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Short Communication

Diagnostic performance of an automated chemiluminescence immunoassay for SARS-CoV-2 IgG and IgM antibodies detection: A real life experience

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ABSTRACT

Background: Recently many serological assays for detection of antibodies to SARS-COV-2 virus were introduced on the market. Aim of this study was to assess the diagnostic performance of an automated CLIA for quantitative detection of anti-SARS-CoV-2 IgM and IgG antibodies.
Methods: A total of 354 sera, 89 from consecutive patients diagnosed with COVID-19 (43 mild, 32 severe and 13 critical) and 265 from asymptomatic and negative on rRT-PCR testing healthcare workers, were evaluated for IgM and IgG anti-SARS-CoV-2 antibodies with MAGLUMI immunoassay.
Results: The overall sensitivity and specificity were 86.5% (95%CI: 77.6–92.8) and 98.5% (95% CI:96.2–99.6), respectively. PPV, PPN, LR+, LR- and OR were 95.1 (95%CI: 87.8–98.6), 95.6 (95% CI: 92.4–97.7), 57.3 (95%CI: 21.6–152.1), 7.3 (95%CI: 4.31–12.4) and 418.6 (95%CI: 131.2–1335.2), respectively. The levels of SARS-CoV-2 IgM and IgG antibodies were 1.22 ± 1.2 AU/mL and 15.86 ± 24.83 AU/mL, 2.86 ± 2.4 AU/mL and 69.3 ± 55.5 AU/mL, 2.47 ± 1.33 AU/mL and 83.9 ± 83.9 AU/mL in mild, severe and critical COVID-19 groups, respectively. A significant difference in antibody levels between mild and severe/critical subjects has been shown.
Conclusions: The CLIA assay showed good diagnostic performance and a significant association between antibody levels and severity of the disease was found.

1. Introduction

Coronavirus infectious disease 2019 (COVID-19) was first reported in Wuhan (China) in December 2019, and it is caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) [1]. After the initial reports of disease outbreak in China, COVID-19 has spread worldwide, and on March 11, 2020 the World Health Organization (WHO) defined the spread of COVID-19 as a pandemic [2].

A timely and accurate diagnosis is very important both to start an appropriate treatment and to limit the spread of the virus,

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supporting case ascertainment and tracking of outbreak [3]. Currently COVID-19 is diagnosed through detection of the SARS-CoV2 virus in upper and lower respiratory specimens by molecular tests, such a real-time-reverse-transcription polymerase chain reaction (rRT-PCR) which was rapidly available following the identification of the virus [3,4]. However, even if some Authors showed that rRT-PCR on throat or nasopharyngeal swabs brought out negligible false negative results [5], other reported that the performance of RT-PCR depends on many factors such as viral load, site and timing of specimen collection [1,6]. Since false negative results may to lead to a misdiagnosis, facilitating the circulation of contagious individual and the spread of the virus, anti-SARS-CoV-2 antibody detection could represent a useful tool to reduce false negative results. However, in general antibodies are not detectable prior to one week from the symptoms onset, and this may represent an important limit of serological diagnosis. On the other hand, serological assays could to be very important to determine the rate of infection in an affected area and to evaluate who is immune and who not.

Recently, many quantitative enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays (CLIA) as well as qualitative chromatographic rapid immunoassays for anti-SARS-CoV-2 antibody detection were introduced on the market, in some cases not preceded by valuable studies assessing their analytical and diagnostic accuracy.

The aim of this study was to assess in a real life experience the diagnostic performance of a novel automated CLIA for quantitative detection of anti-SARS-CoV-2 IgM and IgG antibodies, compared with a rRT-PCR assay.

2. Material and methods

2.1. Patients

A total of 354 sera, 89 from consecutive patients diagnosed with COVID-19 at the Santa Maria degli Angeli Hospital in Pordenone (Italy) confirmed by RT-PCR using nasopharyngeal swabs, and 272 from healthcare workers who underwent one or more nasopharyngeal swabs in the course of preventive surveillance, were collected. Seven healthcare workers resulted positive at the rRT-PCR test and showed mild symptoms (i.e. fever <38 °C, nasal obstruction, cough, anosmia). As a consequence they were included in the mild COVID-19 group. All the other 265 healthcare workers were asymptomatic and negative at the rRT-PCR test. All the sera were stored at -20 °C until use.

Of the 89 patients, 43 (mean age = 51 yrs, range: 28–72; male = 13)were classified as mild (nonpneumonia or mild pneumonia), 32 (mean age = 70 yrs, range: 48–91; male = 22) as severe (dyspnea, respiratory frequency $\geq$ 30/min, blood oxygen saturation $\leq$ 93%) and 13 (mean age = 66, range: 44–81; male = 12) as critical (respiratory failure, septic shock, and/or multiple organ dysfunction or failure) COVID-19. Sera of patients by mild, severe and critical symptoms were collected 24.8 ± 4.7, 19.2 ± 8.7 and 26.2 ± 9.7 days after onset of the symptoms, respectively. In healthcare workers sera were collected jointly with the last nasopharyngeal swab.

The study was approved by local Institutional Board and conducted according to the principles of the Declaration of Helsinky.

2.2. Methods

2.2.1. Molecular assay

Viral RNA was extracted from patient specimens using the multi-channel automated liquid handling system Microlab™NIMBUS™(Hamilton, USA). Quatitative real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) was performed using the CFX™ with Allplex™ 2019-nCoV assay (Seegene, South Korea) for the single or multiple detection of three target genes (E gene, RdRP gene and N gene). The specimens were considered positive if the cycle threshold (Ct) value for at least one of the three genes was $\leq$40. No specimens with E gene positivity alone was found in this study.

2.3. Serological assay

SARS-CoV-2 antibodies IgM and IgG were measured in all sera using MAGLUMI™ 2019-nCoV IgM, v2.0, 2020–03 and 2019-nCoV-IgG, v1,2, 2020–02, respectively. They are two indirect chemiluminescence immunoassays, carried out on the fully-automated analyzer MAGLUMI 800 (SNIBE-Shenzhen New Industries Biomedical Engineering Co., Ltd Shenzhen, china). According to manufacturer's declaration, SARS-CoV-2 antigens coated to magnetic beads are recombinant Nucleocapside (N) ad Spike (S) S1 + S2 proteins. The amount of anti-SARS-CoV-2 antibodies is positively correlated to the relative light units measured by the analyzer, and the results are expressed as arbitrary units (AU). The manufacturer's cut-offs for IgM and IgG are 1.0 and 1.1 AU/mL, respectively. Using these cut-offs, the manufacturer claimed that calculated clinical sensitivities and specificities for IgM and IgG were 78,6% and 97.5%, and 91.2% and 97.3%, respectively.

2.4. Statistical analysis

We did statistical analyses using MedCalc software (Mariakerke; Belgium). Cohen's Kappa test was used to evaluate the agreement between results obtained with rRT-PCR and serological assays. Considering rRT-PCR as "reference method" sensitivity, specificity, predictive value of positive (PPV) and negative (PPN) test, likelihood ratio of positive test (LR+) and of negative (LR-), and Odds ratio (OR) of serological assay were calculated. Comparison of continuous variable was performed using Mann-Whitney *U* test. A p value less than 0.05 was judged statistically significant.

3. Results

The positive rates of SARS-CoV-2 IgM and IgG antibodies in serum samples of the different studied groups are shown in Table 1. The positive rate of SARS-CoV-2 IgM antibodies was significantly lower in mild COVID-19 group (32.6%) compared to severe (81.0%) and critical (100%) ($p = 0.0001$) COVID-19 groups, whereas no significant difference was shown between patients with severe and critical disease. As concerning SARS-CoV-2 IgG antibodies, the positive rate resulted significantly lower in the mild group (74.1%) compared to the severe group (81%) ($p < 0.02$), but not in respect to the critical group ($p = 0.09$), even if all patient of this group were IgG positive. Probably it is due to the low number of patients enrolled in the critical group.

The levels of SARS-CoV-2 IgM and IgG antibodies were 1.22 ± 1.2 AU/mL and 15.86 ± 24.83 AU/mL, 2.86 ± 2.4 AU/mL and 69.3 ± 55.5 AU/mL, 2.47 ± 1.33 AU/mL and 83.9 ± 83.9 AU/mL in mild, severe and critical COVID-19 groups, respectively. As showed in Fig. 1 there was a significant difference in antibody levels between mild and severe/critical subjects, both for IgM and IgG.

The overall agreement between serology and rRT-PCR assays was 95.5% (95%CI: 92.8–97.4%) and the Cohen's k showed an almost perfect agreement (0.876; 95%CI: 0.817–0.935). Considering asymptomatic healthcare workers (rRT-PCR negative) as negative controls, the overall sensitivity and specificity of serological assay were 86.5% (95%CI: 77.6–92.8) and 98.5% (95%CI:96.2–99.6), respectively. PPV, PPN, LR+, LR- and OR were 95.1 (95%CI: 87.8–98.6), 95.6 (95%CI: 92.4–97.7), 57.3 (95%CI: 21.6–152.1), 7.3 (95% CI: 4.31–12.4) and 418.6 (95%CI: 131.2–1335.2), respectively.

4. Discussion

The recent outbreak and rapid spread of the novel coronavirus SARS-CoV-2 make the diagnostic methods the frontline strategy for recognizing the infection, tracking the cases and finally contain the epidemic spread. Serological tests may have an important role in particular for surveillance or epidemiological purpose [7]. However, diagnostic performance and predictive value of SARS-CoV-2 serological testing have not been systematically evaluated and large scale studies are lacking. In our study we evaluated in real life the diagnostic performance of a CLIA assay for IgM and IgG detection, both in sera of COVID-19 diagnosed patients and in healthcare workers who underwent periodical nasopharyngeal swabs in the course of surveillance controls. In the last group only four people showed antibody positivity (1 for IgM and 3 for IgG). Since these cases were asymptomatic, viral RNA resulted negative in all the specimens collected at different times, and the positivity was not confirmed with a second method (data not shown), they were considered as false positive. However, the overall specificity (IgM + IgG) of the assay remains very high (98.5%). Also the overall sensitivity (IgM + IgG), compared with the results of rRT-PCR, is high (86.5%), showing good diagnostic accuracy of the CLIA method as previously shown in other studies [8,9]. It is important to remark that the antibody positive rate, as well as the antibody levels are significantly higher following severe infection compared to mild one. These results confirm data obtained in other studies [10,11] using ELISA assays and they are similar to those reported earlier for MERS-CoV [12,13]. Concerning the low rate of IgM antibody presence in mild COVID-19 patients, we can hypothesize, since serological test was done 24.8 ± 4.7 days after onset of symptoms, the possible disappearance of IgM antibodies prior to testing, though we cannot exclude that this antibody class never developed in these patients in the first place. The latter hypothesis is very likely for IgG antibodies. Tan et al. [11] showed that weak responders for IgG antibodies had a significantly higher viral clearance rate than strong responders, likewise reported for SARS [14], and this may explain the difference in antibody response in our study. However, we cannot exclude the importance of the viral load and of the difference in the adaptive immune-response between mild and severe/critical patients [15].

Although Viral DNA detection by rRT-PCR assay remains the gold standard for the diagnosis of COVID-19, on the basis on our data, high values of specific antibodies may represent a potential marker of risk of a more severe course of disease.

However, this study presents some limitations: a) the number of patients enrolled, in particular in the critical group, is low b) data were not confirmed with other quantitative methods, c) we did not study the antibody kinetic, in particular of IgM. Therefore, future studies are needed to better understand the antibody response profile of SARS-CoV-2 infection and to correctly interpret the clinical meaning of serology findings.

5. Conclusion

The CLIA assay evaluated in this real life study showed good diagnostic performance and may be a useful tool for assessing the immunological response in sera of COVID-19 patients. If other large, longitudinal studies were to confirm the correlation between antibody levels and severity of disease, the use of quantitative methods for antibody detection will become highly recommended.

Table 1
Number and percent of anti-SARS-CoV-2 IgM and IgG positivities in patients and healthcare workers.

	N°	Age (mean; range)	M/F	Days after onset of symptoms (mean; SD)	SARS-CoV2 IgM pos (%)	SARS-Cov-2 IgG pos (%)
Mild COVID-19	43	51; 28-72	13/30	24.8 ± 4.7	14/43 (32.6%)	32 (74.1%)
Severe COVID-19	32	70; 48-91	22/10	19.2 ± 8.7	26/32 (81.0%)	31/32 (96.8%)
Critical COVID-19	13	66; 44-81	12/1	26.2 ± 9.7	13/13 (100%)	13/13 (100%)
Asymptomatic swab negative Healthcare workers	265	44; 25-65	46/219	–	1/265 (0.4%)	3/265 (1,1)

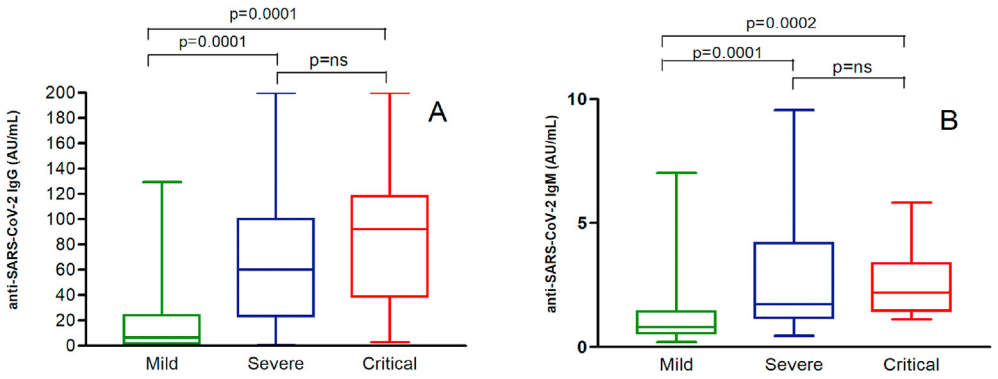

Fig. 1. Level s of anti-SARS-CoV-2 IgG (A) and IgM (B) distributed for different grade of COVID-19 severity.

Finally, in sero-prevalence studies, it should be kept in mind that not all patients, particularly those recovering from mild disease, have developed antibodies.

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