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Research Article

The Role of Serological Testing to Support Diagnosis of Covid-19 During the Pandemic

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Abstract

Evidence on the diagnostic performance of serological tests in the early phase of COVID-19 is lacking.

The aim of our study was to gather evidence on such testing in real-life practice. During the year 2020, we retrospectively analyzed the data records of 637 consecutive patients who underwent serological qualitative tests and reverse transcriptase polymerase chain reaction (RT-PCR) for suspected COVID-19. All sera were first tested by rapid qualitative detection of anti-SARS-CoV-2 antibodies and if positive for IgG and/or IgM qualitative test were subsequently tested for quantitative detection. One hundred and four (16.3%) were positive for anti-SARS-CoV-2 IgG and/or IgM antibodies by the rapid qualitative test and 59 (9.2%) by the RT-PCR, diagnosed COVID-19. Thirty-two out of the 59 patients with positive RT-PCR were negative for IgG and/or IgM antibodies by the rapid qualitative test. A weaker association was found between positive RT-PCR and the positive single qualitative test. The multiple positivity of qualitative and/or quantitative serological tests was strongly associated with diagnosis of COVID-19 (65.2%-100%).

Keywords: *RT PCR, specific anti-SARS-CoV-2 IgG and IgM, serological tests*

Introduction

Diagnosis of coronavirus disease 2019 (COVID-19) is primarily confirmed by Nucleic Acid Amplification Technique (NAAT) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is mainly represented by the reverse transcriptase polymerase chain reaction (RT-PCR) [1-3]. The use of serological tests is less complex and costly and presents a shorter turnaround time than NAAT. IgM can be detected in the patient samples from 10 to 30 days after SARS-CoV-2 infection while IgG appears at day 20 onward [4]. Unlike a classic viral infection, the humoral response following the SARS-CoV-2-infection is characterized by exclusive entity and kinetics. In a recent study, Padoan et al. studied the kinetics of anti-SARS-CoV-2 antibodies [5], concluding that IgM and IgG tend to appear 6-7 days after symptoms onset. Moreover, notwithstanding the epidemiologic potential to evaluate a population's immunization state

[6,7], it becomes more and more evident, the role of SARS-CoV-2 antibodies in diagnosis of suspected cases with negative RT-PCR as well as significantly increase diagnostic sensitivity for COVID-19 patients [8,9].

Nevertheless, systematic data are lacking on the diagnostic performance and predictive value of serological test positivity in the early phase of COVID-19. For this reason, the aim of our study was to investigate the predictive role of serological assays, both qualitative and quantitative tests, and their best interpretation in the real-life practice.

Material and Methods

During the year 2020, we retrospectively analyzed data records of 637 consecutive patients (313 females and 324 males) with mean age ± SD 69.1 ± 20.6 years, admitted to the Emergency Department and Internal Medicine Ward of San Giuseppe Hospital, Empoli (Italy) with symptoms suggestive of COVID-19, who had undergone RT-PCR oro-nasopharynx swabs and qualitative test for anti-SARS-CoV-2 IgG and IgM antibodies. All sera have been subsequently tested for quantitative detection, if positive for IgG and/or IgM by the qualitative test. From 2020 March 12 to 2020 May 31, Internal Medicine ward was transformed in a 96-bed non intensive COVID-19 area. Criteria for hospitalization in COVID area were a) any patient with dyspnoea or increase respiratory rate (≥ 30 breaths per min); b) any patient with oxygen saturation ≤ 94% on room air or decrease in saturation to <90% with ambulation; c) overall clinical concern by Emergency Department attending for risk of outpatient failure based on vital signs, clinical examination and/or high risk for complications from severe COVID-19 based on co-morbidity. Of the 637 patients undergone to oro-nasopharynx swab and serological test, two hundred and twenty-eight patients (35.7%) were under 65 years, ten patients (1.5%) were under 18 years. The median time from symptoms onset to Emergency Department admission, oro-nasopharynx swab and serological tests was 5 days (IQR 2-7). Five hundred and seventy-eight patients underwent oro-nasopharynx swab and serological test in Emergency Department, while fifty-nine in Internal medicine ward.

The qualitative detection of SARS-CoV-2 RNA in human nasopharyngeal swab, was determined by an *in vitro* diagnostic (IVD) (RT-PCR) test (Allplex™ 2019-nCoV Assay, Seegene).

Detection limit (sensitivity) of Allplex™ 2019-nCoV Assay was 100 RNA copies/reaction. The assessment of its performances by the manufacturer demonstrates a specificity of 100%. Test specificity was determined by cross-reactivity of Allplex™ 2019-nCoV Assay using 49 standard materials and pathogenic organisms.

The 2019-nCoV primer and probe set(s) are designed to detect RdRp gene, E gene, and N gene according to the World Health Organization (WHO) recommendations.

RNA extraction was performed according to the STARMag 96 × 4 Universal Cartridge Kit Seegene using Seegene STARlet. A cycle threshold value (Ct value) <40 for all three target genes was defined as a positive result.

All sera were first tested by JusChek + qualitative test, a membrane-based immunoassay able to detect antibodies to 2019-nCoV, Acro Biotech, Inc, using nucleocapsid (N) protein (Manufacturer's data: sensitivity IgG: 100%, IgM 85%; specificity IgG 98%, IgM 96%). Sera positive for IgG and/or IgM were subsequently tested for quantitative detection by a chemiluminescent method, Shenzhen Yhlo Biotech Co. Ltd, using Nucleocapsid (N) and Spike (S) proteins (sensitivity IgG: 76.7%, IgM 73.3%; specificity IgG 100%, IgM 92.2%) [10].

All quantitative antibody tests "iFlash-2019-nCoV IgG and IgM" were performed by iFlash1800 fully automatic CLIA analyzer from YHLO biotechnology Co (LTD, Shenzhen, China) [10,11]. The amount of anti-SARS- CoV-2 antibodies IgM and IgG is positively correlated with the relative light units (RLU) measured by the chemiluminescence analyzer. iFlash1800 CLIA analyzer automatically calculates the concentration (AU/mL) based on the calibration curve. Cut off value proposed by manufacturer is 10 AU/mL both for IgM and IgG antibodies; hence, samples with hence samples with IgM and IgG concentration more than equal to 10 AU/mL are considered positive (reactive).

Statistical analysis

Statistical analysis was performed using SPSS software (version 24.0, SPSS Inc., Chicago, IL, USA). The concordance rate between COVID-19 status and positive IgG and/or IgM tests (qualitative, quantitative, and their different combinations) was calculated by dividing the number of true positive antibodies tests with the total number of positive antibody tests. The combination of the tests was considered positive if all tests had positive results.

This study was approved by the Local Ethic Committee (Authorisation number: 17260_oss).

Results

Of the 637 patients, 59 (29 females) with mean age ± SD 63.4 ± 19.9 years, were diagnosed with COVID-19 (9.2%) on the basis of clinical criteria and positive pharyngeal swab. At first, out of 59 patients, 2 were negative for RT-PCR but positive for anti-SARS-CoV-2 antibodies. Later, these two patients were tested positive for RT-PCR with a following second swab and COVID-19 diagnosis was confirmed. All patients positive for anti-SARS-CoV-2 IgG and/or IgM antibodies by rapid qualitative and negative RT-PCR were retested with RT-PCR 24 h after the first sample resulting negative again. One hundred and four were positive for anti-SARS-CoV-2 IgG and/or IgM antibodies (16.3%) by a rapid qualitative test and 59 (9.2%) for the viral RNA by RT-PCR. Out of 59 patients, RT-PCR positive, 32 were negative for anti-SARS-CoV-2 IgG and/or IgM antibodies by rapid qualitative test while 27 were positive for anti-SARS-CoV-2 IgG and/or IgM antibodies with a concordance rate between the two tests of 40.7%.

Quantitative serological test was assayed in cases positive for qualitative test. The concordance rate was calculated as the ratio between true positive tests (according to the COVID-19 status) and the total number of positive tests. A weaker concordance was found between COVID-19 and the positive single qualitative test (IgG qualitative test: 17.5%; IgM qualitative test: 12.2%) compared to the double positive qualitative test (IgG qualitative test and IgM qualitative test: 65.2%) or to the qualitative and quantitative tests combinations, except for the IgM double tests (qualitative and quantitative), where no positive cases were found (Table 1). The strongest association was observed for the combination of qualitative IgM and IgG and quantitative IgM anti-SARS-CoV-2 antibodies (100%).

Table 1. Cross-tabulation between results of positive anti-SARS-CoV-2 (IgM and IgG) tests and diagnosis of COVID-19

	COVID-19 status		**Concordance rate between positive tests and COVID-19 status**
Anti-SARS-CoV-2 positive test	No	Yes	
IgG qlt	33	7	17.5%
IgM qlt	36	5	12.2%
IgM qlt and IgG qlt	8	15	65.2%
IgM qlt and IgM qnt	5	0	0.0%
IgG qlt and IgG qnt	3	6	66.7%
IgM qlt, IgG qlt and IgG qnt	1	4	80.0%
IgM qlt, IgG qlt and IgM qnt	0	3	100.0%
IgM qlt, IgG qlt, IgM qnt and IgG qnt	1	5	83.3%
qlt: qualitative test; qnt: quantitative test			

Discussion

Though the gold standard test for diagnosis of COVID-19 is still the RT-PCR performed on oro-nasopharynx swab samples, SARS-CoV-2 infection can also be detected indirectly by measuring the host immune response via serological testing. The methods currently used for serological tests are enzyme linked immunosorbent assays (ELISA), lateral flow immunoassays (LFIA) and chemiluminescent immunoassays (CLIA). A systematic review and meta-analysis showed that sensitivity of these methods ranges from 66% (49.3-79.3%) of LFIA to 97.8% (46.2-100%) of CLIA, while specificity

ranges from 96.6% (94.3-98.2%) of LFIA to 97.8% (62.9-99.9%) of CLIA [12]. However, the role of serological testing to support RT-PCR in the acute phase of COVID-19 diagnosis is challenging [5]. Evidence shows that after the onset of symptoms, the time required to detect IgM and IgG antibodies against SARS-CoV-2 is 5-10 days and ≥ 10 days, respectively [13]. Later on, the concentration of both antibody classes increases, the chance of false-negative results decreases. Therefore, early detection of antibodies is possible only in a limited number of patients. For 82 patients in acute phase of COVID-19 diagnosis, Guo L. et al. found that the positive rate of IgM antibodies was 75.6% and the detection efficiency by IgM was higher after 5.5 days from symptoms onset [9]. In another study enrolling 41 patients in acute phase of COVID-19, Qu J et al. found that the positive rate of IgM and IgG by using a CLIA method was 87.8% and 97.6% respectively. Most of the enrolled patients were admitted to the hospital after 8 days. The highest IgG concentration was reached on day 30, while the highest IgM concentration was on day 18 [14].

In another study comprised of 112 COVID-19 patients, Zhang G et al. found antibody positivity in 93.7% (0.9% IgM alone, 41.1% IgG alone, 51.8% IgM plus IgG) [15]. In our experience almost half of COVID-19 patients were positive for IgM and/or IgG antibodies. The median time from symptoms onset to hospital admission was 5 days. This could explain the lower positivity rate found in our study compared with the abovementioned studies [9,14,15]. Our study found that in patients with symptoms, clinical history, radiological and laboratory findings suspected for COVID-19 multiple positivity by qualitative and/or quantitative serological tests significantly increase the probability of COVID-19. A two-step algorithm (orthogonal approach) where individuals who initially test positive were tested with a second test represents one of the testing strategies recommended by Center for Disease Control (CDC) to improve PPV of a test result [16]. Xu et al., showed that the orthogonal testing strategy could be a useful tool that can be used to help identify potentially false positive SARS-CoV-2 IgG serology results (78/98) of positive SARS-CoV-2 IgG results confirmed with a second line test [17]. Pflüger et al. supported that orthogonal test strategies can enhance clinical specificity, evaluating the clinical performance of five automated immunoassays on a set of clinical samples with positive predictive values ranged from 19.5%-100% and 38.0%-100% (for ≤ 10 days or >10 days after start of symptoms, respectively) [18].

In our case the combination of qualitative and quantitative tests was associated with the strongest probability of having COVID-19. This could prove to be very important in clinical practice. For patients with high clinical suspicion of COVID-19 awaiting RT-PCR results, multiple positivity by rapid serological tests could reduce misdiagnosis, thereby allowing the appropriate allocation of patients and the start of proper treatment. Similar results were found by Guo L. et al. who showed that the combination of ELISA IgM positivity with RT-PCR significantly increases the diagnostic probability of COVID-19 compared to RT-PCR alone from 51.9% to 98.6% [9]. Our study has limitations mainly due to small sample size. However, to our knowledge, this is the first study that combines different qualitative and quantitative tests with RT-PCR in the early diagnosis of COVID-19 showing the importance of correctly interpreting different serological tests combined with RT-PCR. Since our results showed a weaker association between SARS-CoV-2 infection and the single positive qualitative test, compared to the double positive qualitative test or the double, triple, and quadruple qualitative and quantitative tests, the significance of the rapid qualitative test should be evaluated according to the isotype detected and to its combination with the quantitative test. Moreover, the main limitation of this study was that we couldn't analyze the concordance rate of negative serological results among qualitative and quantitative tests, given the fact that it has not been possible to deepen them. In the next future further studies are needed to confirm our results.

Conflict of Interests

The Authors declare that there are no conflicts of interest to be disclosed.

References

1. Hanson KE, Caliendo AM, Arias CA, Englund JA, Hayden MK, et al. (2020) Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Serologic Testing. Clin Infect Dis ciaa1343.
2. World Health Organization (2020) Clinical management of COVID-19: interim guidance.
3. Dodig S, Čepelak I, Čepelak Dodig D, Laškaj R (2020) SARS-CoV-2 - a new challenge for laboratory medicine. Biochem Med (Zagreb) 30: 030503.
4. Li K, Huang B, Wu M, Zhong A, Li L, et al. (2020) Dynamic changes in anti-SARS-CoV-2 antibodies during SARS-CoV-2 infection and recovery from COVID-19. Nat Commun 11: 6044.
5. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M (2020) Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med 58: 1081-1088.
6. Espejo AP, Akgun Y, Al Mana AF, Tjendra Y, Millan NC, et al. (2020) Review of Current Advances in Serologic Testing for COVID-19. Am J Clin Pathol 154: 293-304.
7. Infantino M, Damiani A, Gobbi FL, Grossi V, Lari B, et al. (2020) Serological Assays for SARS-CoV-2 Infectious Disease: Benefits, Limitations and Perspectives. Isr Med Assoc J 22: 203-210.
8. Zhao J, Yuan Q, Wang H, Liu W, Liao X, et al. (2020) Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 71: 2027-2034.
9. Guo L, Ren L, Yang S, Xiao M, Chang D, et al. (2020) Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis 71: 778-785.
10. Infantino M, Grossi V, Lari B, Bambi R, Perri A, et al. (2020) Diagnostic accuracy of an automated chemiluminescent immunoassay for anti-SARS-CoV-2 IgM and IgG antibodies: an Italian experience. J Med Virol 92: 1671-1675.
11. Mairesse A, Favresse J, Eucher C, Elsen M, Tré-Hardy M, et al. (2020) High clinical performance and quantitative assessment of antibody kinetics using a dual recognition assay for the detection of SARS-CoV-2 IgM and IgG antibodies. Clin Biochem 86: 23-27.
12. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, et al. (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370: m2516.
13. Fang FC, Naccache SN, Greninger AL (2020) The Laboratory Diagnosis of Coronavirus Disease 2019-Frequently Asked Questions. Clin Infect Dis 71: 2996-3001.
14. Qu J, Wu C, Li X, Zhang G, Jiang Z, et al. (2020) Profile of Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis 71: 2255-2258.
15. Zhang G, Nie S, Zhang Z, Zhang Z (2020) Longitudinal Change of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies in Patients with Coronavirus Disease 2019. J Infect Dis 222: 183-188.
16. Centers for Disease Control and Prevention (CDC) (2019) Interim Guidelines for COVID19 antibody testing.
17. Xu G, Emanuel AJ, Nadig S, Mehrotra S, Caddell BA, et al. (2020) Evaluation of Orthogonal Testing Algorithm for Detection of SARS-CoV-2 IgG Antibodies. Clin Chem 66: 1531-1537.
18. Pflüger LS, Bannasch JH, Brehm TT, Pfefferle S, Hoffmann A, et al. (2020) Clinical evaluation of five different automated SARS-CoV-2 serology assays in a cohort of hospitalized COVID-19 patients. J Clin Virol 130: 104549.