Immunoglobulin G and immunoglobulin M positivity in relation to coronavirus disease 2019 severity

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Objective
The aim of this study was to evaluate the seropositivity of rapid immunoglobulin G (IgG) and immunoglobulin M tests in patients recovered from coronavirus disease 2019 (COVID-19).

Background
Rapid severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen-detection tests can give results in less than 30 min to detect SARS-CoV-2.

Patients and methods
This case–control study was performed in Toluca, Mexico, from September to October, 2020. Rapid test was performed in patients older than 18 years who were diagnosed with COVID-19. Quantitative data were presented as mean ± SD and qualitative in percentages. Pearson correlation was performed between the COVID-19 clinical severity scale and the waist and BMI variables.

Results
Twenty-five patients were included (mean age, 45.24 years). Through the Pearson correlation, a positive relation was found between COVID-19 clinical severity score and waist circumference (R^2 = 0.161, P = 0.046844) and between the first and BMI (R^2 = 0.157, P = 0.049918). The mean COVID-19 clinical severity score of the patients was 23.12 ± 15.23. Sixty-eight percent of positive results were obtained for the detection of IgG antibodies. In patients who exceed a score of 18 in clinical manifestations, all results for the detection of IgG antibodies against SARS-COV-2 were positive after several months.

Conclusion
Patients with comorbidities had a higher predisposition to a more severe COVID-19 disease, the highest intensity of signs and symptoms, the highest immunological response, and the highest probability of having antibodies against SARS-COV-2 over time.

Keywords:
coronavirus disease 2019 severity, immunoglobulin G rapid test, immunoglobulin M rapid test, severe acute respiratory syndrome coronavirus 2

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Introduction

The adaptive immunity appears in response to the new beta coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It occurs within the first 7–10 days of infection [1–3], although others report that the memory antibody-positive quantification in most patients occurs between 7 and 14 days after their diagnosis [4,5].

Determining the long-term immune memory of B and T cells is critical. As such, an expansion of B cells and memory plasma cells is detected at the onset of infection [2,6], but long-lasting humoral immunity against SARS-CoV-2 might not be present with mild illness [7]. In general, serum immunoglobulin M (IgM) and immunoglobulin A titers decrease after 28 days, and immunoglobulin G (IgG) titers reach the peak in about 49 days, although their presence has been observed up to 60 days with later progressive decrease [8]. Simultaneously, SARS-CoV-2 activates specific memory CD4(+) T cells in the first week of infection.

CD8(+) T cells peak within two weeks after the beginning of infection, but remain detectable at lower levels for 100 or more days. Moreover, CD4(+) T and CD8(+) T cells have been identified in up to 100 and 70%, respectively, of patients recovering from coronavirus disease 2019 (COVID-19) [9,10]. Although severe COVID-19 is characterized by high viral titers, the response of chemokines, unregulated innate inflammatory cytokines, prolonged lymphopenia, and antibody-dependent or dominant cytokine-dependent enhancement of

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CD4(+) seems to contribute to the acute severity of COVID-19 [1].

In addition, the antibody-production magnitude and the T lymphocytes' response degree may differ and be discordant between each of those infected, depending on factors such as severity, viral load, the immune system, environmental factors, and inherent to the host [11]. For example, a study of 61 000 people in Spain showed that 5% of the population had formed antibodies against the Spike (S) protein and nucleus proteins and that approximately one-third of the people diagnosed were asymptomatic [12]. Other studies report a high prevalence of antibody levels, depending on the severity of the disease, saying that if there are fewer symptoms, it is also possible to detect lower antibody levels [13].

Rapid antigen-detection tests can be used in the first level of care and can give results in less than 30 min, which would be viable alternatives to molecular tests to confirm COVID-19 cases, allow adequate case management, and guide public health decisions, such as quarantine or self-isolation. Furthermore, rapid antibody tests are an attractive option because they are easy to use and do not need complex processing, but as a point against them, only a few tests show satisfactory performance [14,15]. It is clear, from medical literature, that seroconversion takes time, and is variable and dependent on the severity of the disease and the individual's immune system, but in any case, antibody levels subsequently decrease over time [16]. The aim was to evaluate the seropositivity of rapid IgG and IgM tests in patients recovered from COVID-19.

Patients and methods

This case–control study was performed in Toluca, Mexico, from September 1 to October 31, 2020. Patients older than 18 years were diagnosed with COVID-19 using a computed tomography COVID-19 Reporting and Data System (CO-RADS) more than or equal to 4, positive qPCR in nasopharyngeal swab, or with high-suspicion clinical criteria for COVID-19 according to the Guide for the Care of the Critical Patient with COVID-19 Infection of the Mexican College of Critical Medicine (COMMEC). Patients suffer from chronic kidney disease on renal-replacement therapy, HIV, and receiving immunosuppressive therapy for some type of cancer or rheumatic disease. Patients who, once the study protocol has started, and refuse to continue participating, were discarded from the final analysis.

To register every patient information, the research instrument depicted in Fig. 1 was used. Somatometry [weight (kg) and height (m)] was performed, to finally get the BMI and the waist–hip ratio. For surveillance approach, the IgG and IgM serological tests were carried out using the Novel Coronavirus 2019 (nCoV) IgG/IgM test kit (colloidal gold), Genrui Biotech Inc. Geya Technology Park, Guangming District, 518106, Shenzhen, China.

The protocol was approved by the Research Ethics Committee of the 'Mónica Pretelini Sáenz' Maternal-Perinatal Hospital (HMPMPS), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, with the following registration number: 2020-11-710. Informed consent was asked from all participants.

Statistical analysis

Quantitative data were presented as mean ± SD and qualitative in percentages. The Kolmogorov test was used to verify the distribution of continuous variables and then a Pearson correlation was performed between the COVID-19 clinical severity scale and the waist and BMI variables. The online Social Science Statistics program (https://www.socscistatistics.com) was used, and in all cases, a P value less than or equal to 0.05 was considered statistically significant.

Results

Twenty-five patients were included after having met the high-suspicion diagnostic criteria, from which only 14 (56%) could refer having been in contact with a COVID patient; the rest of them had no idea where they could have been infected. The main characteristics of the patients are shown in Table 1. The mean age of the patients was 45.24 (44.45 in men and 45.85 in women), range 23–65. Two patients required hospitalization (a woman and a man), who suffered from obesity grades I and II, respectively.

According to the BMI, patients were classified as normal weight: two (8%), overweight: 11 (44%), obesity grade I: eight (32%), obesity grade II: three (12%), and obesity grade III: one (4%). The Kolmogorov test confirmed a normal distribution for waist circumference, BMI, and COVID-19 clinical severity score, thus, the Pearson correlation was performed with the next results: between COVID-19 clinical severity score and waist circumference (R^2 = 0.161, P = 0.046) and between the first and BMI (R^2 = 0.157, P = 0.061) (Table 2). It is important to emphasize that the correlation between the severity of symptoms and BMI/waist circumference was moderate (by Cohen's criteria).

The mean COVID-19 clinical severity score of the patients was 23.12 ± 15.23. Being the frequency of

Figure 1

Name				Age	Gender	Date
Case		Date of onset of symptoms			Date of follow-up	Exposed to a COVID case?
Adress						
Weight (kg)	Height (m)	BMI	Waist (cm)	Hip (cm)	WHR	
CT			Criteria of Clinical suspicion for COVID		Interpretation	
CO-RADS 1			Fever ≥ 38°C	5	Green: ≤ 11	Mild
CO-RADS 2			Dry Cough	5	Yellow: 11-29	Moderate
CO-RADS 3			Headache	5	Red: > 30	Severe
CO-RADS 4			Dyspnea	20		
CO-RADS 5			Anosmia	3		
			Diarrhea	3		
qPCR	Positive	Negative	Myalgias	1		
			Arthralgia	1		
Serum IgG and IgM antibodies		IgG levels	Odynophagia	1		
Positive	Negative	IgM levels	Rhinorrhea	1		
			Conjunctivitis	1		
			TOTAL			
Risk Factors			Chest radiograph with bilateral infiltrates			Yes / No
T2DM	>5 Years	5 - 10 Years	>10 Years	In good control	Uncontrolled	
Systemic Hypertension	>5 Years	5 - 10 Years	>10 Years	In good control	Uncontrolled	
	Treatment with ARBs	YES	NO	REQUIRED HOSPITALIZATION		TIME
Smoking	>5 Years	5 - 10 Years	>10 Years	YES	NO	
Alcoholism	>5 Years	5 - 10 Years	>10 Years	USED OXYGEN		TIME
Asthma	>5 Years	5 - 10 Years	>10 Years	YES	NO	
COPD	>5 Years	5 - 10 Years	>10 Years	REQUIRED MECHANICAL VENTILATION		TIME
SEVERITY OF SYMPTOMS				YES	NO	
DEPRESIVE	Mild	Moderate	Severe	BOUGHT PULSE OXIMETER		Yes / No
ANXIETY	Mild	Moderate	Severe	TELEMEDICINE MEDICAL ATTENTION		Yes / No
Do you think you have sequels from COVID-19? Describe them						
SARS COV-2 antibodies results						
IgG	Positive					
IgM	Negative					

ARBs: Angiotensin receptor blockers, COPD: Chronic obstructive pulmonary disease, CT: Computed tomography, T2DM: Type 2 diabetes mellitus.

Research instrument.

the signs and symptoms as follows: myalgia: 20 (80%), dry cough: 19 (76%), headache: 16 (64%), arthralgia: 16 (64%), anosmia: 14 (56%), fever: 12 (48%), diarrhea: 12 (48%), odynophagia: 12 (48%), rhinorrhea: 12 (48%), dyspnea: 10 (40%), and conjunctivitis: five (20%).

Only 11 qPCR tests were performed to detect asymptomatic cases and carriers, of which seven were positive and four negative (Table 3). Of the 25 cases that underwent the rapid antibody-detection test, 17 were positive and eight negative for IgG and 56% of the total had been exposed to confirmed cases.

The main risk factors for severe COVID-19 illness were as follows: poorly controlled type-2 diabetes mellitus (T2DM) in two patients with CO-RADS

Table 1 Characteristics of the patients

Variables	All (mean±SD)	Men (mean±SD)	Women (mean±SD)
Age (years)	45.24±13.1	44.45±14.72	45.85±12.20
Height (m)	1.62±0.09	1.69±0.075	1.57±0.071
Weight (kg)	81.18±14.86	85.86±14.67	77.5±14.46
BMI (kg/m^2)	30.72±4.2	29.87±2.92	31.39±5.08
Waist (cm)	101.08±13.31	103.36±7.28	99.28±16.46
Hip (cm)	108.6±13.45	106.27±8.43	110.42±16.46
WHR	0.93±0.06	0.97±0.07	0.8974±0.045

WHR, waist-hip ratio.

Table 2 Pearson correlation

Variables	R^2	P
COVID-19 severity scale and waist circumference	0.161	0.046
COVID-19 severity scale and hip circumference	0.112	0.100
COVID-19 severity scale and BMI	0.143	0.061
COVID-19 severity scale and WHR	0.048	0.292

COVID-19, coronavirus disease 2019; WHR, waist-hip ratio.

Table 3 Clinical and complementary diagnostic tools

Case	Clinical criteria	CT	Chest radiograph	qPCR	Serum antibodies		Rapid test	
					IgM	IgG	IgM	IgG
1	+	NR	NR	–	NR	NR	–	+
2	+	NR	+	NR	NR	NR	–	–
3	+	+	NR	+	NR	NR	–	+
4	+	NR	NR	NR	0.62	4.29	–	+
5	+	NR	NR	–	NR	NR	–	+
6	+	+	NR	NR	NR	NR	–	+
7	+	NR	NR	NR	5.12	3.41	–	–
8	+	NR	NR	NR	NR	NR	–	–
9	+	NR	NR	NR	NR	NR	–	–
10	+	NR	NR	NR	0.39	13.39	–	–
11	+	NR	NR	NR	NR	NR	–	+
12	+	NR	NR	NR	10.19	7.54	–	+
13	+	+	NR	+	NR	NR	–	+
14	+	NR	NR	+	NR	NR	–	–
15	+	NR	NR	+	NR	NR	–	+
16	+	NR	NR	NR	NR	NR	–	–
17	+	NR	NR	–	NR	NR	–	–
18	+	NR	NR	NR	NR	NR	–	+
19	+	NR	NR	+	NR	NR	–	+
20	+	NR	NR	NR	NR	NR	–	+
21	+	NR	NR	NR	NR	NR	–	+
22	+	NR	NR	NR	27.84	22.7	–	+
23	+	NR	NR	+	NR	NR	–	+
24	+	NR	NR	+	NR	NR	–	+
25	+	+	+	–	NR	NR	–	+

–, negative; +, positive; CT, computed tomography; IgG, immunoglobulin G; IgM, immunoglobulin M; NR, not realized.

5 and punctuation of 45 and 43 in the severity score; both patients remained hospitalized for 10 days without requiring invasive mechanical ventilation, only supplemental oxygen with a reservoir mask.

Six patients used supplemental oxygen during the acute phase of the illness and the convalescent period ranging from 1 to 2 weeks in mild-to-moderate illness, with a mean of 23.5 days with supplemental nasal oxygen.

The most used diagnostic tools by the population was qPCR from nasopharyngeal swab: 11, followed by serum antibodies: five, computed tomography: four, and chest radiography: two. Of 25 SARS-CoV-2 antibody rapid-detection tests, 68% of positive results were obtained for IgG antibody detection. In patients who exceed a score of 18 in clinical manifestations, all results for the detection of IgG antibodies against SARS-CoV-2 were positive after several months of suffering from the disease.

A patient who was positive in the qPCR and who suffered severe anxiety symptoms as the only manifestation during his home isolation gave a negative result in the IgG and IgM antibody detection. It is hypothesized that, in this case, the patient did not develop antibodies, perhaps due to the promptness of the treatment established or for having had a nonsevere condition.

The longest seropositivity duration with the rapid test for IgG antibodies against SARS-CoV-2 were 195, 178, and 173 days, the first corresponded to a 41-year-old female patient with obesity grade II, T2DM of 3 years of evolution; the second corresponded to a 59-year-old health worker with T2DM and systemic arterial hypertension, both of more than 15 years of evolution with regular treatment with oral hypoglycemic agents and with an angiotensin-receptor antagonist II, overweight with central obesity; the latter was a 55-year-old patient without comorbidities. It is important to mention that the three patients had a high score in clinical manifestations, the first was hospitalized for 10 days and used supplemental oxygen for 30 days, the second remained at home with 'very severe' symptoms described by the patient, and the last patient, a woman, presented CO-RADS 5 pneumonia, carditis, and used oxygen for 15 days with complete recovery.

The acute clinical manifestations described in COVID-19 disease are widely varied, in this report, in order of frequency, the main symptoms were myalgia 20 (80%), dry cough 19 (76%), headache 16 (64%), arthralgia 16 (64%), anosmia 14 (56%), rhinorrhea 12 (48%), odynophagia 12 (48%), diarrhea 12 (48%), fever 12 (48%), dyspnea 10 (40%), and conjunctivitis five (20%).

The main sequelae and frequencies described by the patients were fatigue eight (32%), myalgias four (16%), alopecia four (16%), hyposmia three (12%), anosmia two (8%), headache one (4%), insomnia one (4%), dyspnea one (4%), and ageusia one (4%). Fifty-two percent of the patients recovered *ad integrum* even though the majority presented a high degree of clinical

symptoms, without finding a significant relationship with the presence of comorbidities.

Discussion

In this study, the two hospitalized patients had the highest measures of waist circumference with 133 and 100 cm, being a woman and a man respectively, and they showed also a high score of clinical manifestations, as well as being classified as severe cases during their hospitalizations. Based on the Pearson correlation, a clear positive relationship was found between the anthropometric variable waist circumference and COVID-19 clinical severity score.

In relation with the immunological response, when the pandemic began in February, the authors Li *et al*. [17] suggested that the combined IgM–IgG assay has better utility and sensitivity compared with a single IgM or IgG test, and that it could be used for rapid detection of symptomatic or asymptomatic patients, in hospitals, clinics, and testing laboratories.

In a previous study evaluating saliva for qPCR, direct RT-qPCR, reverse-transcription loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19, the authors concluded that the rapid antigen test alone is not recommended for an initial COVID-19 diagnosis due to its low sensitivity [18]. Therefore, it was decided to use the test with two antibodies, IgG and IgM, for a better reliability.

In this survey, the rapid test negativeness, corresponding to the IgM antibody detection, was the expected situation, since all the patients who assisted to the sample test already had days of convalescence and the IgM antibody rises in the acute phase of the disease, reflecting the first cellular immune response against SARS-CoV-2; it is well known that this antibody concentration begins to decrease after the 14th day of the onset of symptoms.

In a study in Thailand, the sensitivity and specificity of the rapid SARS-CoV-2 antigen test were 98.33% (95% confidence interval, 91.06–99.96%) and 98.73% (95% confidence interval, 97.06–99.59%), respectively. One false-negative test result was from a sample with a high-threshold qPCR (Ct) cycle, while five false-positive test results were from preoperative patient samples [19].

During the health crisis due to the COVID-19 pandemic, multiple studies suggested that obesity could exacerbate the acute respiratory syndrome. In a study by Petersen *et al*. [20] that included 30 patients confirmed for COVID-19 by qPCR from a medical center in Berlin, Germany, retrospectively analyzed, hospitalized in an ICU, and under mechanical ventilation, an increase in visceral fat per square decimeter, as well as each additional centimeter of abdominal circumference, was associated with an increased risk of requiring mechanical ventilation in a hospitalized patient. This is in line with the results of this survey as demonstrated with the Pearson test.

In a study carried out by Freuer *et al*. [21], taking into account the fat distribution as a hospitalization-susceptibility risk factor due to SARS-CoV-2 infection, a positive association was found between BMI, abdominal circumference, and the trunk fat ratio by means of a statistical analysis of Genome-Wide Association Study; as such, BMI was strongly associated with the susceptibility to acquire the disease and being hospitalized. Being in accordance with the type of patients that was approached in our study, in which the data also demonstrated this tendency to susceptibility, since most of the included subjects were classified as overweight or in obesity grade I, added to the highest punctuation in the COVID-19 clinical severity score.

Regarding the results related to the rapid-detection tests, a relationship was found between the positive IgG results, symptom duration, and a greater number of signs and symptoms presented according to the obtained scores, suggesting that the higher the viral load, the higher probability that positive IgG results will be obtained, this has also been commented on in multiple reviews and that have stated that a low viral load may not develop long-term immunity against SARS-CoV-2.

To check the IgG and IgM seropositivity, it is necessary to know the viral load, that is, the viral microorganism quantity that has invaded the host. In our study, a relationship was found between IgG seropositivity, the duration of this response, and the symptom severity. In a study carried out by Seow *et al*. [22] in which 74 sequential samples were taken from 65 individuals with confirmed SARS-CoV-2 infection and 31 healthcare providers around 94 days postinfection, they found that the higher the concentration of both IgG and IgM antibodies, the higher the degree of severity and duration of immunity through IgG.

Regarding symptoms, in Italy, a study demonstrated that during their hospitalization, 72.7% of the participants had evidence of interstitial pneumonia, of these, 21 (15%) patients received noninvasive ventilation and seven (5%) patients received invasive ventilation.

In the acute phase of the disease, the most common symptoms were fatigue (80%), dyspnea (68%), dry cough (70%), loss of appetite (58%), myalgia (58%), arthralgias (56%), headache (50%), dysgeusia (49%), diarrhea (28%), rhinitis (30%), and chest pain (32%). Patients were evaluated on an average of 60.3 (SD, 13.6) days after the onset of the first COVID-19 symptom; at the time of evaluation, only 18 (12.6%) were completely asymptomatic, 32% had one or two symptoms, and 55% had three or more, none of the patients had signs or symptoms of acute illness at the time of evaluation. They presented as persistent symptoms such as fatigue (53.1%), dyspnea (43.4%), arthralgias (27.3%), and chest pain (21.7%) [23]. In comparison with our study, the most frequent symptom in the acute phase was myalgia in 80% and dry cough in 76%, these data might vary due to the intrinsic characteristics of each population. About the chronic symptoms in our population, fatigue (32%), alopecia (16%), and myalgia (16%) persisted as the most representative, and as discussed above, socioeconomic, demographic, anthropometric, and racial factors could explain at least partially the differences among different reports.

This study has some limitations, besides the small sample, rapid tests should not be considered diagnostic tests, since there are intrinsic factors of the patient that can generate a false negative, the most important and frequent having a high concentration of serum cholesterol, that prevents the reaction from taking place in the immunochromatoplate of the rapid test, by precipitation of antibodies, a factor that does not intervene in the detection of serum antibodies. The second one, a false negative secondary to a low concentration of blood antibodies due to the time since the onset of symptoms and the day of the rapid-test application.

Conclusion

Rapid tests for the antibody detection against SARS-CoV-2 are a useful tool to detect the new coronavirus in recovered patients and in their primary caregivers, it is a technically easy test to perform, the result is obtained in 15 min, but it should not be used for diagnostic purposes. The low sensitivity in patients with dyslipidemia must always be taken into account when requesting the determination of IgG and IgM antibodies against SARS-CoV-2.

Patients with comorbidities had a higher predisposition to a more severe COVID-19 disease, and the highest the intensity of signs and symptoms, the highest the immunological response and the highest the probability of having antibodies against SARS-CoV-2 over time.

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Conflicts of interest

There are no conflicts of interest.

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