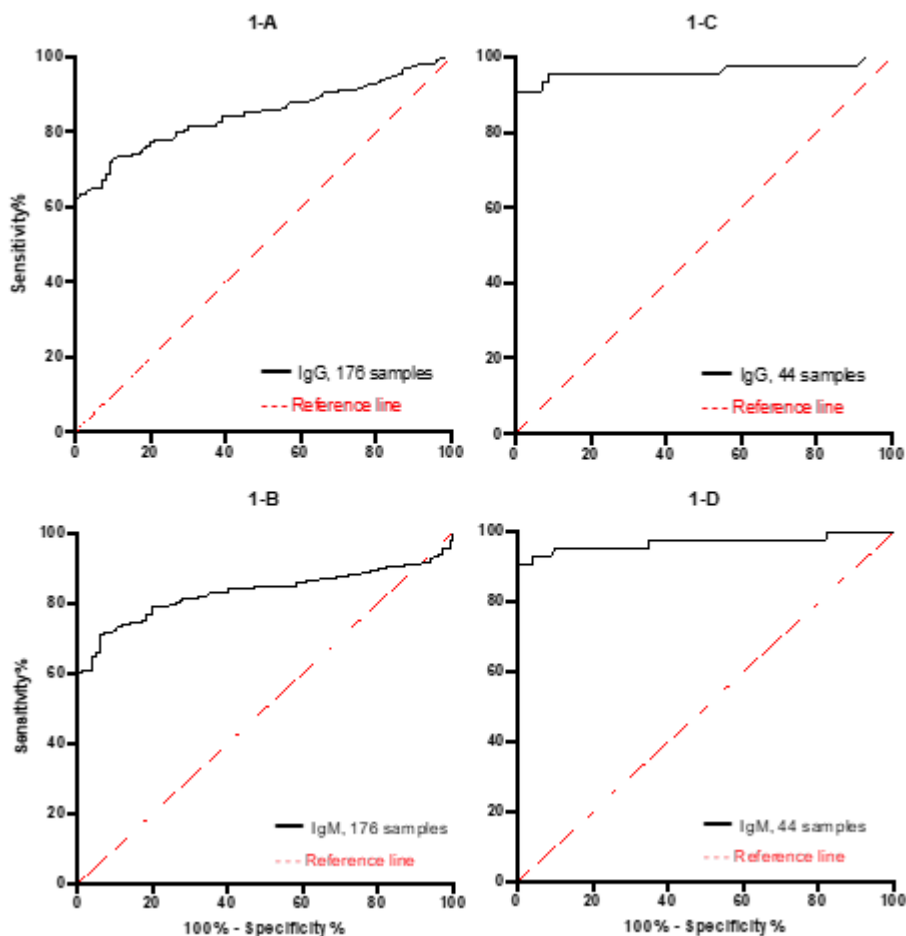
Figure 1-A: Receiver operating characteristic (ROC) curve for IgG levels obtained from 176 samples at different stages of infection. Area under the ROC curve (AUC) was estimated = 0.846 (CI= 0.801-0.891), $p < 0.0001$. The latter AUC suggests excellent discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups.

Figure 1-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), $p < 0.0001$. The latter

AUC suggests excellent discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups.

Figure 1-C: ROC curve for IgG levels obtained from 44 samples between 15-25 days after symptoms onset. AUC was estimated = 0.963 (CI= 0.916 to 1.000), $p < 0.0001$. The latter AUC suggests outstanding discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups.

Figure 1-D: ROC curve for IgM levels obtained from 44 samples between 15-25 days after symptoms onset. AUC was estimated = 0.970 (CI= 0.931 to 1.000), $p < 0.0001$. The latter AUC suggests outstanding discriminatory power of the test between the COVID-19 patients and the COVID-19 free groups.



Tables

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.

COVID-19 patients (n=125; F=58)	Deceased (n=17; F=9)	Remained at hospital by 30 Avril 2020 (n=7; F=0)	Recovered patients (n=101; F=49)
Demographics			
Mean age (95% CI) Male= 63.5 (60.1-66.8) Female= 67.2 (62.4-72.1) Overall= 65.2 (62.3-68.1) years	Male= 79.0 (67.9-90.1); Female= 84.9 (75.3-94.5); Overall= 82.1 (75.5-88.7)	Male & overall= 62.6 (55.1-70.0); Female= NA	Male= 61.2 (57.5-64.9); Female= 64.0 (58.9-69.0); Overall= 62.5 (59.5-65.9)
18-39 years (F)	0 (0)	0 (0)	3 (5)
40-59 years (F)	1 (0)	3 (0)	22 (15)
60-79 years (F)	4 (3)	4 (0)	23 (17)
≥80 years (F)	3 (6)	0 (0)	4 (12)
Time from symptoms onset to hospital admission (days) Mean (95% CI) Median (IQR)	3.8 (1.6-6.0) 2.0 (1.0-7.0)	6.6 (5.7-7.5) 7.0 (6.0-7.0)	6.6 (5.9-7.4) 6.0 (3.0-8.0)
Time from hospital admission to final outcome (days)	11.1 (7.8-14.5)	> 29.4 (26.2-32.7)	9.6 (8.5-10.6)

Mean (95% CI)	11.0 (6.0-18.5)	> 28.0 (27.0-31.0)	8.0 (5.0-12.5)
Median (IQR)			
Time from symptom onset to final outcome (days)			
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 (4-10x10⁹/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 (65.3); I=21 (20.8); D=14 (13.9)
Neutrophil count (1.6-7x10⁹/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 (0.8-5x10⁹/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 (150-450x10⁹/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 umol/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)
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)
Anti-viral/bacterial/fungal treatments during hospitalization			

Hydroxy chloroquine (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 (27.7)
Lung diseases (Asthma, BPCO): n (%)	2 (11.8)	0 (0.0)	8 (7.9)
Leucopenia (immunosuppressive treatments (auto-immune 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)

95% CI= 95% confidence interval; IQR= Interquartile range; ALT= Alanine aminotransferase; AST= Aspartate Aminotransferase; GGT= Gamma Glutamyl Transpeptidase; ALP= Alkaline Phosphatase; LDH= Lactate Dehydrogenase; T.Bili= Total Bilirubin; SCr= Serum Creatinine; Urea= Blood Urea Nitrogen; CRP= C-Reactive Protein; hs-Tpn-T= High-sensitive cardiac troponin T; pro-BNP= B-type natriuretic peptide; Flu = Influenza A & B; RSV= Respiratory Syncytial Virus; ARDS= Acute respiratory distress syndrome; ECMO= Extracorporeal membrane oxygenation; GGO= ground glass opacity; N= Normal, I= Increased, D= Decreased; NA= Not applicable; NR= Not realized. Considering there were not enough subjects (< 20 patients) in deceased and remained at hospital subgroups, we did not realize any statistical analysis.

Table 2: Association between clinical/biological characteristics and the immune response in COVID-19 patients.

Factors	Positive immune response (n= 100)	Negative immune response (n= 25)	<i>p</i> value
Deceased: n	8	9	0.001
Sampling period (days)			
Mean (95% CI)	12.8 (11.9-13.6)	7.6 (6.2-9.1)	<0.001
Median (IQR)	13.0 (10.0-16.0)	8.0 (4.0-10.3)	
Symptoms onset to hospital admission (days)			
Mean (95% CI)	6.8 (6.1-7.6)	3.9 (2.5-5.3)	0.001
	7.0 (4.0-9.0)	2.0 (1.0-7.5)	

Median (IQR)			
Hospital admission to final outcome (days)			
Mean (95% CI)	11.2 (9.7-12.6)	9.8 (7.4-12.2)	0.51
Median (IQR)	9.5 (6.0-14.0)	9.0 (5.0-14.0)	
Age (years): Mean (95% CI)			
	63.7 (60.9-66.4)	71.4 (62.2-80.6)	0.03
Sex: n of male			
	57	9	0.07
Abnormal biochemistry markers: n			
	100	25	NS
Abnormal CBC markers: n			
	44	13	NS
Comorbidities: n			
	89	18	NS
Hydroxychloroquine: n			
	87	21	0.75
Antibiotics: n			
	45	7	0.17
Corticosteroids: n			
	8	2	>0.99
Immunocompromised: n			
	9	5	0.15

95% CI= 95% confidence interval; IQR= Interquartile range; NS= not statistically significant; biochemistry markers= CRP, hepatic and renal function markers; CBC markers= Hemoglobin, white blood cells, neutrophil, lymphocyte and platelet count; Immunocompromised= Included chemotherapy and immunosuppressive treatment.

Table 3: Median (IQR) of subgroups Ab levels and overall/subgroups DS_n, NPV, AUC and test accuracy.

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)
Antibody (AU/mL)						
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)
	DS _n % (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)	96.15 (90.76-98.45)	60.61 (55.92-65.11)
	Accuracy % (95% CI)	86.78 (79.42-92.25)	80.00 (72.70-86.08)	90.68 (85.10-94.69)	97.22 (93.04-99.24)	76.45 (70.99-81.33)
	AUC (CI 95%)	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)
	DS _n % (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)

IgG/IgM	Accuracy % (95%CI)	84.30 (76.57-90.27)	80.00 (72.70-86.08)	89.44 (83.63-93.73)	97.22 (93.04-99.24)	74.64 (69.08-79.66)
	AUC (CI 95%)	0.52 (0.34-0.70)	0.74 (0.64-0.84)	0.92 (0.87-0.98)	0.97 (0.93-1.00)	0.83 (0.78-0.88)
	DSn% (95%CI)	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)
	NPV% (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)
	Accuracy % (95%CI)	87.60 (80.38-92.89)	84.67 (77.89-90.02)	92.55 (87.34-96.09)	98.61 (95.07-99.83)	81.16 (76.04-85.60)
	AUC (CI 95%)	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)

SO= Symptoms onset; IQR= Interquartile range; DSn= Diagnostic sensitivity; NPV= Negative predictive value, AUC= Area under the receiver operating characteristic curve; IgG/IgM= both IgG and IgM at the same time.

All *p* values were < 0.0001, except for IgG (*p*= 0.43), IgM (*p*= 0.77) and IgG/IgM (*p*= 0.50) obtained by computing 0 to 4 days results after the onset of symptoms.

Table 4: Analytical assessments of the CLIA method. NCL= Negative control level; PCL= Positive control level; LOB= Limit of blank; LOD= Limit of detection; LOQ= Limit of quantification, Ab= Antibody.

	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
Turness	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
% Cary-over		0.01	0.90
Linearity (dilution titer took into account)	Means of pool 1' Ab levels (AU/mL) x1; recovery %	0.07; 0.1	0.51; 3.4
	Means of pool 2' Ab levels (AU/mL) x4; recovery %	215.71; 207.3	33.73; 224.8

Means of pool 3' Ab levels (AU/mL) x2; recovery %	161.33; 155.0	21.42; 142.8
Means of pool 4' Ab levels (AU/mL) x1.33; recovery %	128.92; 123.9	16.28; 108.5
Means of pool 5' Ab levels (AU/mL) x1; recovery %	104.07; 100	15.00; 100
