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Complete manuscript title: **Survival analysis of time to SARS-CoV-2 PCR negativization to optimize PCR prescription in health workers: the Henares COVID-19 healthcare workers cohort study.**

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What is already known about this subject?

Health professionals should not return to work until they will no longer transmit the disease. The most extended protocol requires the health worker to be free of COVID-19 symptoms and to have at least one negative PCR for SARS-CoV-2.

What are the new findings?

In this study with a development cohort of 159 healthcare workers, and using survival analysis, we defined the variables that influence PCR time to negativization. Median time to negativization was 25 days from symptom onset. Workers with dry cough and dyspnea needed nearly one week more to negativizate.

How might it impact on clinical practice in the foreseeable future?

In future waves, this information could help to choose the most efficient time to perform PCR.

Survival analysis of time to SARS-CoV-2 PCR negativization to optimize PCR prescription in health workers: the Henares COVID-19 healthcare workers cohort study.

AimObjectives: Reverse-transcriptase polymerase-chain-reaction (RT-PCR) is considered the gold standard for diagnosing coronavirus disease 2019 (COVID19). Infected healthcare workers don´t go back to work until RT-PCR has demonstrated that the virus is no longer present in the upper respiratory tract. The aim of this study is to determine the most efficient time to perform a RT-PCR prior to their reincorporation.

Material and methods: Cohort study of those health workers with RT-PCR confirmed COVID-19. Data was collected using the medical charts of the health workers and completed with a telephone interview. Kaplan-Meier curves were used to determine the influence of several variables in the time to RT-PCR negativization. The impact of the variables in survival was assessed using the Breslow test. A Cox regression model was developed including the associated variables.

Results: 159 subjects with a positive RT-PCR out of 374 workers with suspected COVID-19 were included. Median time to negativization was 25 days from symptom onset (interquartilic range 20 to 35 days). Presence of Ig G, dyspnea, cough and throat pain were associated with significant longer times to negativization.

Cox logistic regression was used to eliminate confounding variables. Only dyspnea

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Cox logistic regression was used to eliminate confounding variables. Only dyspnea and cough remained in the model as significant

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determinants of prolonged negativization times. Adjusted hazard ratios were 0.68(0.48-096)) for dyspnea and 0.61(0.42-0.88) for dry cough.

Conclusions: RT-PCR during the first three weeks leads to a high percentage of positive results. In the presence of respiratory symptoms, negativization took nearly one week more. Those who developed antibodies needed longer times to negativizate.

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Introduction

In December 2019, an outbreak of atypical pneumonia occurred in Wuhan (China). Days later a new coronavirus was identified as the responsible agent. In a few weeks, due to the lack of immunity and the high transmissibility of the virus, the infection had spread worldwide causing thousands of deaths.(1, 2)

Spain has one of the highest number of confirmed infections.(3) The Henares catchment area was one of the most severely hit by the pandemic, and a significant number of healthcare professionals became infected. The occupational medicine department was overwhelmed and unable to cope with this severe and unexpected crisis and a new specific task force composed by both physicians from different specialties and nurses was set up to help manage the pandemic. In April of 2020 the Henares COVID cohort health workers study was initiated with the purpose of investigating this new disease.

It is easier to garner comprehensive information from cohorts of healthcare workers than general population, since they define more precisely their symptoms, have a more direct access to diagnostic technology and are usually keener to participate because they are constantly dealing with patients and understand the need for thorough studies. Thereby several previous cohort studies have followed healthcare workers (4, 5). In the case of COVID-19 this population has a demonstrated higher incidence of infection, thus making it easier to gather a significant sample.

In order to keep their workplace as safe as possible for both staff and patients, health professionals should not return to work until they will no longer transmit the disease (6). Several protocols have been proposed. Our center adhered to the protocol recommended by the CDC at that time, requiring at least two negative reverse transcriptase-polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 in a nasopharyngeal swab prior to return to work. In order to maintain an adequate workforce, it is important to allow healthcare workers to return as soon as possible, but, especially if there is limited capacity for RT-PCR testing, it is necessary to optimize its performance. Therefore, the aim of this study was to estimate the most efficient time-frame to do a RT-PCR prior to return to work.

Material and methods

Patients and study design

The Henares COVID-19 healthcare workers cohort study was designed as a combined prospective and retrospective cohort study. This cohort included all those healthcare workers who consulted with clinical manifestations compatible with COVID 19 (Fever and/or pseudo-flue syndrome or/and digestive symptoms or/and chemosensory disorders) at Hospital Universitario del Henares from 11th of March 2020 and the 31st of April 2020. This center is a secondary care hospital, located in the region of Madrid, with a catching area of 175,000 inhabitants.

To be included in the cohort patients had to have at least one confirmatory upper respiratory tract RT-PCR for SARS-CoV-2. A survival analysis was performed to determine time to negativization, which was calculated as the difference between the date of the first of the two consecutive negative RT-PCR and the date of patient-estimated symptom onset. Patients with at least two weeks of follow up were included in the study.

The electronic chart of the workers was reviewed and data transcribed to an Excel sheet by one of the authors of the study. An additional personal phone interview conducted by the same doctor who introduced the data was scheduled for each patient, to resolve missing or contradictory data. Then patients were followed in a prospective fashion until PCR negativization took place, registering the results of each PCR. Data were analyzed using SPSS software program, version 15.0 (SPSS, Chicago, Illinois). The study was approved by the Clinical Research Ethics Committee of Universidad Francisco de Vitoria and by the Research Committee of University Hospital of Henares, and all the workers expressed their consent signing an authorization form or affirmatively replying to an email.

Demographic data including age, date of birth, gender, professional activity, height and weight at the onset of symptoms, date of symptom onset, blood type, smoking status and date of positive PCR for SARS-CoV-2 were collected. The presence of the following comorbidities was also recorded: high blood pressure, treatment with angiotensin-converting enzyme inhibitors, diabetes mellitus, ischemic heart disease, stroke, lung disease (asthma or obstructive pulmonary disease). All these risk factors were collected as binary variables except tobacco exposure, which was stratified as mild (1-9 cigarettes per day), moderate (10-19 cigarettes per day) and severe (more than 20 cigarettes per day). Binary data related to 14 clinical manifestations was also collected: fever, rhinorrhea, throat pain, cough dry/productive, headache, myalgia, dyspnea, tachycardia, hyposmia/hypogeusia, asthenia, digestive manifestations (diarrhea, nausea or vomiting), conjunctivitis, dermatological manifestations. The maximum body temperature reached was also registered in those patients who referred fever. When the patient referred dyspnea, a chest radiography was performed. The result of the chest radiography and the most aggressive clinical management required (ambulatory, conventional hospitalization or intensive care unit hospitalization) as well as the date of PCR negativization were also collected.

Statistical analysis was performed with SPSS 15.0 software (IBM Corp, Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation if normally distributed, and as median and interquartile range (IQR) if non-normally distributed. A two-tailed $P < 0.05$ was considered statistically significant.

Bivariate analysis of time to RT-PCR negativization was performed through the study of Kaplan-Meier curves and statistical significance was performed using the Breslow test. Demographic variables (age, gender and BMI) and those clinical variables that were associated in the Breslow test were included in a multivariate analysis using Cox proportional hazards models. A backward-conditional method was chosen, with significance levels of 0.05 for inclusion and 0.1 for exclusion.

Results

399 healthcare workers were attended for suspected COVID 19, between the 11th of March 2020 and the 31st of April 2020 (Figure 1). In 374 cases, patients had clinical manifestations compatible with COVID and a sample for RT-PCR of the upper respiratory tract was obtained. Of these, in 159 cases the presence of SARS Cov-2 was confirmed with RT PCR; these patients were included in the COVID-19 PCR positive cohort. (Figure 1) Demographic characteristics are summarized in table 1. Mean age was 41.3 years, and women made up almost four-fifths of the sample. As regards profession, 35.2% were nurses, 32.7% physicians and 22.6 % heath technicians. This

distribution mirrors the composition of the hospital staff (41% registered nurses, 28% medical doctors, and 29 % health technicians).

Most of the sample (74.2%) was composed by healthy subjects. Only 20.8% had one medical condition, and 3.1% had two conditions. Asthma was the most common comorbidity (9.4%), followed by high blood pressure (5%). Only 10.2% of the members of the cohort were smokers.

Chest radiography demonstrated the presence of pneumonia in 27 patients. Most of the patients received only symptomatic treatment. Only 22 patients (17%) received specific treatment for COVID 19. Only eleven patients were admitted into the hospital; 10 of them needed conventional hospitalization and one needed intensive care hospitalization.

74% of the patients in the cohort reported hyperthermia, mostly low fever: only 24% of patients reported a maximal temperature above 38.0ºC. Cough was the most common symptom; 65.4% referred dry cough while 7.5% referred productive cough. Headache, muscle pain, asthenia and some degree of hyposmia or taste alteration was present in a similar number of subjects (Table 1).

	Studied variables
Demographic variables	(n=159)
Age (mean; SD)	Mean=41.3; SD=11.7
Women	126 (79.2%)
Body mass index (BMI)	Mean=24.8; SD=4.7
Comorbidities	
High blood pressure	8 (5%)
Diabetes mellitus	3 (2%)
Ischemic heart disease	0
Ictus	0
Tobacco exposure	0 cig: 143 (89%) 1-10 cig: 12 (8%) 10-20 cig: 4 (2.5%)
Asthma	15(9.4%)

Clinical manifestations	
Fever	No fever: 41 (25.8%) Low fever: 81 (56.9%) Moderate fever: 34 (21.4%) High fever: 3 (1.9%)
Maximum body temperature	38ºC SD=0.7ºC
Rhinitis	40 (25.2%)
Hyposmia/hypogeusia	111 (69.8%)
Throat pain	59 (36.9%)
Cough (dry/productive)	104 (65.4%)/ 12 (7.5%)
Headache	104 (65.4%)
Myalgia	107 (67.3%)
Dyspnea	60 (37.5%)
Tachycardia	28 (17.6%)
Asthenia	94 (59.1%)
Digestive manifestations (diarrhea, nausea or vomiting).	73 (45.9%)

Table 1. Demographic data, main comorbidities and clinical manifestations during the course of disease.

Median time to negativization was 25 days from symptom onset (interquartile range 23 to 27 days). The influence of several symptoms on the speed of negativization was analyzed using Kaplan Meier curves (Figure 2). The Breslow test was used to determine the influence of these symptoms. Negativization was slower in patients who manifested dry cough (p=0.001), dyspnea (p=0.020) or throat pain (p=0.016). Negativization was also slower in those who developed Ig G (p=0.010) (Table 2)

	Present	Absent	Breslow test
Dry cough	28 (22-36)	23 (17-28)	P=0.01
Dyspnea	28 (21-39)	24 (19-33)	P=0.02
Throat pain	29 (21-40)	24 (19-33)	P=0.016
Ig G	25 (20-34)	18 (15-26)	P=0.01

Table 2. Median, interquartile intervals and statistical signification (Breslow test) of those variables that influenced the speed of negativization using Kaplan-Meier curves. Time is expressed in days from disease onset.

Cox regression analysis was performed, introducing age, gender, BMI and the three respiratory symptoms that were associated with the speed of negativization. Using the backward approach, two variables remained in the model (Dry cough and dyspnea). The adjusted hazard ratios were 0.61 (0.42-0.88), p=0.008 for dry cough and 0.68 (0.48-0.96), p=0.027 for dyspnea, suggesting that the presence of one of these symptoms reduced the speed of negativization between 30% and 40%.(Table 3).

Factor	Hazard ratio (95% confidence interval)	p
Model (n=159)		
Dyspnea	0.677 (0.479-0.958)	0.027
Dry cough	0.606 (0.419-0.876)	0.008

Table 3. Cox regression model.

Discussion

RT-PCR, despite its limitations, is considered by most experts the gold standard for the diagnosis of COVID 19.(7) Managing healthcare workers represents additional challenges, because these workers may transmit the disease to vulnerable patients. Our current understanding of the viral kinetics in COVID-19 is still incomplete, but recent findings suggest that infectivity and transmissibility may be lower after the initial illness, even with a positive PCR.(8) Recently, some groups have suggested that return to work might be based on the evolution of cycle threshold and not in the dichotomized results of PCR. This, together with each center`s capacity for performing PCR, has led to significant heterogeneity in return to work protocols. During the initial weeks of the pandemic, with a more limited knowledge of the disease, our guidelines considered that workers should not go back to work until they are symptom-free and the virus is no longer detected by PCR testing in the upper respiratory tract, in accordance with the initial recommendation of the CDC.(9)The strain the pandemic is exerting on healthcare systems requires their staff to rejoin their units as soon as possible. Due to the possible limitations on the number of PCRs that can be performed, it would be very useful to know the most efficient time after diagnosis to schedule a PCR prior to their reincorporation. Furthermore, acquiring a greater knowledge of the impact of the pandemic in this population is important, because as Friese et al stated in a recent article: *The health and well-being of our healthcare workers determine our nation's health, security, and economic prosperity*.(10)

Most articles on COVID-19 and health workers have discussed how to prepare (11), protect(10) or to screen (12, 13) them. To the best of our knowledge this is the first study that uses survival analysis to study RT-PCR negativization. A better understanding of the temporal behavior of RT-PCR could make healthcare worker management more efficient.

We have observed that the disease appears abruptly and patients are able to pinpoint when they got sick. However, the cure is more gradual and in current practice patients undergo PCR when they feel better, not when they are completely cured. RT-PCR is usually performed when the patient`s respiratory symptoms have resolved. However we think that time intervals measured from symptom onset can be more precise, and that this information should be taken in consideration to decide when the control RT-PCR is scheduled.

Our workers needed a median time of 25 days from the onset of symptoms to negativizate RT-PCR. Gender, age and body mass index did not influence this process, despite their proven prognostic value. Neither did blood type.

Although RT-PCR should be planned depending on the clinical scenario, our results suggest that doing a RT-PCR before three weeks of symptom onset yields low effectiveness. In the presence of respiratory symptoms (dyspnea, dry cough and throat pain), this test should be delayed one more week. This information may also be useful to guide the most appropriate time to return to work in less favored settings where the use of PCR is limited.

A remarkable finding of our study is that the speed of negativization was slower in those who develop antibodies. The role of humoral response in the healing process has not been established yet, indeed a recent Cochrane review casts a lot of uncertainty on the utility of convalescent plasma.(14) It seems reasonable to infer that if humoral immunity were the main mechanism for virus elimination those who develop antibodies would have eliminated the virus faster. This finding should be interpreted with caution as the serology test we employed was a qualitative test. Those patients that harbor the virus for a longer time are more prone to develop antibodies.

Our work has some strengths and limitations. Survival analysis is the best way to estimate the expected duration of a biological or non-biological phenomenon. To the best of our knowledge this is the first study that has used survival analysis to evaluate PCR negativization in a group of healthcare workers. The main limitation is that information about the number of amplification cycles was not considered important at the time and thereby this information is not included in our analysis. As a recent publication has highlighted, future studies should also consider RT-PCR amplification cycle threshold , which may be better correlated with viral load.(15) Nevertheless, we detected some clinical variables that were correlated with longer times to negativization and may be considered in the future for the development of return to work protocols.

Other limitations are derived from the characteristics of our patients. Our sample is mainly composed of healthy, middle-age subjects, with a strong female predominance, and comorbidities were very uncommon. Thereby our sample is not ideal for evaluating the influence of comorbidities in this process. Not only young age and the unbalanced gender distribution are linked to a better prognosis. The mere fact that the studied subjects are workers makes the prognosis better, because workers represent the fittest part of a population. This bias, the so called healthy worker effect, is common to most cohort occupational studies.(16) Nevertheless, our results probably mirror accurately the composition of most healthcare systems, so our conclusions are applicable to most healthcare worker populations. Indeed a recent survey from the Center for Disease control (CDC) revealed a very similar distribution.(17)

A further limitation is that most of our patients were managed without chest radiography and laboratory tests, although mild patients are usually managed thus and most healthcare workers do not develop severe forms of COVID.

We conclude that time from disease onset can be an objective variable which may help to schedule control RT-PCT in infected healthcare workers. Performing this test before 25 days after disease onset yields low effectiveness. However, in those patients who have been free of respiratory symptoms the test might be scheduled sooner, while in those who have developed respiratory symptoms it should be delayed. In our cohort patients who developed antibodies were ill one week longer than those who did not develop antibodies.

Contributorship

Julio González-Martín-Moro planned, coordinated, participated in data collection, made the statistical analysis of the data, wrote de manuscript and submitted it.

Marta Chamorro Gómez, Galicia Davila Fernández, Ana Elices Apellaniz. Ana Fernández Hortelano, Elena Guzmán Almagro, Angela Herranz Varela, Carlos Izquierdo Rodríguez, Beatriz Molina Montes, Gema Vanessa Sánchez Moreno, participated in data collection and study design.

Alberto Mohedano Gómez and Inés Contreras, participated in the study design, and writing and correcting the manuscript.

Julio José González López participated in the statistical analysis of data and writing the manuscript.

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Competing interests

None of the authors have **conflict of interest**

Data Sharing/Data availability

Original data are available upon reasonable request.

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Figure 1. Recruitment algorithm

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Figure 2. Kaplan-Meier survival curves of the four variables that were associated with the speed of negativization (A. Development of IgG, B. Dry cough, C. Dyspnea, D. Throat pain)

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