

Study of the serological profile of Charles Nicolle Hospital personnel in Tunis affected by covid-19

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ABSTRACT

Introduction : Studying the human immune response to SARS-CoV-2 infection, as well as the factors influencing inter-individual variability in this response, is crucial to improving our understanding of the disease and guiding strategies to combat the virus. Our aim was to determine the immunological status of healthcare workers at Charles Nicolle Hospital in Tunis who had been diagnosed with SARS-CoV-2 infection, as well as the factors influencing the variability of their humoral response.

Methods: This was a retrospective and descriptive study, conducted in collaboration between the Department of Occupational Medicine and Work Capacity and the Immunology Laboratory at Charles Nicolle Hospital in Tunis. The study included healthcare workers from the same hospital who had been diagnosed with SARS-CoV-2 infection between September 2020 and March 2021 and who subsequently underwent post-infection serological testing.

Results: 551 healthcare workers were included in the study. Regardless of antibody isotype, anti-SARS-CoV-2 seroconversion was detected in 434 healthcare workers, representing 78.7% of cases. Following multivariate analysis, the factors independently associated with seroconversion were age, the presence of symptoms like fever, anosmia and the time between symptom onset and RT-PCR diagnosis ($p < 10^{-3}$). Factors inversely associated with seroconversion were smoking ($p = 0.002$) and a history of asthma ($p = 0.044$).

Conclusion: Seroconversion is partial and variable among patients infected with SARS-CoV-2. Given the widespread rollout of vaccination and the continued emergence of new variants, it is important to study the kinetics of the serological profile in these patients and the level of protection against COVID-19.

Key-words: COVID-19 – Health personnel - serology.

INTRODUCTION

The emergence of the SARS-CoV-2 virus has raised several questions about the microbiological characteristics, transmissibility, and clinical features of the disease caused by this virus. Studying the human immune response, its efficacy, the duration of its protective effect and the factors influencing the inter-individual variability of this response is a vital element in gaining a better understanding of this disease and guiding strategies to fight this virus.

Several studies of the humoral response against SARS-CoV-2 have shown variable rates of seroconversion [1-4]. This raises several questions about the factors that might influence the inter-individual variability of this response against this virus.

Health personnel (HP) represent a high-risk population for SARS-CoV-2 infection. Several cohorts have assessed seroprevalence in this population [5-7]. However, few studies have analyzed the variations in the humoral immune response of HP infected with this virus and the factors associated with this response.

In Tunisia, since the second wave of the pandemic in September 2020, the number of cases of infection with SARS-CoV-2 has increased considerably, and successive waves have been observed both in the general population and in HP [8]. Few studies have investigated post-infection immunization against SARS-CoV-2 among HP in Tunisia.

Thus, the determination of the rate of immunized HP and the monitoring of the clinical course of the disease in these patients would be of a great benefit. In this context, the aim of this study was to determine the immunological status of HP infected with the SARS-CoV-2 virus and to investigate the factors influencing the variability of the humoral immune response against this virus in this population.

METHODS

This was a descriptive and retrospective study. This study included HP from Charles Nicolle Hospital (HCN) of Tunis infected with SARS-CoV-2 virus between September 2020 and March 2021, who underwent to post-infection serological tests after their returning to work. HP with incomplete medical records at return to-work (RTW) medical visit were excluded from the study.

Clinical and therapeutic data were collected from the medical records of the included HP who had received phone follow-ups during isolation and a RTW medical visit. Blood samples for serological tests for anti-SARS-CoV2 antibodies were taken after the RTW medical visit and were analyzed in the HCN immunology laboratory.

Data collection included socio-demographic characteristics (age, gender, habits (smoking, alcohol, physical activity)), medical data (medical history, long-term medical treatment, weight, height and Body Mass Index (BMI)) and clinical data related to COVID-19 disease.

COVID-19 diagnosis was confirmed by nasopharyngeal swab RNA tests for SARS-CoV-2. These tests were taken in case of symptoms suggestive of COVID-19 and/or close contact with a case infected with SARS-CoV-2. In the latter case, sampling was indicated within 4 to 7 days of exposure.

We also collected the various symptoms reported by patients during phone follow-ups at their sick leave (fever, cough, anosmia, ageusia...).

HP infected with COVID-19 were called 21 days to 1 month after the onset of symptoms for serological sampling. Blood samples were taken between October 9th, 2020 and April 28th, 2021 during the RTW medical visit. Serological tests were analyzed in the immunology laboratory using the commercial kits available during our study period. Some kits detect the presence of antibodies directed against the receptor binding domain (RBD) of the Spike (S) protein (anti-Spike antibodies) while other kits test for antibodies directed against the virus nucleocapsid (anti-Nucleocapsid antibodies).

These semi-quantitative tests analyze the fluorescence emitted at the end of their action. This fluorescence is proportionally correlated to the quantity of antibodies present in the serum/blood sample. The intensity of the fluorescent signal is determined as a cut-off index value (COI) or test value (i) (corresponding to the relative fluorescence value (RFV) for each sample/RFV obtained for the standard. The threshold value for the positivity of COI or (i) was one. The presence of seroconversion is defined by the positivity of antibodies (anti-N and/or anti-S), whatever the isotype (Ig M and/or Ig G).

There may be a selection bias of participants as they are volunteers. The phone questionnaire could generate reporting bias.

The data for this study were entered and analyzed using Statistical Package for the Social Science (IBM SPSS Statistics 22.0).

This study, as an observation of the outcomes of serological tests, is exempt from Institutional Review Board approval [9] and met the following criteria: The information obtained is recorded by the investigator in such a manner that the identity of the Human Subjects cannot be readily ascertained, directly or indirectly through identifiers linked to the subjects.

Participation in the study was voluntary and the HP were informed of the objectives of the study. Anonymity was respected during data entry and analysis.

RESULTS

A total of 551 HP diagnosed with SARS-CoV-2 between September 2020 and March 2021 was included in the study. The study population was predominantly female (75%) with a M/F sex ratio of 0.25. The mean age of the participants was 41.6 ± 10.3 years [extremes: 24; 63 years]. The 30 to 39 age group was the most represented (34.4%). The professional category of the participants was: nurses (31.2%), health technicians (27.4%), doctors (19.6%), manual workers (13.1%), and administrative staff (6.5%). They belonged to medical departments in 36.5%, surgical departments in 34.8%, emergency department in 9.8%, laboratories in 8.3% and administration in 5.8% of cases.

Pathological medical history was reported by 53% of the participants. Long-term medical treatment was reported in 35.4%. The main medical conditions were hypertension (10.9%), asthma (10.3%), osteoarticular disorders (9.1%), and diabetes (7.1%). The BMI was 26.5 kg/m^2 (IQR: $23.9\text{-}29.4 \text{ kg/m}^2$) [extremes: 16.4; 47.7 kg/m^2]. The study population was overweight in 33.6%. Active smoking and regular physical activity were reported in 9.3% and 32.1% respectively.

Nasopharyngeal sampling for RT-PCR was indicated for symptoms suggestive of Covid-19 in 93.8% and for contact tracing in 6.2%. The median delay between the onset of symptoms and RT-PCR was two days, up to 14 days after the onset of symptoms in asymptomatic subjects. The main symptoms reported by HP during phone follow-ups at their sick leave were asthenia (80.5%), headache (72.7%), cough

(68.4%), aches and pains (65.7%) and anosmia (60.2%).

Hospitalization in a Covid-19 unit was indicated for respiratory symptoms in 2.4% (N=13). The median sick leave was 14 days (IQR: 12-17 days), with extremes from 7 to 152 days. This duration was less than or equal to 14 days in 55.9%.

Serological tests were performed after RTW, with a median delay from the onset of symptoms of 28 days (IQR: 24-32 days) [extremes: 8; 194 days]. Anti-N and/or anti-S seroconversion was found in 78.7% (N=434) as shown in Table I.

Table I: Serological test results by antigen and antibody isotype.

Isotype	Anti-N ab		Anti-S ab	
	Number	Positive	Number	Positive
Isolated IgM	1	0	7	5 (71,4%)
Isolated IgG	10	9 (90%)	192	147 (76,5%)
IgM and IgG	233	102 (43,7%) Ig M only:5 Ig G only:84	9	3 Ig M only:2 Ig G only:1
IgM and/or IgG	143	94 (63,9%)	0	0
Total	387	294 (75,9%)	208	158 (75,9%)

ab: antibodies

The median COI for anti-N Ig M was 1.45 (IQR: 0.3-4.4) [extremes: 0; 11] (N=234). The median COI for anti-N Ig G was 13.07 (IQR: 1.7-16.5) [extremes: 0; 176] (N=243).

The median COI for anti-S Ig G was 3.6 (IQR: 1.08-9.8) [extremes: 0; 36.2] (N=201).

The statistical analysis of the factors associated with seroconversion is shown in Table II.

After multivariate analysis, the factors independently associated with seroconversion were age ($p=0.021$), the presence of symptoms such as fever, anosmia, cough and the delay between the onset of symptoms and diagnosis by RT-PCR ($p<10^{-3}$). Factors inversely associated with seroconversion were smoking ($p=0.002$), and having a history of asthma ($p=0.044$).

Table II: Statistical analysis of factors associated with seroconversion.

Variables	NS	Covid-19 SC (%)	p	a OR	a IC
Demographic characteristics and lifestyle					
Age (years)	39.8±9.2	42.1±10.5	0.030	1.032	1.005-1.059
Gender	Men Women	76.5 79.3	0.528		
Active smoking	Yes No	60.7 81.8	0.002	0.444	0.211-0.934
Physical activity	Yes No	79.6 78.4	0.528		
Medical data					
BMI (Kg/m ²)	<25 ≥25 and <30 ≥30	77.4 77.2 81.7	0.599		
Medical history	Yes No	77.3 80.6	0.206		
History of asthma	Yes	68.4	0.044	0.391	0.183-0.838
Characteristics of the COVID-19 disease					
Symptoms	Yes	80.9	<10 ⁻³	2.043	0.715-5.838
Anosmia	Yes No	81.1 70.3	0.013	1.254	0.654-2.443
Headaches	Yes No	82 71.9	0.016	1.230	0.684-2.215
Cough	Yes No	82 71.8	0.007	1.808	1.057-3.092
Generalized stiffness	Yes No	82.1 72	0.007	1.094	0.616-1.944
Anosmia	Yes No	86.5 66.8	<10 ⁻³	2.326	1.164-4.648
Diarrhea	Yes No	79.9 77.7	0.528		
Agueusia	Yes No	86.5 71.7	<10 ⁻³	2.326	0.625-2.646
Dyspnea	Yes No	80.4 77.7	0.450		
Fever	Yes No	86.2 74	0.001	1.891	1.056-3.389
Sore throats	Yes No	76.2 80.3	0.268		
Chest pain	Yes No	81.9 77.3	0.213		
vertigo	Yes No	82.5 77.8	0.253		
Nausea/vomiting	Yes No	80.8 78.5	0.645		
Abdominal pain	Yes No	81.8 78.8	0.619		
Lowback pain	Yes No	87.3 77.7	0.078		
O ₂ desaturation (SpO ₂ <94%)	Yes No	85.7 78.3	0.356		
NS PS					
Delay onset of symptoms -serological analysis (days)	32.7±29.2	32.4±18.4	0.870		
Delay onset of symptoms - RT-PCR (days)	1.25±2.5	2.26±2.3	<10 ⁻³	1.227	1.072-1.405
Length of time off work (days)	14.3±4	15.2±8.2	0.211		

a OR: adjusted odd ratio; **a IC :** adjusted confidence interval; **NS:** Negative serology; **PS:** Positive serology; **SC:** Seroconversion

DISCUSSION

In this study, we described the serological responses of 551 HP infected with SARS-CoV-2 between September 2020 and March 2021 and the factors influencing these responses. Regardless of isotype, anti-SARS-CoV-2 seroconversion was found in 78.7% of cases. After multivariate analysis, the factors associated with seroconversion were age, the presence of symptoms such as fever, anosmia, cough and the delay between the onset of symptoms and diagnosis by RT-PCR ($p < 10^{-3}$). Factors inversely associated with seroconversion were smoking ($p = 0.002$) and having a history of asthma ($p = 0.044$).

After RTW, serological tests were performed within a median delay from the onset of symptoms of 28 days (IQR: 24-32 days) [extremes: 08; 194 days]. This delay was dictated by the study design, which depended primarily on the duration of sick leave and the availability of HP for serological sampling after RTW. In the literature, the median delay to obtain anti-SARS-CoV2 seropositivity was 11 days (8-14) [3]. Indeed, in a French study of HP with mild forms of COVID-19 disease, anti-

SARS-CoV-2 antibodies were detectable from the 13th day after the onset of symptoms [10]. Regardless of isotype, anti-SARS-CoV-2 seroconversion was found in 78.7%. This rate is near to those reported in the literature, varying between 63.8% and 100% [3, 4, 11-14], compared to 15.4% in asymptomatic subjects [6]. It has been assumed that such inter-individual disparities are linked to both host-dependent factors and immunization conditions. In this context, we were interested in analyzing the demographic and clinical data associated with the occurrence of seroconversion in our series.

Older age was associated with seroconversion in our study. Similarly, Ganz-Lordetal, showed that seronegative people were on average 10 years (95%CI 3 to 17) younger than seropositive people [15]. The observed association between age and seroconversion might be explained in this way; it has been shown that age is a known predictive factor for COVID-19 severity, and that the production of anti-SARSCoV-2 Ig G is greater in severe forms [15, 16]. Nevertheless, our population did not include subjects aged over 65 (retired) who may be immuno-senescent and poorly reactive.

Surprisingly, having a history of asthma was inversely associated with seroconversion in our results. Fafi-Kremer et al, who studied the factors associated with the neutralizing activity of antibodies, showed that the absence of asthma was significantly associated with neutralizing serum activity ($ID_{50} > 100$) ($p = 0.02$) [10]. Other studies on asthma and COVID-19 mainly evaluated the association between asthma and the severity of COVID-19 disease. However, in the study by Lieberman-Cribbin et al, no association was found between asthma and mortality in patients with COVID-19 [18]. Similarly, respiratory allergies were associated with a reduced risk of COVID-19 infection and the occurrence of severe forms. This could be explained by a low expression of ACE2 receptors in nasal and bronchial airway epithelial cells according to Bakakos [19] and Jackson et al [20].

The presence of fever, anosmia, and cough was statistically associated with seroconversion in our series. This view is supported by Thiruvengadam et al [16] who found that the presence of these symptoms at the time of

diagnosis was a predictive factor for seroconversion (a OR=3.23; 95%CI=2.01 to 5.17). However, this finding has not been observed in other cohorts. In the study by Liu et al., the authors found no significant difference in terms of seroconversion between patients with and without these symptoms [13]. Despite these controversies, it is accepted that exposure to high viral loads is potentially immunogenic. This corroborates the fact that severe forms of COVID-19 with a systemic response are probably more associated with the production of anti-SARS-CoV-2 antibodies.

To our knowledge, our work represents the first Tunisian survey to study the anti-SARS-CoV-2 serological status in HP. Our study is one of the few series to analyze the relationship between seroconversion and associated factors such as demographic characteristics, comorbidities, and symptoms during the sick leave. In addition, this study included patients with various forms of COVID-19 severity. Another strength of the study is that we have avoided memory bias, due to regular phone follow-ups of the patients' symptoms.

Some limitations of this study should be considered linked in particular to the heterogeneity in performance of the serological tests and the type of the antibodies sought. Indeed, the serological tests were performed using the commercial kits available during our study period. Thus, the concomitant search for anti-N and anti-S antibodies was not performed for all subjects. In addition, the delay between the onset of symptoms and the serological analysis was relatively variable.

CONCLUSION

Overall, despite the limitations of our study, it is clear that seroconversion is partial and variable between patients infected with SARS-CoV-2. However, seroconversion should be considered as a biological proof of infection for medico-legal reasons, which would allow COVID-19 to be declared an occupational disease among HP in Tunisia if the RT-PCR is negative.

Given the widespread use of vaccinations and the continuing emergence of new variants of the virus, it is important to study the kinetics of the serological profile in these patients and the level of protection against COVID-19, taking into account the type and number of doses of vaccine received. In addition, research on cellular immunity is interesting to better elucidate

the human immune response against this virus and its various mutant strains.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no conflict of interest.

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