Prevalence and risk factors of SARS-CoV-2 antibody responses and asymptomatic carriers among a cohort of 2,455 healthcare workers of a general hospital during the Covid-19 Pandemic

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Abstract

Observational and prospective study to establish an immunological map of SARS-CoV-2 exposure of 2,455 healthcare workers (HCW) at the Maresme Health Consortium (CSdM), Catalonia, during COVID-19 pandemic, with serum sampling for antibody determination. In pre-vaccination serum samples, we performed an initial chemiluminescence immunoassay (CLIA) to detect: IgG+IgM+IgA and, in those with positive results, a differential analysis of IgM+IgA vs IgG by ELISA to detect an acute or past infection. A PCR was performed in nasopharyngeal swabs in positive IgM+IgA to confirm the non-diagnosed carriers of the virus. In post-vaccination samples, we assessed IgG SARS-CoV2 SPIKE-TRIMERIC and total antibodies to the nucleocapsid (N) protein of SARS-CoV-2. The individual occupational, social and health risk factors were assessed by a self-administrated epidemiological survey at each serum sampling period. Relatively large sample size, high participation rate in all phases of the study with four blood samples over time, high vaccination acceptance, and follow-up of the study cohort for 17 months (6-7 months before and 10-11 months after vaccination), represent important strengths of the study. As this is a study with an open cohort (with entrances and exits), it has made it possible to obtain an accurate picture of the epidemiological characteristics of the pandemic among CSdM HCW in real life. However, this open cohort design has made it difficult to assess risk factors that, on the other hand, have shown some changes over time due to progressive implementation of protective and preventive measures. Moreover, this is not a clinical trial but an observational study, so it is not the best design to assess the efficacy of a vaccine. However, it allows us to assess its effectiveness in terms of immunological response in real conditions.

Introduction

A new coronavirus was identified in 2019. It first appeared in Wuhan, China, and caused a number of cases of pneumonia. The virus was named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The World Health Organization (WHO) named the disease caused by the virus as Covid-19. The mass, cross-cutting and mass spread of SARS-CoV-2 caused a pandemic with a major impact on the health systems of most affected countries.

The main route of transmission of SARS-CoV-2 is through direct person-to-person contact. It is known that a cohort vulnerable to infection caused by frequent and close contact with patients with COVID-19 are health workers. In order to prevent the transmission of SARS-CoV-2 from patients to health personnel, strict occupational health and safety regulations are necessary. SARS-CoV-2 infection usually leads to seroconversion between 11 and 14 days later. It should be noted that the infection causes many asymptomatic or oligosymptomatic cases, a key piece that needs to be taken into account to prevent the spread and risk of infection among the family and work of these people.
Due to the situation developed by the aforementioned virus, it is necessary to evaluate the "invisible iceberg" of mild infections, in order to determine in an effective and viable way, the real severity of the disease, the true rate of specific hospitalization, intensive care admission and death rates. This information is critical in order to: a) assess the development of group (herd) immunity, b) gauge the response developed to this pandemic, and c) determine the factors and work practices most directly associated with seroconversions and the effectiveness of protective measures. This knowledge, which can be derived from the disease, the viral cycle and the immune response of the infected, is essential to be able to curb this pandemic (large and small scale) and to reduce the risk of infection.

The general objective of this study is to establish an immune map of SARS-CoV-2 exposure of professionals working in the Maresme Health Consortium and to assess the occupational, social and health risk factors associated with SARS infection. -CoV-2 in order to minimize the risk of infection.

Reagents

Equipment

a. Screening by chemiluminescence Immunoassay (CLIA; Elecsys Cobas, Roche Diagnostics) to detect SARS-CoV-2 nucleocapsid-protein total antibodies. As a marker of seropositivity - June 2020, October 2020, April 2021 and November 2021

b. In total antibodies positive serum samples, ELISA (COVID-19 ELISA IgG G1032; COVID-19 ELISA IgM+IgA MA1032, Vircell Microbiologists) to differentiate IgG from IgM+IgA - June 2020 and October 2020

c. In the case of positive IgM+IgA, a nasopharyngeal swab for real-time reverse transcriptase-polymerase chain reaction (TaqPath COVID-19 CE-IVD RT-PCR (Thermo Fisher Scientific, Pleasanton, CA, USA) to detect asymptomatic carriers - June 2020 and October 2020

d. In post-vaccination samples, quantitative serological assay to determinate SARS-CoV-2 spike-protein antibodies (DiaSorin LIAISON, TrimericS IgG assay) to determine seroconversion - April 2021 and November 2021

Procedure

1. Serological study based on blood extraction following the strategy of the Reference Laboratory of Catalonia (LRC):
a. Screening the entire population with total chemiluminescence immunoassay (CLIA) result (IgA, IgM, IgG) to track down negatives

b. ELISA for IgM, IgA, and IgG differentiated positive results.

c. PCR smears nasopharynx on all IgM and IgA + (to determine asymptomatic cases).

2. Universal Cohort Study. All the staff working at CSdM, including the staff of the subcontracted companies.

3. Periodic Analysis: at 0/90/180/270/360 days.

4. Treatment of samples for preventive purposes in the field of work, clinical and clinical research.

5. Related information. Participants will be required to complete an electronic questionnaire with information on symptoms, treatments, and other data of interest (occupational, social, and health factors). To take the test, the questionnaire must be answered in advance.

6. Approval of the Ethics Committee and protocol registered in the ClinicalTrials.gov website: study protocol, Patient Information Sheet and Informed Consent approved by the Drugs Research Ethics Committe of Consorci Sanitari del Maresme under code 56/20. Protocol registered in ClinicalTrials.gov website under code NCT04425759.

7. Internal coordination with CSdM Clinicians and Researchers. Coordination of the strategy with clinicians and researchers of the CSdM. Inclusion of a group of in-house researchers.

8. External coordination with IGTP Researchers. Coordination of the serological and epidemiological strategy with clinicians and researchers from IrsiCaixa and IGTP to ensure an optimal approach for the design, execution and interpretation of the results of the study. Inclusion of a group of external researchers.

9. Recruitment: the questionnaire will be located on the CSdM employee portal, which requires access to the portal located on the CSdM intranet, with the password used by the worker, to access their employment data and links to your worker number. In order to be able to participate, the professionals will have to give their consent, through the Informed Consent which will also be found on the intranet, where they will have to tick those boxes that they want or not, to accept in relation to their participation.

11. Custody of the questionnaire data. Clinical information will be managed with the maximum guarantee of security in accordance with current legislation by the research team. Participants will be included in a coded form and no identifying data will be added. Only designated researchers will be able to access this record. Once the procedure for including data within the Database has been completed, the data will be exported in the most convenient format for statistical exploitation to the CSdM. The selected company will then delete the Database from its system.
12. **Blood draw.** The antibody determination request will be made through the laboratory software (EYRA) minutes prior to extraction. This requires that administrative staff, under the authorization of the physician responsible for occupational health, generate such a request. In this application the worker will be identified by the SL letters followed by his worker number. To verify the identity of the worker, the worker must bring his ID. The administrator, at the time of generating the application will have a card reader so that you can immediately and unequivocally know the number of workers. The management staff will deliver the printed application to the interested party.

13. **Extraction.** A second identity check must be made at the extraction point of the extraction nurse by reading the bar code printed on the extraction application. The computer software will return the worker’s name. A single 10 ml serum tube will be removed, which the nurse will identify with the printed label prior to application.

14. **Sample processing.** In the CSdM laboratory an aliquot of the sample will be frozen at -80°C and the other part will be sent to the laboratory of MasBlau (Reference Laboratory of Catalonia) for processing. The presence of total antibodies by ECLIA electrochemoluminescent immunoassay will be determined on a Cobas-E self-analyzer. This anti-SARS-CoV-2 assay uses a recombinant protein that represents the N antigen of the virus nucleocapsid. Both negative and positive results will be reported. Serums with detectable results will be subjected to other tests. ELISA tests to determine the class of immunoglobulin detected, only IgM and IgG will differ. The results will be incorporated into the laboratory system within a maximum of 2 working days.

15. **Delivery of individual results to workers.** From computer will develop a tool so that each worker can access through the portal of the worker to the intranet the result of the serology. The results can appear together with a comment that interprets them, generated through logic, also by computer science and occupational health. This will avoid unnecessary consultations with Occupational Health.

16. **Serum collection.** A collection of samples will be made with the rate of extraction of the biological sample taken. This collection will have a specific line of research: Seroprevalence of COVID antibodies in CSdM workers, no other studies will be performed that are not included in this field. The specific consent of the participant will be required to store his sample. Samples will not be transferred in any case to third parties. In the case of wanting to make use of the samples, for purposes that are outside the initially agreed scope, it will be required to pass the CEIm and ask the participant again for his consent to transfer the sample. The indefinite collection of samples gives the possibility of new clinical and additional research studies that can help control the pandemic. In particular, the appearance of new quantitative tests and the determination of neutralizing antibodies will make it possible to determine precisely the degree of AC and the duration of the acquired immunity as soon as the methods are available in the Reference Laboratory.

17. **Method of data aggregation and analysis.** Questionnaires and serum samples will be linked by worker number and this set (labor, social, medical data and serological test result) will be coded. The
epidemiological and risk factor statistical analysis will be carried out under the direction of the IP to meet the objectives of the study in the Research Unit of the CSdM Health Foundation.

17.1 Descriptive study. A description of the main baseline characteristics of the study sample in means and standard deviations (for numerical variables) and percentages (for categorical variables) was performed. Seroprevalence against SARS-Cov-2, expressed as percentages and 95% confidence intervals (CI), and the prevalence of non-diagnosed carriers in June 2020, October 2020, April 2021 and November 2021 were estimated.

17.2 Cross sectional (June 2020) and longitudinal (June-April 2021) analyses of the risk factors for a positive serological test result.

17.3 Assessment of factors related to non-diagnosed carriers in June 2020 using the same above-mentioned statistical tests (Chi square or Fisher exact test and logistic regression analysis).

17.4 Evaluation of immunological response after vaccination by describing the prevalence of HCW with antibodies against SARS-Cov-2 spike protein (as percentage and 95% CI) in April 2021 and November 2021, description of the levels of antibodies against SARS-Cov-2 spike protein, and analysis of the factors associated with the levels of antibodies against SARS-Cov-2 spike protein using the t-test or the Mann Whitney U test.

17.5 Comparison of the levels of anti spike protein antibodies between April 21 and November 21 using the Wilcoxon test for paired data.

18. Description of the labor use of the results in accordance with current regulations. The results will help to improve working procedures with solid criteria for the safety of professionals and patients, thus strengthening the health system to deal with the pandemic. The results will be managed in a coded way and will be presented in an aggregated way, as a “serological map”, a work tool that provides information to improve the working conditions and protection of professionals against infection.

Troubleshooting

Time Taken

Recruitment period: June 2020 - November 2021

Extraction and analysis of the data: November 2021 - January 2022

Manuscript preparation: January 2022 - May 2022

Figures
Figure 1

Flow chart of HCW participants in the seroprevalence study. S+Q=Serological test + Questionnaire; OS=Only Serological test; OQ: Only Questionnaire; S = Serological test (with and without questionnare).