Original research

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

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ABSTRACT

Objectives Reverse transcriptase PCR (RT-PCR) is considered the gold standard in diagnosing COVID-19. Infected healthcare workers do not 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 RT-PCR prior to healthcare workers' reincorporation.

Materials and methods This is a cohort study of healthcare workers with RT-PCR-confirmed COVID-19. Data were collected using the medical charts of healthcare workers and completed with a telephone interview. Kaplan-Meier curves were used to determine the influence of several variables on the time to RT-PCR negativisation. The impact of the variables on 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. The median time to negativisation was 25 days from symptom onset (IQR 20–35 days). Presence of IgG, dyspnoea, cough and throat pain were associated with significant longer time to negativisation. Cox logistic regression was used to adjust for confounding variables. Only dyspnoea and cough remained in the model as significant determinants of prolonged negativisation time. Adjusted HRs were 0.68 (0.48–096) for dyspnoea and 0.61 (0.42–0.88) for dry cough.

Conclusions RT-PCR during the first 3 weeks leads to a high percentage of positive results. In the presence of respiratory symptoms, negativisation took nearly 1 week more. Those who developed antibodies needed longer time to negativisate.

Key messages

What is already known about this subject?
- Health professionals should not return to work until they no longer transmit the disease.
- The most extended protocol requires health workers to be free of COVID-19 symptoms and to have at least one negative PCR test for SARS-CoV-2.

What are the new findings?
- In this cohort study of 159 healthcare workers using survival analysis, we defined the variables that influence PCR time to negativisation.
- The median time to negativisation was 25 days from symptom onset.
- Workers with dry cough and dyspnoea needed nearly 1 week more to obtain negative results.

How might this impact on policy or clinical practice in the foreseeable future?
- In future waves, this information could help to choose the most efficient time to perform PCR.

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 numbers 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 were infected. The occupational medicine department was overwhelmed and unable to cope with this severe and unexpected crisis, and a new specific task force composed of both physicians from different specialties and nurses was set up to help manage the pandemic. In April 2020 the Henares COVID-19 cohort healthcare 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 from the general population since they define their symptoms more precisely, 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 demonstrated a higher incidence of infection, thus making it easier to gather a significant sample.

In order to keep the workplace as safe as possible for both the staff and the patients, healthcare professionals should not return to work until they no longer transmit the disease.[6] Several protocols have been proposed. Our centre adhered to the protocol recommended by the Center for Disease Control (CDC) at that time, requiring at least two negative reverse transcriptase PCR (RT-PCR) tests 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 to work as soon as possible; however, especially if there is limited capacity for RT-PCR testing, it is necessary to optimise its performance. Therefore, the aim of this study was to estimate the most efficient timeframe to perform RT-PCR prior to return to work.

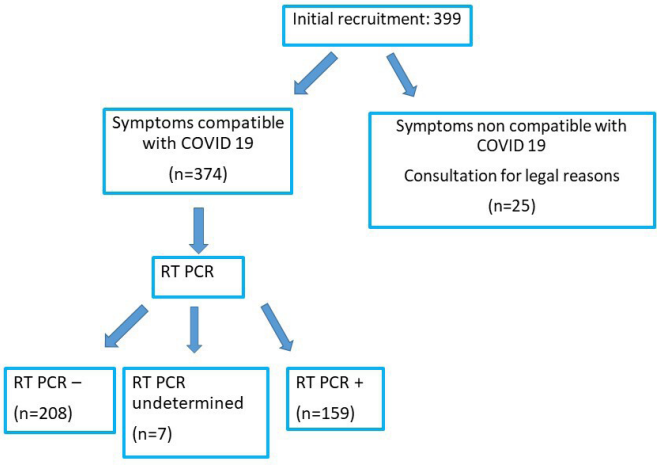

Figure 1 Recruitment algorithm. RT-PCR, reverse transcriptase PCR.

MATERIALS 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 healthcare workers who consulted with clinical manifestations compatible with COVID-19 (fever and/or pseudo-influenza syndrome and/or digestive symptoms and/or chemosensory disorders) at Hospital Universitario del Henares from 11 March 2020 to 31 April 2020. This centre 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 negativisation, which was calculated as the difference between the date of the first of the two consecutive negative RT-PCR tests and the date of patient-estimated symptom onset. Patients with at least 2 weeks of follow-up were included in the study.

The electronic chart of the workers was reviewed and data were 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. Patients were then followed in a prospective fashion until PCR negativisation took place, registering the results of each PCR. Data were analysed using SPSS V.15.0 software program. All workers expressed their consent signing an authorisation form or affirmatively replying to an email.

Demographic data including age, date of birth, gender, professional activity, height and weight at 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, ischaemic heart disease, stroke and lung disease (asthma or obstructive pulmonary disease). All these risk factors were collected as binary variables except tobacco exposure, which was stratified into 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 were also collected: fever, rhinorrhoea, throat pain, dry/productive cough, headache, myalgia, dyspnoea, tachycardia, hyposmia/hypogeusia, asthenia, digestive manifestations (diarrhoea, nausea or vomiting), conjunctivitis and dermatological manifestations. The maximum body temperature reached was also registered in patients who referred fever. When the patient referred dyspnoea, a chest radiography was performed. The results of the chest radiography and the most aggressive clinical management required (ambulatory, conventional hospitalisation or intensive care unit hospitalisation) as well as the date of PCR negativisation were also collected.

Statistical analysis was performed with SPSS V.15.0 software. Continuous variables were presented as mean±SD if normally distributed and as median and IQR if non-normally distributed. A two-tailed $p<0.05$ was considered statistically significant.

Bivariate analysis of time to RT-PCR negativisation was performed through the study of Kaplan-Meier curves and statistical significance was evaluated using the Breslow test. Demographic variables (age, gender and body mass index) 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

There were 399 healthcare workers attended for suspected COVID-19 between 11 March 2020 and 31 April 2020 (figure 1). In 374 cases, patients had clinical manifestations compatible with COVID-19, 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). The demographic characteristics are summarised in table 1. The 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% were physicians and 22.6% were health 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 of 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

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

Studied variables	N=159
Demographic variables	
Age, mean (SD)	41.3 (11.7)
Women, n (%)	126 (79.2)
Body mass index, mean (SD)	24.8 (4.7)
Comorbidities, n (%)	
High blood pressure	8 (5)
Diabetes mellitus	3 (2)
Ischaemic heart disease	0
Ictus	0
Tobacco exposure	0 cigarette: 143 (89)
	1–10 cigarettes: 12 (8)
	10–20 cigarettes: 4 (2.5)
Asthma	15 (9.4)
Clinical manifestations, n (%)	
Fever	No fever: 41 (25.8)
	Low fever: 81 (56.9)
	Moderate fever: 34 (21.4)
	High fever: 3 (1.9)
Maximum body temperature	38°C (SD=0.7°C)
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)
Dyspnoea	60 (37.5)
Tachycardia	28 (17.6)
Asthenia	94 (59.1)
Digestive manifestations (diarrhoea, nausea or vomiting)	73 (45.9)

for COVID-19. Only 11 patients were admitted to the hospital; 10 of them needed conventional hospitalisation and 1 needed intensive care hospitalisation.

Of the patients in the cohort, 74% reported hyperthermia, mostly low fever; only 24% of the patients reported a maximal temperature above 38.0°C. 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 were present in a similar number of subjects (table 1).

The median time to negativisation was 25 days from symptom onset (IQR 23–27 days). The influence of several symptoms on the speed of negativisation was analysed using Kaplan-Meier curves (figure 2). The Breslow test was used to determine the influence of these symptoms. Negativisation was slower in patients who manifested dry cough (p=0.001), dyspnoea (p=0.020) or throat pain (p=0.016). Negativisation was also slower in those who developed IgG (p=0.010) (table 2).

Cox regression analysis was performed, introducing age, gender, body mass index and the three respiratory symptoms that were associated with speed of negativisation. Using the backward approach, two variables remained in the model (dry cough and dyspnoea). The adjusted HRs were 0.61 (0.42–0.88; p=0.008) for dry cough and 0.68 (0.48–0.96; p=0.027) for dyspnoea, suggesting that the presence of one of these symptoms reduced the speed of negativisation between 30% and 40% (table 3).

DISCUSSION

RT-PCR, despite its limitations, is considered by most experts the gold standard for the diagnosis of COVID-19.[7] Managing

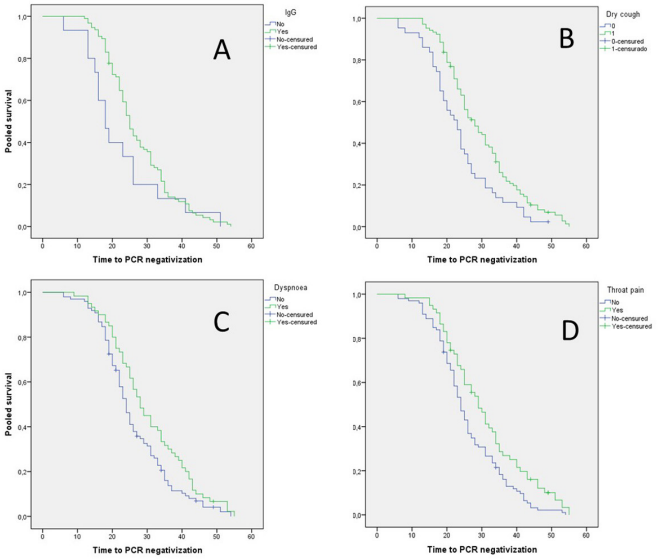

Figure 2 Kaplan-Meier survival curves of the four variables that were associated with speed of negativisation: (A) development of IgG, (B) dry cough, (C) dyspnoea and (D) throat pain.

healthcare workers represents additional challenges because these workers may transmit the disease to vulnerable patients. Our current understanding of the viral kinetics of 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 on the dichotomised results of PCR. This, together with each centre's capacity to perform 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 test 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 PCR prior to their reincorporation. Furthermore, acquiring a greater knowledge on the impact of the pandemic in this population is important because, as Friese *et al*[10] stated in a recent article, *the health and well-being of our healthcare workers determine our nation's health, security and economic prosperity*.

Most articles on COVID-19 and health workers have discussed how to prepare,[11] protect[10] or screen[12 13] them. To the best of our knowledge, this is the first study to use survival analysis to study RT-PCR negativisation. A better understanding of the

Table 2 Median, interquartile intervals and statistical significance (Breslow test) of variables that influenced the speed of negativisation, using Kaplan-Meier curves

	Present	Absent	Breslow test (p value)
Dry cough	28 (22–36)	23 (17–28)	0.01
Dyspnoea	28 (21–39)	24 (19–33)	0.02
Throat pain	29 (21–40)	24 (19–33)	0.016
IgG	25 (20–34)	18 (15–26)	0.01

Time is expressed in days from disease onset.

Table 3 Cox regression model

Factor	HR (95% CI)	P value
Model (N=159)		
Dyspnoea	0.677 (0.479 to 0.958)	0.027
Dry cough	0.606 (0.419 to 0.876)	0.008

temporal behaviour 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 into consideration when deciding when to schedule RT-PCR.

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

Although RT-PCR should be planned depending on the clinical scenario, our results suggest that performing RT-PCR within 3 weeks from symptom onset yields low effectiveness. In the presence of respiratory symptoms (dyspnoea, dry cough and throat pain), this test should be delayed one more week. This information may also be useful in guiding the most appropriate time to return to work in less favoured settings where the use of PCR is limited.

A remarkable finding of our study is that the speed of negativisation was slower in those who developed antibodies. The role of humoral response in the healing process has not yet been established; 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 developed antibodies would have eliminated the virus faster. This finding should be interpreted with caution as the serology test we employed was a qualitative test. Patients who harbour the virus for a longer time were 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 negativisation 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 was 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 negativisation and may be considered in the future in 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-aged 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, but 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 accurately mirror the composition of most healthcare systems, so our conclusions are applicable to most healthcare worker populations. Indeed a recent survey from the 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 this way, and most healthcare workers do not develop severe forms of COVID-19.

We conclude that time from disease onset can be an objective variable which may help to schedule RT-PCR in infected healthcare workers. Performing this test within 25 days from disease onset yields low effectiveness. However, in patients who have been free of respiratory symptoms, the test might be scheduled sooner, while it should be delayed in those who have developed respiratory symptoms. In our cohort, patients who developed antibodies were ill 1 week longer than those who did not develop antibodies.

Contributors JGM-M planned, coordinated, participated in data collection, performed statistical analysis of data, and wrote and submitted the manuscript. MCG, GDF, AEA, AFH, EGA, AHV, CIR, BMM and GVSM participated in data collection and study design. AMG and IC participated in study design, and in writing and correcting the manuscript. JJGL participated in the statistical analysis of data and in writing the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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