Serological follow up of positive SARS-CoV-2 cases

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Abstract

Objective. To describe the humoral response in a cohort with mild and asymptomatic SARS-CoV-2 infection previously identified in a community-based serological survey. **Materials and methods.** This study was an observational follow up of 193 subjects previously identified with positive anti-SARS-CoV-2 antibodies invited for a second test 112 days after the first sampling. All completed a standardized electronic questionnaire. IgM/IgG antibodies were determined using a qualitative IgM/IgG chemiluminescent immunoassay. **Results.** Among the 193 eligible subjects, a total of 174 (90%) attended the follow-up visit, and their serum samples were tested. Of the samples, 171 (98.3%) were still positive, and 3 (1.7%) were negative. Also, the cut-off index (COI) value of the immunoassay significantly increased from the first to the second test (P <0.001). **Conclusions.** Our findings support a sustained humoral response in individuals with mild and asymptomatic SARS-CoV-2 infection up to 112 days after a positive serologic baseline test, accompanied by increasing antibody titers.

Keywords: anti-SARS-CoV-2; SARS-CoV-2; Covid-19; antibodies; persistence; follow up

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Resumen

Objetivo. Describir la respuesta humoral en una cohorte con una infección leve o asintomática por SARS-CoV-2, previamente identificada en una encuesta serológica comunitaria. **Material y métodos.** Se realizó un seguimiento observacional de 193 individuos previamente identificados con anticuerpos IgM/IgG anti-SARS-CoV-2 invitados 112 días después de una determinación serológica inicial. Todos los participantes completaron un cuestionario electrónico estandarizado. Se determinaron los anticuerpos IgM/IgG mediante un inmunoensayo quimioluminiscente cualitativo. **Resultados.** De entre los 193 sujetos elegibles, 174 (90%) acudieron al seguimiento. De las muestras, 171 (98.3%) eran positivas y 3 (1.7%) negativas. Además, el valor de COI del inmunoenasayo se incrementó al comparar la primera y segunda determinación (P <0.001). **Conclusiones.** Los presentes resultados apoyan una respuesta humoral sostenida en individuos con infección por SARS-CoV-2 con síntomas leves o asintomática hasta 112 días después de una prueba serológica positiva, acompañada de incremento en los títulos de anticuerpos.

Palabras clave: anti-SARS-CoV-2; SARS-CoV-2; Covid-19; anticuerpos; persistencia; seguimiento

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As SARS-CoV-2 infection has spread worldwide, cross-sectional serological surveys have provided initial data about exposure to the SARS-CoV-2 virus at a particular epidemic time, place, and the population studied.[1,2] Currently, follow-up studies that focused on profiling both the cellular and humoral response are emerging with new evidence. Since SARS-CoV-1 and MERS-CoV share a genetic and clinical background with SARS-CoV-2, it may be possible to expect a similar immune response in terms of seroconversion, timing, and duration.[3] SARS-CoV-2 interacts with target cells by binding the ACE2 receptor. Viral entry in the lung cells results in the typical Covid-19 associated symptoms of fever, nasal discharge, cough, and sore throat. Since the ACE2 receptor is expressed in several tissues, patients may experience additional gastrointestinal and cardiovascular symptoms.[4] Cytokine dysregulation, coagulopathy, ischemic complications, and multiorgan dysfunction comprise a broad Covid-19 spectrum of clinical manifestations. Exposure to SARS-CoV-2 leads to an immunologic response, including antibody production against viral proteins, while antibodies' duration and protection capacity remain unknown. Meanwhile, vaccines development (viral vector-based and mRNA-based) represented a critical effort during the last year, and vaccination is ongoing worldwide, including in our country. However, viral variants of clinical concern arising from natural selection might compromise current vaccine efficacy, and additional redesign and adjustment may be required.[5,6] To date, there is a lack of evidence about the efficacy and long-term duration of the humoral response in the natural SARS-CoV-2 infection. This information will be of prime interest in defining epidemic dynamics, maintain current preventive strategies, and guide vaccination programs.[7]

Several studies have highlighted a persistent and robust humoral response in most people infected with SARS-CoV-2,[8-13] while others have reported a decline in humoral immunity that may not be long-lasting.[14-19] These controversial and inconclusive results are based on studies with small sample sizes, variable follow-up times, and different immunoassays. Furthermore, they included specific populations, such as health-care workers with a wide range of disease severity, symptoms, and underlying conditions.

Accordingly, in this study, we aimed to determine the persistence of IgM/IgG antibodies against SARS-CoV-2 among 193 previously detected seropositive subjects in a community-based serological survey, at least 112 days from infection.

Materials and methods

The Institutional Ethics Committee approved the study protocol and written informed consent was obtained from all participants who attended. In this follow-up study, the 193 government employees previously identified with positive anti-SARS-CoV-2 antibodies were invited to a second test around 112 days after the first sample.

Briefly, positive anti-SARS-CoV-2 cases (n= 193) were identified in a community level serological survey performed with 3 268 participants in Guadalupe, Nuevo Leon, Mexico, during July 2020.[20] This serological follow-up occurred during a Phase 3 epidemic (World Health Organization, WHO). According to local health authorities, at the time of the follow up tests (October 31, 2020), Nuevo Leon and Guadalupe reported 82 021 and 10 987 PCR confirmed cases, respectively. Of note, participants did not report current symptoms during the baseline and follow-up antibody tests. All answered a standardized electronic questionnaire, and follow-up samples were collected between October 29-31, 2020.

Blood collection and anti-SARS-CoV-2 immunoassay

Blood (5 mL) was collected after fasting (4-8 h) and sent to the Metabolic Research Laboratory at the Endocrinology Division of the Dr. Jose Eleuterio Gonzalez University Hospital. Serum was obtained after centrifugation at 3 500 rpm for 5 min at room temperature. Similar to the first anti-SARS-CoV-2 assessment, we used the Elecsys Anti-SARS-CoV-2 chemiluminescent immunoassay (Roche, Germany, Ref. 09203079190) to qualitatively assess IgM/IgG against the SARS-CoV-2 nucleocapsid protein in each sample according to the manufacturer's instructions and recommended quality control. Briefly, double-antigen sandwich immune complexes were formed in the presence of anti-SARS-CoV-2 antibodies in the serum sample. After adding streptavidin-coated microparticles and binding the complexes, the mixture was transferred to a measuring cell. Electrochemiluminescence was induced by applying a voltage. Consequently, as the antibody titer increased, the signal yield increased. Chemiluminescent detection was performed in a Cobas e801 automated analyzer (Roche, Germany). A positive result is obtained when a cut-off index (COI) ≥1 is met. All samples were analyzed within 24 h after collection. The manufacturer reports an overall specificity of 99.8% and a sensitivity of 99.5% (≥ 14 days). Positive (100%) and negative (99.7 95CI% 98.9-99.9) predictive values were reported by Chan, *et al*. elsewhere.[21]

Statistical analyses

Descriptive statistics were used to summarize the data. Categorical variables were described as frequencies and percentages. Normal data distribution was determined using kurtosis and skewness measures. Mean and standard deviation or median and interquartile range were used for parametric and non-parametric numerical variables, respectively. We used the Wilcoxon signed-rank test to compare the quantitative result (COI) of the first and second antibody tests. The Mann-Whitney U test was used to compare COI values differences by age category, gender, obesity, and presence or absence of persistent Covid-19-related symptoms. We used SPSS V22 software* for all statistical analyses, and a P-value <0.05 was considered significant. Graphics were prepared using R package.[22]

Results

Demographic and clinical characteristics

Among the 193 eligible subjects, 174 (90%) attended the follow-up visit around 112 days after the baseline test. The rest of the participants were unable to complete their follow-ups due to work shifts and schedules (n= 13) or programmed vacations (n= 6). Demographic and clinic characteristics are described in table I. Overall, there were 104 (59.8%) men and 70 (40.2%) women with a mean age of 40 ± 10.6 years. Overweight and obesity were reported in 42.5 and 43.1% of the population, respectively. Only 20.1% of the volunteers reported having contact with a Covid-19 confirmed case. No previous Covid-19 related symptoms were reported by 60% of the participants during the IgM/IgG baseline test and, during follow-up. At least one Covid-19-related symptom was self-reported by 28.2% of the volunteers, mainly anosmia and dysgeusia (supplementary table A).[23] According to the national Covid-19 study protocol, only 23% (n= 40) of the patients underwent an RT-PCR test for SARS-CoV-2 in their healthcare setting based on the suspected symptoms.

Follow up assessment of IgM/IgG antibodies

A total of 174 serum samples were tested in the follow-up. Among these, 171 (98.3%) were still positive, and only 3 (1.7%) were negative to IgM/IgG antibodies (table II). Also, the COI value (electrochemiluminescence signal) of the immunoassay significantly increased from the first

* International Business Machines Corporation. SPSS V22 software. USA: IBM, 2013.

Table I

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE POPULATION. NUEVO LEÓN, MÉXICO, 2020

Characteristic	*Total sample (n= 174)*
Males, n (%)	104 (59.8)
Age, yrs	40.2 ± 10.6
Age group (%)	
18-34, n	61 (35.1)
35-49, n	76 (43.7)
50-65, n	34 (19.5)
>65, n	3 (1.7)
Weight,* kg	81.5 (72- 92.25)
Height, m	1.66 ± 0.1
BMI,* kg/m^2	29.0 (26.2- 33.2)
BMI categories (%)	
Normal, n	25 (14.4)
Overweight, n	74 (42.5)
Obesity I-III, n	75 (43.1)
Comorbidities (%)	
Hypertension, n	18 (10.3)
Diabetes type 2, n	14 (8.0)
Obesity, n	75 (43.1)
City (%)	
Guadalupe, n	111 (63.8)
Monterrey, n	11 (6.3)
Juárez, n	29 (16.7)
Other, n	23 (13.2)
Occupation (%)	
Police, firefighters and public safety, n	63 (36.2)
Office/Management, n	33 (19)
Maintainance/Janitors, n	20 (11.5)
Healthcare workers, n	8 (4.6)
Other, n	50 (28.7)
Contact with a Covid-19 confirmed case	35 (20.1)
PCR status (%)	
Never performed, n	134 (77)
Negative, n	19 (10.9)
Positive, n	20 (11.5)
Indetermined, n	1 (0.6)

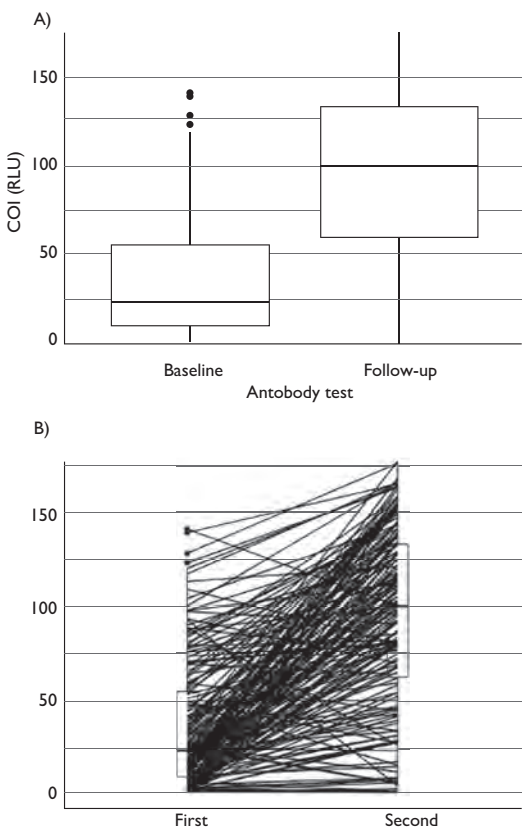
* Median (IQR)

to the second test (P<0.001) (figure 1). The COI value was not different when analyzed by gender (P= 0.52), age >45 years (P= 0.17), obesity (P= 0.07), or self-reported

Table II
Follow up assessment of anti-SARS-CoV-2 antibodies. Nuevo León, México, 2020

	n (%)	*COI (RLU)**
Positive baseline test	174 (100)	23.3 (9.1-55.5)
Positive follow up test	171 (98.3)	100 (63.9-133)‡
Negative follow up test	3 (1.7)	0.651 ± 0.46

* Median (IQR) of electrochemiluminescence signal; COI: Cut off index; RLU: Relative luminescence units
‡ Wilcoxon signed rank test *P*<0.001, baseline vs. follow up positive COI values



COI: Cut off index; RLU: Relative luminescence units

Figure 1. A) Quantitative luminescence values at first and second IgM/IgG tests. COI is expressed in RLU. Wilcoxon signed rank test p < 0.001. B) Individual changes from baseline to follow-up in antibody levels. Nuevo León, México, 2020

Table III
COI value by demographic and clinical characteristics. Nuevo León, México, 2020

Characteristic	*COI (RLU)*	*p-value**
Sex		
Males	100.5 (59.6-133)	0.52
Females	86.2 (55.7-134)	
Age (years)		
< 45	95.7 (46.2-128.5)	0.17
> 45	108 (76.7-137.5)	
Covid-19 symptoms		
Asymptomatic	99.8 (48.4-135)	0.56
Symptomatic	99.4 (76-132.5)	
Comorbidities		
Non-obese	95.7 (42.7-128)	0.07
Obese	102 (77.2-141)	

* Mann-Whitney test
COI: Cut off index; RLU: Relative luminescence units. RLU are expressed as median (IQR)

persisted symptoms (P= 0.56) (table III). Non-reactive subjects to the follow-up test reported no known contact with a Covid-19 confirmed case, and only one had mild and unspecific symptoms. They were young adults (<45 years), not obese, and had a medium risk of infection by occupational exposure (supplementary table B).[23]

Discussion

We found that about 98.3% of the subjects with SARS-CoV-2 infection still presented IgM/IgG antibodies 112 days after a positive baseline test. A SARS-CoV-2 infection could happen weeks before initial sampling. Moreover, we observed a statistically significant increase in the quantitative luminescence signal between the baseline and the follow-up test, reflecting increasing antibody titers. Therefore, there was a persistent, robust, and sustained humoral response up to 112 days after the first antibody detection in subjects with mild and asymptomatic SARS-CoV-2 infection. Our results are the first serology follow-up study reported in Mexico.

Since SARS-CoV-2 was first described a year ago, there is limited evidence about the elicited immunological response, and ongoing studies focus on defining the long-term duration of humoral immunity after symptomatic and asymptomatic natural infection. Ibarrondo and colleagues described a rapid decay of anti-SARS-

CoV-2 antibodies in mild Covid-19 at 37 and 86 days in 34 subjects.[14] These findings may support a concern about a limited duration of the humoral immunity in people with mild illness. Similarly, Liang and colleagues evaluated 76 healthcare workers in a prospective observational follow-up study of Covid-19 survivors from Wuhan (28% males, 41.3 ± 13.8 years). They found that IgM and IgG were negative in 87 and 13% three-months after discharge.[15] Additional evidence in asymptomatic subjects supporting the antibody decline is provided by Milani and colleagues. In this study, about 80% of asymptomatic subjects did not present immunoglobulins against SARS-CoV-2 after eight weeks after a positive PCR test.[16] However, only 31 subjects were evaluated after the initial antibody test during a relatively short follow-up period, and only antibodies against the spike protein were assayed. Also, the decline of anti-SARS-CoV-2 antibodies at 60 days among healthcare workers, who may be at a high exposure risk, was reported in three individual studies.[17,18,24] IgG levels in 93.3% (28/30) of the asymptomatic group declined during the convalescent phase, eight weeks after a positive PCR test and discharge from hospital isolation, according to the study by Long and colleagues. The median percentage decrease was 71.1% (32.8-88.8) for IgG levels in asymptomatic subjects.[25]

Our results contrast with the reports mentioned above. Conversely, our findings showed a positive IgM/IgG test in 171 of 174 subjects evaluated at the 112-day follow-up visit. The discrepancy in the persistence of antibodies could be explained by the different methods of serological assays (enzyme-linked immunosorbent assays, immunochromatography, or Luminex) and by a difference in the antigenic targets. Furthermore, in our study, the quantitative immunoassay result (COI) was significantly higher compared to the baseline signal. The study population mainly consisted of asymptomatic patients who were not eligible for a PCR test under the Covid-19 study protocol in Mexico. Also, we performed a qualitative and highly sensitive luminescent assay available as a routine test in our daily practice. Since we identified seropositive subjects using an IgM/IgG baseline test, it may be likely that patients had been exposed to SARS-CoV-2 at least several weeks before the first screening. These data suggest that cases had sustained antibody-mediated immunity. Given the conflicting results observed, additional prospective studies with longer follow-up periods in particular populations are needed.

The relationship between Covid-19 severity and the resulting antibody titers is currently unclear. Previous observations indicate that severe Covid-19 may result in a strong humoral response. Wu and colleagues suggested that the humoral immune response to SARS-CoV-2 in symptomatic Covid-19 patients is rather prototypical for viruses having an early expansion phase followed by an intermediate contraction phase and a sustained memory phase, similar to other common cold viruses.[26] We speculate that the persisting and increasing antibody levels determined in our study population may be due to the early expansion phase previously described. However, further assessment and characterization of the persisting antibodies will be needed to define a long-lasting humoral response.

In agreement with our findings, Malfertheiner and colleagues detected an IgG response in 77.8% of healthcare workers up to 12 weeks using the Elecsys Anti-SARS-CoV-2 test kit.[10] Interestingly, 22% of Covid-19 patients did not show humoral evidence of infection within 60 days. Marklund and colleagues showed that patients with severe Covid-19 seroconvert earlier and develop higher IgG titers than patients with mild illness.[11] Also, another study at Mount Sinai Health System recalled 121 plasma donors at various baseline titer levels and found IgG evidence against the viral spike protein at 82 days post-symptom onset.[13] Between days 40 and 199, 90% seropositivity in previous SARS-CoV-2-PCR positive subjects was reported by Figueiredo-Campos and colleagues in the longest follow-up study currently available.[9] Iyer and colleagues reported little to no decrease in IgG antibodies over 75 days since symptom onset in 343 North American patients (93% hospitalized) infected with SARS-CoV-2.[8] All authors agreed that not all patients develop traceable levels as we did in exceptional cases.

Some limitations should be taken into account. Antibodies were determined around 100 days after the baseline IgM/IgG test, and longer follow-up times may be evaluated. We used an immunoassay exclusively designed to detect antibodies against the nucleocapsid virus protein located in the viral core that leads to the production of non-neutralizing antibodies.[13,27] However, recent observations by Batra and colleagues suggest that antibodies against N protein could be a useful prognostic factor for the clinical course of the disease.[28] As for strengths, we followed a large sample size of cases with SARS-CoV-2 infection. All of them did not refer current symptoms during the baseline test. Also, we had a high follow-up rate (90%) at 112 days, and a longer follow-up of these patients would be likely as they are an institutionalized population.

In conclusion, we determined the antibody persistence up to 112 days in previously seropositive cases of SARS-CoV-2 infection. Additional research is needed to profile the complete immunological response in the long term because of the future implications in the serological survey data and immunity-based strategies.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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