COVID-19 among healthcare workers in a Southern Brazilian Hospital and evaluation of a diagnostic strategy based on the RT-PCR test and retest for SARS-CoV-2

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Abstract. – **OBJECTIVE:** Healthcare workers are at risk for COVID-19 contamination. It is important to protect them in order to reduce nosocomial transmission and maintain the assistance capacity of health systems. To evaluate the diagnostic test and retest strategy with RT-PCR for SARS-CoV-2 and factors associated with the diagnosis of COVID-19 among healthcare workers.

PATIENTS AND METHODS: Cross-sectional study carried out in a Brazilian hospital. From April 27 to June 16, 2020, symptomatic healthcare workers underwent an RT-PCR test on upper respiratory tract specimens as soon as possible and, if negative, it was repeated close to the 5th day of symptom evolution. Working areas were divided into assistance areas dedicated or not dedicated to COVID-19 and non-assistance areas. The type of activity was divided into assistance or non-assistance activity.

RESULTS: 775 individuals were evaluated. 114 were diagnosed with COVID-19, of whom 101 followed the testing protocol. A second RT-PCR identified five (4.9%) of the positive cases. Working in an area dedicated to patients with COVID-19 was more prevalent among positive cases (35.1% x 19.8%, p=0.001) as well as working in an assistance activity (80.7% x 70.8%, p=0.031).

CONCLUSIONS: A second RT-PCR test after the 5th day of symptom evolution showed limited diagnostic improvement. The adoption of a single test-based strategy, carried out at the right time after the onset of symptoms, allows the optimal use of resources. Working in a COVID-19 dedicated area and in direct contact with patients is related to a higher prevalence of COVID-19 among symptomatic healthcare workers.

Key Words:
COVID-19, RT-PCR, Healthcare worker.

Introduction

After the outbreak of the COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), several strategies have been used to deal with it[1]. A common issue is the need to reduce the very rapid interpersonal transmission rate of the disease[2].

Healthcare workers are at special risk for contracting the virus[3-7]. Promoting the protection of this population is essential to reduce nosocomial transmission and maintain the assistance capacity of healthcare systems[8]. Preventive and controlling approaches involve the use of personal protective equipment (PPE)[9-12], early and accurate identification and isolation of individuals transmitting the virus[13-16], definition of specific action protocols, among others[17-19].

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Given the non-specificity of clinical manifestations, laboratory tests for viral identification are very important to accurately diagnose COVID-19[20,21]. The Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) for viral RNA identification in biological samples is considered the gold standard test. There are different protocols for this test, each of them with its technical specificities and diagnostic characteristics[22,23].

The use of RT-PCR in clinical practice should take into account the possibility that its diagnostic performance may be modified by factors, such as the origin of the biological sample and the duration of symptoms at the time it is collected. Material from the upper airways is most commonly used, especially in an outpatient setting, and nasopharyngeal samples may have a higher diagnostic value than those from the oropharynx[24-26]. Among healthcare workers with a short time of symptom evolution, there is a similarity in the diagnostic capacity of testing combined samples from the oropharynx and nasal cavities in relation to those from the nasopharynx alone[27]. Although there may be a greater diagnostic gain, sputum samples require an appropriate environment for their collection and are not usually performed[28-30]. As for the best time to perform the test, previous studies[31-33] indicate that positive results can be obtained already in the first days of symptoms and that the viral load in upper airway samples is higher in this period, especially in the first week.

Another very important issue regarding the RT-PCR test is the occurrence of an initial negative result in individuals with COVID-19 disease. Studies[34,35] have reported rates of up to 30%, which means that an initial negative result does not exclude the possibility of disease, especially in a suggestive clinical and epidemiological context. The sequential use of more than one RT-PCR test in a highly probable clinical setting, as a way to increase the diagnostic capacity of the test, has been described[36-38].

The present study evaluated the diagnostic strategy of testing and retesting RT-PCR in upper airway samples among healthcare workers with symptoms suggestive of COVID-19. In addition, the profile of symptoms in the clinical presentation was assessed as well as the distribution of the disease among professionals working in different sectors and activities.

Patients and Methods

This is a cross-sectional observational study conducted at the Hospital de Clínicas de Porto Alegre University Hospital. The institution is a reference center for the treatment of patients with COVID-19 in Rio Grande do Sul State, the Southernmost State of Brazil.

Among the measures to combat nosocomial transmission, the institution promoted actions, such as the mandatory use of PPE, use of remote working in some areas, organizational changes to avoid face-to-face meetings and agglomerations, allocation of patients with COVID-19 in specific areas and increase in the number of intensive care beds. Also, an outpatient clinic was implemented dedicated to the evaluation of healthcare workers suspected of having contracted COVID-19, according to symptoms or exposure history, regardless of the activity performed or working area.

From April 27 to June 16, 2020, this outpatient clinic performed RT-PCR testing in upper respiratory tract specimens for most individuals with symptoms potentially attributable to COVID-19. Symptoms that acted as an indication for testing were those present in flu-like conditions, such as fever, cough, sore throat, diffuse body pain, and also upper airway symptoms, such as coryza and nasal obstruction, gastrointestinal tract symptoms, such as diarrhea, abdominal distention, nausea and vomiting, systemic symptoms, such as body pain and fatigue, as well as changes in smell or taste perception, headache, chest pain, dyspnea, or any other symptom at the discretion of the attending physician. Subjects whose RT-PCR test was negative and collected before the fifth day of symptom evolution underwent a new test on the fifth day or as close as possible to that. The diagnosis of COVID-19 was made if the test was positive in a symptomatic subject. If a healthcare worker was diagnosed with COVID-19 at another institution, he or she were required to inform the Occupational Medicine Service team as soon as possible.

The RT-PCR test was performed according to the protocol recommended by the Center for Disease Control and Prevention (CDC 2020), using molecular detection with primer for two regions of the viral nucleocapsid gene (N1 and N2) and also for the P gene of human RNase. The samples were collected by an oropharyngeal swab and bilateral nasal mid-turbinate swab, stored both in the same sterile tube containing saline, which was stored in a refrigerator at a temperature between 4ºC and 6ºC and promptly transported for processing to a qualified institutional laboratory.

Working areas were divided into assistance areas exclusively dedicated to COVID-19 patients, assistance areas not exclusively dedicated to COVID-19 patients and non-assistance areas. Institutional practice determined that as soon as a patient was diagnosed with COVID-19, he or she was relocated to a COVID-19 unit. For analysis, professional activities were divided into assistance and non-assistance activities. Professionals classified in the assistance activity category were doctors, nurses, nursing technicians, social workers, nutritionists, physiotherapists, and others with direct and repetitive contact with patients. Professionals considered in the non-assistance activity category were receptionists, security guards, carpenters, nutrition assistants, cooks, and any other without direct and repetitive contact with patients.

Clinical, demographic and occupational data were obtained from electronic medical records. Data referring to initial clinical evaluations of healthcare workers from April 27 to June 16, 2020, were included, without restrictions as to the profession, area of activity, working area, age or comorbidity. In cases in which the same professional had been evaluated more than once due to different symptomatic episodes, data were included only from the episode with a positive RT-PCR test result, preferably, or from the first episode. Individuals who tested positive for SARS-CoV-2 in a period prior to analysis were excluded. Subjects who tested positive at another institution did not have their data included in the evaluation of the clinical presentation profile nor in the RT-PCR diagnostic features analysis.

Continuous variables were expressed as mean and standard deviation, or median and interquartile range, as appropriate, and compared using Student's *t*-test or Mann-Whitney test, according to the assumptions of each test. The normality of the quantitative variables was evaluated by inspection of the histogram and the Shapiro-Wilk test. Categorical variables were described in absolute frequency and percentages and compared using the chi-square test or Fisher's exact test, according to the assumptions of each test. A significance level of 5% was adopted and 95% confidence intervals with respect to point estimates were calculated. Considering the scope of the study, sample size calculations were not performed.

The study protocol was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre under number 3080132400005327 and financial support was provided by the Research Incentive Fund of Hospital de Clínicas de Porto Alegre (Brazil).

Results

In June 2020, the institution had 6267 active professionals, of whom 41 had already been diagnosed with COVID-19 before April 27. Among the professionals evaluated from April 27 to June 16, 775 were included in the study. Twenty-six initially considered for analysis were excluded: sixteen for having a positive RT-PCR result prior to the study period, one for having a positive result only at a third test, and nine with negative tests, six for having been tested while asymptomatic, two for having incomplete medical records (one for not filling out a specific questionnaire to assess exposure and occupational factors and one for error in the medical record), and one for undergoing the first assessment after a prolonged period of onset.

One hundred and fourteen new cases of COVID-19 were identified based on a positive RT-PCR test, resulting in a period prevalence among symptomatic healthcare workers of 14.7%. Of these, 13 underwent testing and a first clinical evaluation at another institution (Figure 1).

The demographic and occupational characteristics of the 775 individuals evaluated are shown in Table I. Comparison between individuals with a positive and negative RT-PCR test identified a lower average age among the former (39.7 x 42.5 years, difference of 2.9 years, 95% CI 0.9 to 4.8, p=0.004). There was no difference between groups regarding the frequency of female sex (78.1% x 79.7%, p=0.707) and age of 60 years or more (1.8% x 4.8%, p=0.149).

Most of the evaluated individuals worked in an assistance area not dedicated to COVID-19 (54.4% of RT-PCR positive individuals *vs.* 62.6% of RT-PCR negative, p=0.097). Healthcare workers with a positive RT-PCR test reported working in assistance activity more frequently than those with negative tests (80.7% *vs.* 70.8%, p=0.031), as well as working in an area dedicated to patients with COVID-19 (35.1% *vs.* 19.8%, p=0.001). The period prevalence of COVID-19 among evaluated individuals, according to working area, was 23.4% in areas dedicated to COVID-19, 13% in non-dedicated areas and 9.4% in non-assistance areas. The prevalence rate of positive RT-PCR among workers who reported working in areas dedicated to COVID-19 *vs.* not working in these areas was 1.90 (95% CI 1.35 to 2.69). Regarding the activity performed, 16.4% of the individuals who reported exercising assistance activity tested positive for SARS-CoV-2, while among the re-

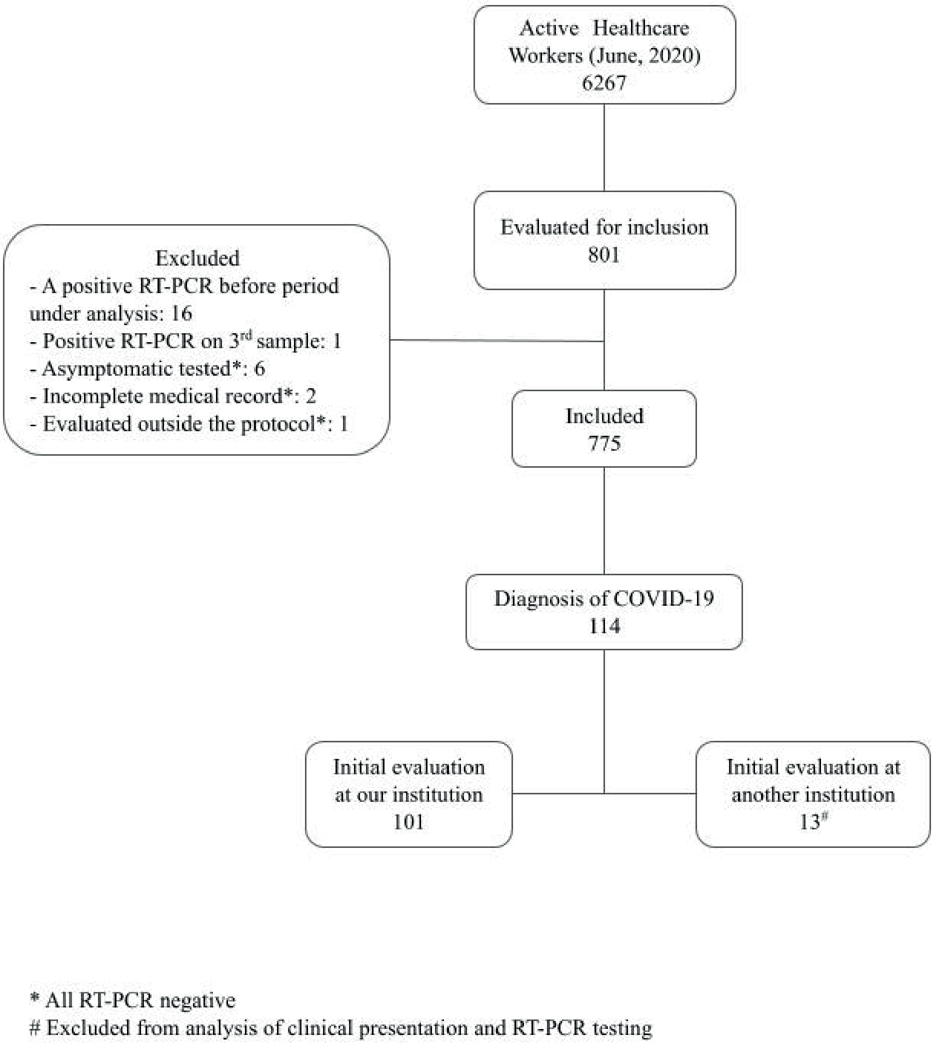

Figure 1. Flowchart of included and excluded patients.

maining the prevalence was 10.2%. The prevalence rate of positive RT-PCR tests among professionals who reported exercising assistance activity *vs.* those who denied this level of contact was 1.60 (95% CI 1.04 to 2.49).

Among the 762 healthcare workers who followed the diagnostic evaluation protocol with a test and retest RT-PCR, 101 were diagnosed with COVID-19 by one test and only 5 (4.9%) by a second test. There was no difference in the duration of symptoms by the time of the first test between individuals identified with a single or a second test (Table II).

The clinical presentation of these 762 individuals is described in Table III. The profile of symptoms was mild in most individuals with a positive test, with only 10.9% reporting some degree of dyspnea. Subjects diagnosed with COVID-19 presented a higher prevalence of fever (30.7% *vs.* 7.7%, p<0.001), altered perception of smell (22.8% *vs.* 3.6%, p<0.0001), altered perception of taste (22.8% *vs.* 6.1%, p<0.0001), headache (71.3% *vs.* 55.1%, p=0.002), body pain (58.4% *vs.* 29.3%, p<0.001), fatigue (33.7% *vs.* 20.3%, p=0.003) and nasal obstruction (26.7% *vs.* 13.5%, p=0.001), but less sore throat (44.6% *vs.* 58.9%, p=0.007). Symptoms of cough, coryza, chest pain, diarrhea or abdominal pain, nausea or vomiting and dyspnea showed no statistical difference between groups (Table III). Only 1.7% of the individuals did not present fever or any respiratory symptoms (cough, nasal obstruction, coryza, sore throat, dyspnea) at presentation.

Table I. Demographic and occupational characteristics of healthcare workers with and without a diagnosis of COVID-19, n (%).

Characteristics	Total (n = 775)	COVID-19 undiagnosed (n = 661)	COVID-19 diagnosed (n = 114)	*p**
Women	616 (79.5)	527 (79.7)	89 (78.1)	0.707
Age (years)#	42.1 (9.8)	42.5 (9.7)	39.7 (9.7)	0.004
≥60 years	34 (4.4)	32 (4.8)	2 (1.8)	0.149
Activity with a high degree of contact with patients	560 (72.3)	468 (70.8)	92 (80.7)	0.031
Works in assistance to COVID-19 area	171 (22.1)	131 (19.8)	40 (35.1)	0.001
Works in assistance area not dedicated to COVID-19	476 (61.4)	414 (62.6)	62 (54.4)	0.097
Works in non-assistance area	128 (16.5)	116 (17.5)	12 (10.5)	0.075

**p*-value for comparison between "diagnosed COVID-19" and "undiagnosed COVID-19" groups. #Average (SD).

Table II. Demographic characteristics of positive cases according to diagnosis at the 1st or 2nd RT-PCR collection (only individuals who performed both tests at the institution).

Characteristics	Positive in the 1st test (n = 96)	Positives in the 2nd test (n = 5)
Age (years)-Mean (SD)	39.8 (9.3)	33.2 (11.6)
Women n (%)	76 (79.2)	5 (100)
Time of symptom evolution in the 1st collection (days) – median (Q1-Q3)	2 (1-4)	2 (1-2)

Discussion

In our study, the strategy of sequential testing with RT-PCR in symptomatic healthcare workers showed low diagnostic improvement, identifying only 5 (4.9%) additional cases among 101 subjects diagnosed with COVID-19. A similar result was found at a health institution in the United Kingdom, where the second test detected only 4.2% of cases[39]. On the other hand, in a retrospective study with 610 patients hospitalized in a reference center for COVID-19 in Wuhan, China, who presented clinical and radiological pictures suggestive of the disease, 12.5% of the cases were diagnosed by retesting with RT-PCR in 1 to 2 days after the first test[40]. In another Chinese study, 23% of the subjects were diagnosed after the second test, which was done 1 to 2 days after the first[34]. The lower performance of the second test in our population may be due in part to the low prevalence of the disease in our region at the time and to the unrestricted testing strategy, in which professionals even with mild symptoms and without a clear exposure were tested[41].

The relatively low prevalence of the disease observed among symptomatic health professionals at our institution is close to that reported in some other countries[42]. Nevertheless, a health complex in the United Kingdom that adopted a restricted testing strategy, prioritizing individuals with a greater suspicion of carrying the virus, identified a prevalence of 43% among symptomatic healthcare workers[39].

The clinical profile at presentation indicated mild manifestations of the disease in most individuals with COVID-19, which may be related to the short time between the onset of symptoms and seeking medical care, as well as the low prevalence of people over 60 years of age, a group considered at risk for worse clinical outcomes. The symptoms presented in our population are similar to those reported in the literature, with a high prevalence of headache[43,44], cough, fever, and asthenia[45-47].

The high prevalence of changes in smell and taste among individuals with COVID-19 has been reported in different populations[47-49]. A study with healthcare workers in a French tertiary hospital identified a prevalence of 68% of anosmia and

Table III. Symptoms in the first clinical evaluation of healthcare workers with and without a diagnosis of COVID-19, n (%).

Symptoms	Total (n = 762)	COVID-19 undiagnosed (n = 661)	COVID-19 diagnosed (n = 101)	*p**
Fever	82 (10.8)	51 (7.7)	31 (30.7)	<0.001
Cough	351 (46.1)	302 (45.7)	49 (48.5)	0.668
Sore throat	434 (57.0)	389 (58.9)	45 (44.6)	0.007
Headache	436 (57.2)	364 (55.1)	72 (71.3)	0.002
Smell alteration	47 (6.2)	24 (3.6)	23 (22.8)	<0.001
Change of taste	63 (8.3)	40 (6.1)	23 (22.8)	<0.001
Coryza	362 (47.5)	315 (47.7)	47 (46.5)	0.915
Nasal obstruction	116 (15.2)	89 (13.5)	27 (26.7)	0.001
Diarrhea or bloating	95 (12.5)	87 (13.2)	8 (7.9)	0.149
Nausea or vomiting	64 (8.4)	55 (8.3)	9 (8.9)	0.847
Body pain	253 (33.2)	194 (29.3)	59 (58.4)	<0.001
Chest pain	30 (3.9)	29 (4.4)	1 (1)	0.163
Fatigue	168 (22.0)	134 (20.3)	34 (33.7)	0.003
Dyspnea	83 (10.9)	77 (11.6)	6 (5.9)	0.089

**p*-value for comparison between "diagnosed COVID-19" and "undiagnosed COVID-19" groups.

64% of ageusia[45]. At a Spanish center, healthcare workers diagnosed with the disease also had a high prevalence of changes in smell (68%) and taste (70%)[46]. An Italian study compared the presence of a change in smell between patients with COVID-19 and healthy individuals, with a higher risk of change found among diseased subjects[50]. Although the prevalence of changes in smell and taste among individuals diagnosed with COVID-19 in our study was lower than that reported by these other centers, it was significantly higher than in individuals without COVID-19. This is important because it reinforces the need to value these symptoms promptly in the initial diagnostic evaluation of suspected cases. A limitation of our work was the fact that the evaluation of changes in smell and taste was exclusively subjective, and not by means of objective tests that could allow grading the intensity of symptoms and identification of mild cases[51].

The higher prevalence of COVID-19 among professionals who perform activities that require direct contact with patients in our center is also in accordance with the results reported by other groups[14,45,52-53]. Regarding the sector of activity, we observed that areas dedicated to patients with COVID-19 had a higher prevalence of positive cases among symptomatic individuals. This result differs from that demonstrated in a French hospital, in which the highest prevalence of the disease was among healthcare workers working in areas not dedicated to COVID-19. In a European health complex with a high incidence of COVID-19 among healthcare workers, there was no difference in risk for positivity in the RT-PCR test in relation to the area of work or activity performed[39]. Also, a multicenter case-control study identified that working in an area dedicated to patients with COVID-19 (nursery or intensive care unit) meant a smaller chance of COVID-19 than working in regular wards but identified contact with patients with COVID-19 and with a sick co-worker as factors associated with illness among professionals[53]. This study also identified that the occurrence of exposure outside the work environment was significantly associated with illness among health professionals[54], however we were unable to measure this in our study.

Some of the limitations of our work are intrinsic to its design. The data used for analysis came from medical records and semi-structured forms designed for institutional risk management and medical assistance. Factors such as exposure in other workplaces or outside work could not be assessed.

Conclusions

Our study shows the low diagnostic gain with a testing strategy based on test and retest with RT-PCR in upper respiratory tract specimens among symptomatic healthcare workers from a tertiary Brazilian hospital. This information is extremely important in the context of a shortage of material for carrying out RT-PCR tests as currently needed. For this reason, our institution adopted a single test strategy, preferably performed from the 4th to the 5th days of symptom evolution.

Possibly, the best clinical scenario for sequential use of the RT-PCR test in healthcare workers is among individuals with a high pre-test probability for the disease. The observed difference of the initial symptoms presented by individuals with and without COVID-19, the type of activity performed, and area of work should be taken into account for an easier identification of those most likely to have the disease. These individuals should receive special attention from their health institutions in order to reduce the risk of contagion.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Interests

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References

1) Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. China Novel Coronavirus Investigating and Research Team. A novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382: 727-733.
2) Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020; 323: 1239-1242.
3) Iacobucci G. COVID-19: doctors sound alarm over hospital transmissions. BMJ 2020; 369: m2013.
4) Wang J, Zhou M, Liu F. Reasons for healthcare workers becoming infected with novel coronavirus disease 2019 (COVID-19) in China. J Hosp Infect 2020; 105: 100-101.
5) Lapolla P, Mingoli A, Lee R. Deaths from COVID-19 in healthcare workers in Italy-What can we learn? Infect Control Hosp Epidemiol 2020; 15: 1-2.
6) Lahner E, Dilaghi E, Prestigiacomo C, Alessio G, Marcellini L, Simmaco M, Santino I, Orsi GB, Anibaldi P, Marcolongo A, Annibale B, Napoli C. Prevalence of Sars-Cov-2 infection in Health Workers (HWs) and diagnostic test performance: the experience of a teaching hospital in central Italy. Int J Environ Res Public Health 2020; 17: 4417.
7) Nguyen LH, Drew DA, Graham MS, Joshi AD, Guo CG, Ma W, Mehta RS, Warner ET, Sikavi DR, Lo CH, Kwon S, Song M, Mucci LA, Stampfer MJ, Willett WC, Eliassen AH, Hart JE, Chavarro JE, Rich-Edwards JW, Davies R, Capdevila J, Lee KA, Lochlainn MN, Varsavsky T, Sudre CH, Cardoso MJ, Wolf J, Spector TD, Ourselin S, Steves CJ, Chan AT. Coronavirus Pandemic Epidemiology Consortium. Risk of COVID-19 among front-line health-care workers and the general community: a prospective cohort study. Lancet Public Health 2020; 5: e475-e483.
8) The Lancet. COVID-19: protecting health-care workers. Lancet 2020; 395: 922.
9) Verbeek JH, Rajamaki B, Ijaz S, Sauni R, Toomey E, Blackwood B, Tikka C, Ruotsalainen JH, Kilinc Balci FS. Personal protective equipment for preventing highly infectious diseases due to exposure to contaminated body fluids in healthcare staff. Cochrane Database Syst Rev 2020; 5: CD011621.
10) Khalil MM, Alam MM, Arefin MK, Chowdhury MR, Huq MR, Chowdhury JA, Khan AM. Role of Personal Protective Measures in Prevention of COVID-19 Spread Among Physicians in Bangladesh: a Multicenter Cross-Sectional Comparative Study. SN Compr Clin Med 2020; 1-7.
11) Liu M, Cheng SZ, Xu KW, Yang Y, Zhu QT, Zhang H, Yang DY, Cheng SY, Xiao H, Wang JW, Yao HR, Cong YT, Zhou YQ, Peng S, Kuang M, Hou FF, Cheng KK, Xiao HP. Use of personal protective equipment against Coronavirus disease 2019 by healthcare professionals in Wuhan, China: cross sectional study. BMJ 2020; 369: m2195.
12) Walker J, Fleece ME, Griffin RL, Leal SM, Alsip JA, Stigler WS, Nafziger SD, Marrazzo JM, Lee RA. Decreasing high risk exposures for healthcare-workers through universal masking and universal SARS-CoV-2 testing upon entry to a tertiary care facility. Clin Infect Dis 2020; ciaa1358.
13) Meredith LW, Hamilton WL, Warne B, Houldcroft CJ, Hosmillo M, Jahun AS, Curran MD, Parmar S, Caller LG, Caddy SL, Khokhar FA, Yakovleva A, Hall G, Feltwell T, Forrest S, Sridhar S, Weekes MP, Baker S, Brown N, Moore E, Popay A, Roddick I, Reacher M, Gouliouris T, Peacock SJ, Dougan G, Török ME, Goodfellow I. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. Lancet Infect Dis 2020; 20: 1263-1272.
14) Rivett L, Sridhar S, Sparkes D, Routledge M, Jones NK, Forrest S, Young J, Pereira-Dias J, Hamilton WL, Ferris M, Torok ME, Meredith L; CITIID-NIHR COVID-19 BioResource Collaboration; Gupta R, Lyons PA, Toshner M, Warne B, Bartholdson Scott J, Cormie C, Gill H, Kean I, Maes M, Reynolds N, Wantoch M, Caddy S, Caller L, Feltwell T, Hall G, Hosmillo M, Houldcroft C, Jahun A, Khokhar F, Yakovleva A, Butcher H, Caputo D, Clapham-Riley D, Dolling H, Furlong A, Graves B, Gresley EL, Kingston N, Papadia S, Stark H, Stirrups KE, Webster J, Calder J, Harris J, Hewitt S, Kennet J, Meadows A, Rastall R,

Brien CO, Price J, Publico C, Rowlands J, Ruffolo V, Tordesillas H, Brookes K, Canna L, Cruz I, Dempsey K, Elmer A, Escoffery N, Jones H, Ribeiro C, Saunders C, Wright A, Nyagumbo R, Roberts A, Bucke A, Hargreaves S, Johnson D, Narcorda A, Read D, Sparke C, Warboys L, Lagadu K, Mactavous L, Gould T, Raine T, Mather C, Ramenatte N, Vallier AL, Kasanicki M, Eames PJ, McNicholas C, Thake L, Bartholomew N, Brown N, Parmar S, Zhang H, Bowring A, Martell G, Quinnell N, Wright J, Murphy H, Dunmore BJ, Legchenko E, Gräf S, Huang C, Hodgson J, Hunter K, Martin J, Mescia F, O'Donnell C, Pointon L, Shih J, Sutcliffe R, Tilly T, Tong Z, Treacy C, Wood J, Bergamaschi L, Betancourt A, Bowyer G, De Sa A, Epping M, Hinch A, Huhn O, Jarvis I, Lewis D, Marsden J, McCallum S, Nice F, Curran MD, Fuller S, Chaudhry A, Shaw A, Samworth RJ, Bradley JR, Dougan G, Smith KG, Lehner PJ, Matheson NJ, Wright G, Goodfellow IG, Baker S, Weekes MP. Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. eLife 2020; 9: e58728.

15) Treibel TA, Manisty C, Burton M, McKnight Á, Lambourne J, Augusto JB, Couto-Parada X, Cutino-Moguel T, Noursadeghi M, Moon JC. COVID-19: PCR screening of asymptomatic health-care workers at London hospital. Lancet 2020; 395: 1608-1610.

16) Jones NK, Rivett L, Sparkes D, Forrest S, Sridhar S, Young J, Pereira-Dias J, Cormie C, Gill H, Reynolds N, Wantoch M, Routledge M, Warne B, Levy J, Córdova Jiménez WD, Samad FNB, McNicholas C, Ferris M, Gray J, Gill M; CITIID-NIHR COVID-19 BioResource Collaboration, Baker S, Bradley J, Dougan G, Goodfellow I, Gupta R, Lehner PJ, Lyons PA, Matheson NJ, Smith KG, Torok ME, Toshner M, Weekes MP, Jones NK, Rivett L, Routledge M, Sparkes D, Warne B, Cormie C, Forrest S, Gill H, Kean I, Pereira-Dias J, Reynolds N, Sridhar S, Wantoch M, Young J, Caddy S, Caller L, Feltwell T, Hall G, Hamilton W, Hosmillo M, Houldcroft C, Jahun A, Khokhar F, Meredith L, Yakovleva A, Butcher H, Caputo D, Clapham-Riley D, Dolling H, Furlong A, Graves B, Le Gresley E, Kingston N, Papadia S, Stark H, Stirrups KE, Webster J, Calder J, Harris J, Hewitt S, Kennet J, Meadows A, Rastall R, Brien CO, Price J, Publico C, Rowlands J, Ruffolo V, Tordesillas H, Gill M, Gray J, Hannon G, Brookes K, Canna L, Cruz I, Dempsey K, Elmer A, Escoffery N, Fuller S, Jones H, Ribeiro C, Saunders C, Wright A, Nyagumbo R, Roberts A, Bucke A, Hargreaves S, Johnson D, Narcorda A, Read D, Sparke C, Worboys L, Lagadu K, Mactavous L, Gould T, Raine T, Shaw A, Mather C, Ramenatte N, Vallier AL, Kasanicki M, Eames PJ, McNicholas C, Thake L, Bartholomew N, Brown N, Curran M, Parmar S, Zhang H, Bowring A, Ferris M, Martell G, Quinnell N, Wright G, Wright J, Murphy H, Dunmore BJ, Legchenko E, Gräf S, Huang C, Hodgson J, Hunter K, Martin J, Mescia F, ODonnell C, Pointon L, Shih J, Sutcliffe R, Tilly T, Tong Z, Treacy C, Wood J, Bergamaschi L, Betancourt A, Bowyer G, De Sa A, Epping M, Hinch A, Huhn O, Jarvis I, Lewis D, Marsden J, McCallum S, Nice F, Omarjee O, Perera M, Romashova N, Strezlecki M, Yarkoni NS, Turner L, Bailey B, Chaudhry A, Doughton R, Workman C, Trotter C, David W, Jiménez C, Levy J, Samad FN, Curran MD, Fuller S, Chaudhry A, Shaw A, Bradley JR, Hannon GJ, Goodfellow IG, Dougan G, Smith KG, Lehner PJ, Wright G, Matheson NJ, Baker S, Weekes MP. Effective control of SARS-CoV-2 transmission between healthcare workers during a period of diminished community prevalence of COVID-19. eLife 2020; 9: e59391.

17) Nagesh S, Chakraborty S. Saving the frontline health workforce amidst the COVID-19 crisis: challenges and recommendations. J Glob Health 2020; 10: 010345.

18) Zhang Z, Liu S, Xiang M, Li S, Zhao D, Huang C, Chen S. Protecting healthcare personnel from 2019-nCoV infection risks: lessons and suggestions. Front Med 2020; 14: 229-231.

19) Demartini K, Konzen VM, Siqueira MO, Garcia G, Jorge MSG, Batista JS, Wibelinger LM. Care for frontline health care workers in times of COVID-19. Rev Soc Bras Med Trop 2020; 53: e20200358.

20) Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for COVID-19. Clinical characteristics of Coronavirus disease 2019 in China. N Engl J Med 2020; 382: 1708-1720.

21) Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395: 497-506.

22) Nalla AK, Casto AM, Huang MW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. J Clin Microbiol 2020; 58: e00557-20.

23) Etievant S, Bal A, Escuret V, Brengel-Pesce K, Bouscambert M, Cheynet V, Generenaz L, Oriol G, Destras G, Billaud G, Josset L, Frobert E, Morfin F, Gaymard A. Performance assessment of SARS-CoV-2 PCR assays developed by WHO referral laboratories. J Clin Med 2020; 9: 1871.

24) Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L, Sun Z. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. Int J Infect Dis 2020; 94: 107-109.

25) Wang H, Liu Q, Hu J, Zhou M, Yu MQ, Li KY, Xu D, Xiao Y, Yang JY, Lu YJ, Wang F, Yin P, Xu SY.

Nasopharyngeal swabs are more sensitive than oropharyngeal swabs for COVID-19 diagnosis and monitoring the SARS-CoV-2 load. Front Med (Lausanne) 2020; 7: 334.

26) Patel MR, Carroll D, Ussery E, Whitham H, Elkins CA, Noble-Wang J, Rasheed JK, Lu X, Lindstrom S, Bowen V, Waller J, Armstrong G, Gerber S, Brooks JT. Performance of oropharyngeal swab testing compared to nasopharyngeal swab testing for diagnosis of COVID-19 – United States, January-February 2020. Clin Infect Dis 2020; 2020: ciaa759.

27) Vlek ALM, Wesselius TS, Achterberg R, Thijsen SFT. Combined throat/nasal swab sampling for SARS-CoV-2 is equivalent to nasopharyngeal sampling. Eur J Clin Microbiol Infect Dis 2020; 40: 193-195.

28) Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). Clin Chem Lab Med 2020; 58: 1089-1094.

29) Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020; 323: 1843-1844.

30) Han H, Luo Q, Mo F, Long L, Zheng W. SARS-CoV-2 RNA more readily detected in induced sputum than in throat swabs of convalescent COVID-19 patients. Lancet Infect Dis 2020; 20: 655-656.

31) To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020; 20: 565-574.

32) Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020; 382: 1177-1179.

33) Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020; 323: 2249-2251.

34) Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, Ji W. Sensitivity of chest CT for COVID-19: comparison to RT-PCR. Radiology 2020; 296: E115-E117.

35) Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Del Campo R, Ciapponi A, Sued O, Martinez-García L, Rutjes AW, Low N, Bossuyt PM, Perez-Molina JA, Zamora J. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. PLoS One 2020; 15: e0242958.

36) Huang P, Liu T, Huang L, Liu H, Lei M, Xu W, Hu X, Chen J, Liu B. Use of chest CT in combination with negative RT-PCR assay for the 2019 novel Coronavirus but high clinical suspicion. Radiology 2020; 295: 22-23.

37) Fisher B, Seese L, Sultan I, Kilic A. The importance of repeat testing in detecting coronavirus disease 2019 (COVID-19) in a coronary artery bypass grafting patient. J Card Surg 2020; 35: 1342-1344.

38) Valan AB, Sture C. Negative nasopharyngeal swabs early in the course of COVID-19. Tidsskr Nor Laegeforen 2020; 140.

39) Leeds JS, Raviprakash V, Jacques T, Scanlon N, Cundall J, Leeds CM. Risk factors for detection of SARS-CoV-2 in healthcare workers during April 2020 in a UK hospital testing programme. EClinicalMedicine 2020; 26: 100513.

40) Li Y, Yao L, Li J, Chen L, Song Y, Cai Z, Yang C. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. J Med Virol 2020; 92: 903-908.

41) Hallal PC, Hartwig FP, Horta BL, Silveira MF, Struchiner CJ, Vidaletti LP, Neumann NA, Pellanda LC, Dellagostin OA, Burattini MN, Victora GD, Menezes AMB, Barros FC, Barros AJD, Victora CG. SARS-CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys. Lancet Glob Health 2020; 8: e1390-e1398.

42) Gómez-Ochoa SA, Franco OH, Rojas LZ, Raguindin PF, Roa-Díaz ZM, Wyssmann BM, Guevara SLR, Echeverría LE, Glisic M, Muka T. COVID-19 in healthcare workers: a living systematic review and meta-analysis of prevalence, risk factors, clinical characteristics, and outcomes. Am J Epidemiol 2020; 186: 227-236.

43) Poncet-Megemont L, Paris P, Tronchere A, Salazard JP, Pereira B, Dallel R, Aumeran C, Beytout J, Jacomet C, Laurichesse H, Lesens O, Mrozek N, Vidal M, Moisset X. High prevalence of headaches during COVID-19 infection: a retrospective cohort study. Headache 2020; 60: 2578-2582.

44) Lechien JR, Chiesa-Estomba CM, Place S, Van Laethem Y, Cabaraux P, Mat Q, Huet K, Plzak J, Horoi M, Hans S, Rosaria Barillari M, Cammaroto G, Fakhry N, Martiny D, Ayad T, Jouffe L, Hopkins C, Saussez S; COVID-19 Task Force of YO-IFOS. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. J Intern Med 2020; 288: 335-344.

45) Contejean A, Leporrier J, Canouï E, Alby-Laurent F, Lafont E, Beaudeau L, Parize P, Lecieux F, Greffet A, Chéron G, Gauzit R, Fourgeaud J, L’Honneur AS, Tréluyer JM, Charlier C, Casetta A, Frange P, Leruez-Ville M, Rozenberg F, Lortholary O, Kernéis S. Comparing dynamics and determinants of SARS-CoV-2 transmissions among health care workers of adult and pediatric settings in central Paris. Clin Infect Dis 2020; ciaa977. doi: 10.1093/cid/ciaa977. Online ahead of print.

46) Villarreal IM, Morato M, Martínez-RuizCoello M, Navarro A, Garcia-Chillerón R, Ruiz Á, de Almeida IV, Mazón L, Plaza G. Olfactory and taste disorders in healthcare workers with COVID-19 infection. Eur Arch Otorhinolaryngol 2020; 1-5. doi: 10.1007/s00405-020-06237-8. Online ahead of print.

47) Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, Rodriguez A, Dequanter D, Blecic S, El Afia F, Distinguin L, Chekkoury-Idrissi Y, Hans S, Delgado IL, Calvo-Henriquez C, Lavigne P, Falanga C, Barillari MR, Cammaroto G, Khalife M, Leich P, Souchay C, Rossi C, Journe F, Hsieh J, Edjlali M, Carlier R, Ris L, Lovato A, De Filippis C, Coppee F, Fakhry N, Ayad T, Saussez S. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. Eur Arch Otorhinolaryngol 2020; 277: 2251-2261.

48) Chiesa-Estomba CM, Lechien JR, Radulesco T, Michel J, Sowerby LJ, Hopkins C, Saussez S. Patterns of smell recovery in 751 patients affected by the COVID-19 outbreak. Eur J Neurol 2020; 27: 2318-2321.

49) Haehner A, Draf J, Dräger S, de With K, Hummel T. Predictive value of sudden olfactory loss in the diagnosis of COVID-19. ORL J Otorhinolaryngol Relat Spec 2020; 82: 175-180.

50) D'Ascanio L, Pandolfini M, Cingolani C, Latini G, Gradoni P, Capalbo M, Frausini G, Maranzano M, Brenner MJ, Di Stadio A. Olfactory dysfunction in COVID-19 patients: prevalence and prognosis for recovering sense of smell. Otolaryngol Head Neck Surg 2020; 164: 82-86.

51) Moein ST, Hashemian SM, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: a biomarker for COVID-19. Int Forum Allergy Rhinol 2020; 10: 944-950.

52) Misra-Hebert AD, Jehi L, Ji X, Nowacki AS, Gordon S, Terpeluk P, Chung MK, Mehra R, Dell KM, Pennell N, Hamilton A, Milinovich A, Kattan MW, Young JB. Impact of the COVID-19 pandemic on healthcare workers risk of infection and outcomes in a large, integrated health system. J Gen Intern Med 2020; 35: 3293-3301.

53) Chow EJ, Schwartz NG, Tobolowsky FA, Zacks RLT, Huntington-Frazier M, Reddy SC, Rao AK. Symptom screening at illness onset of health care personnel with SARS-CoV-2 infection in King County, Washington. JAMA 2020; 323: 2087-2089.

54) Lentz RJ, Colt H, Chen H, Cordovilla R, Popevic S, Tahura S, Candoli P, Tomassetti S, Meachery GJ, Cohen BP, Harris BD, Talbot TR, Maldonado F. Assessing coronavirus disease 2019 (COVID-19) transmission to healthcare personnel: the global ACT-HCP case-control study. Infect Control Hosp Epidemiol 2020; 1-7. doi: 10.1017/ice.2020.455. Online ahead of print.