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Genetic Load of SARS-CoV-2 in Aerosols Collected in Operating Theaters

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ABSTRACT After the outbreak of COVID-19, additional protocols have been established to prevent the transmission of the SARS-CoV-2 from the patient to the health personnel and vice versa in health care settings. However, in the case of emergency surgeries, it is not always possible to ensure that the patient is not infected with SARS-CoV-2, assuming a potential source of transmission of the virus to health personnel. This work aimed to evaluate the presence of the SARS-CoV-2 and quantify the viral load in indoor air samples collected inside operating rooms, where emergency and scheduled operations take place. Samples were collected for 3 weeks inside two operating rooms for 24 h at 38 L/min in quartz filters. RNA was extracted from the filters and analyzed using RT-qPCR targeting SARS-CoV-2 genes E, N1 and N2 regions. SARS-CoV-2 RNA was detected in 11.3% of aerosol samples collected in operating rooms, despite with low concentrations (not detected at 13.5 cg/m^3 and 10.5 cg/m^3 in the scheduled and emergency operating rooms, respectively). Potential sources of airborne SARS-CoV-2 could be aerosolization of the virus during aerosol-generating procedures and in open surgery from patients that might have been recently infected with the virus, despite presenting a negative COVID-19 test. Another source could be related to health care workers unknowingly infected with the virus and exhaling SARS-CoV-2 virions into the air. These results highlight the importance of reinforcing preventive measures against COVID-19 in operating rooms, such as the correct use of protective equipment, screening programs for health care workers, and information campaigns.

IMPORTANCE Operating rooms are critical environments in which asepsis must be ensured. The COVID-19 pandemic entailed the implementation of additional preventative measures in health care settings, including operating theaters. Although one of the measures is to operate only COVID-19 free patients, this measure cannot be always implemented, especially in emergency interventions. Therefore, a surveillance campaign was conducted during 3 weeks in two operating rooms to assess the level of SARS-CoV-2 genetic material detected in operating theaters with the aim to assess the risk of COVID-19 transmission during operating procedures. SARS-CoV-2 genetic material was detected in 11% of aerosol samples collected in operating rooms, despite with low concentrations. Plausible SARS-CoV-2 sources have been discussed, including patients and health care personnel infected with the virus. These results highlight the importance of reinforcing preventive measures against COVID-19 in operating rooms, such as the correct use of protective equipment, screening programs for health care workers and information campaigns.

KEYWORDS SARS-CoV-2, coronavirus, virus, aerosol, operating theaters, healthcare, COVID-19

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SARS-CoV-2, a new strain of coronavirus recently identified in humans and the causal agent of COVID-19, has been responsible for more than 518 million cases of disease and about 6.25 million deaths in the world from 31 December 2019 to date (10 May 2022) (1). In Spain, the number of people affected amounts to 12 million cases with 104,883 deaths (2).

The first days after the contagion and incubation of the SARS-CoV-2, there is a period in which those infected with the virus are highly infectious and exhale viral particles into the ambient air. This facilitates the transmission of the virus through droplets exhaled directly by an infected person, which are eventually deposited onto surfaces (1). Therefore, inhalation of virus laden aerosols, especially indoors (2, 3), and contact with contaminated surfaces are the main routes of transmission (4, 5). In fact, genetic traces of SARS-CoV-2 have been detected in air filters collected in hospital environments (6–10) and not only in spaces with COVID-19 patients (7, 11). In fact, higher levels of SARS-CoV-2 genetic material have been detected in hospital corridors than in rooms of infected patients, which has been attributed to the ventilation systems (12). Particularly in the University General Hospital of Castellón, the presence of SARS-CoV-2 has been detected in two laparoscopy filters from operating rooms with a prevalence of 1.5% (13).

Operating rooms are critical environments in which asepsis must be ensured. To do this, hospitals follow rigorous cleaning protocols in spaces, surfaces, and health personnel, to avoid the introduction of pathogenic microorganisms that can lead to an infection in the patient. After the outbreak of COVID-19, additional protocols have been established to prevent the transmission of the SARS-CoV-2 from the patient to the health personnel and vice versa. However, in the case of emergency surgeries, it is not always possible to ensure that the patient is not infected with SARS-CoV-2, assuming a potential source of transmission of the virus to health personnel. In addition, several surgical practices lead to aerosolization of particles, which in infected patients, might be a source of SARS-CoV-2 spreading in operating theaters (14). Furthermore, although vaccines are effective in reducing COVID-19 severe health effects, they cannot avoid transmission of the virus (15), and vaccinated people can still be transmitters of the virus (16), even though they expire a much lower viral load than unvaccinated people (17). Moreover, the effectiveness of vaccines against new strains (18, 19) and the duration of immunity they provide are unknown (20). Given these considerations, COVID-19 infections could occur in operating rooms. Therefore, it is essential to evaluate the presence of genetic material of SARS-CoV-2 in the air of operating rooms to assess the possibility of transmission of COVID-19 in these critical health care settings.

The objective of this work is to evaluate the presence of the SARS-CoV-2 and quantify the viral load in indoor air samples collected inside operating rooms, where emergency and scheduled operations take place. In addition, the presence of SARS-CoV-2 RNA and viral load was investigated in laparoscopic filters used in surgery procedures performed in days where air samples from operation rooms tested positive for SARS-CoV-2. Finally, a comparative analysis of the presence and genetic load in aerosols collected in environmental sample filters and in laparoscopy filters was conducted.

RESULTS AND DISCUSSION

In the present study, samples were taken for 3 weeks in two operating rooms of the University General Hospital of Castellón. This is the reference hospital of the area serving a population of circa to six hundred thousand inhabitants.

Among the 44 air samples taken in the two operating rooms (22 samples in each one), genetic material of SARS-CoV-2 was detected in 5 filters, which represents 11.4% of the samples collected (Table 1). On the contrary, the presence of SARS-CoV-2 genetic material was not detected in any of the three laparoscopic filters available from the surgery procedures conducted on the days where the air samples tested positive for SARS-CoV-2. The internal standard mengovirus was recovered in all samples

TABLE 1 SARS-CoV-2 genetic material (gc/m^3) detected in air filters from operating rooms

Sampling point	% samples with detection	N1 (gc/m^3)	N2 (gc/m^3)	E (gc/m^3)
Emergency operating room	9.09	N.D.[a]	N.D.	N.D. – 10.5 (5.7 ± 6.8)[b]
Scheduled operating room	13.63	N.D. – 5.4	N.D. – 1.9	N.D. – 13.5

[a]N.D., not detected.
[b]Mean ± standard deviation of those samples where the gene was detected.

(50.8% ± 22.4%). In addition, the positive control was detected in all batches, and null detection was recorded in all negative controls.

The viral concentrations observed in the operating rooms in this study are shown in Table 1. The target gene more detected in our samples was gene E, which also reported the highest concentrations. On the other hand, N1 and N2 were detected in only one of the samples.

Our findings are similar to those reported in an emergency department, an intensive care unit, a COVID-19 and a non-COVID-19 ward in a hospital in a Boston, Massachusetts, which is consistent with the type of health care space (8). On the other hand, the levels reported in our study are two to five logs lower than those measured in hospitals with COVID-19 patients in several studies (6, 9, 10, 12, 21–24). Levels of SARS-CoV-2 genetic material in this study are also two to three logs lower than concentrations emitted by infected patients while breathing, talking or singing (25). Therefore, while concentrations are detected in the monitored operating rooms, the levels are considerably lower than those reported in hospital settings in contact with diagnosed COVID-19 patients elsewhere. The low concentrations analyzed in the present study could be related to the number of people infected in the operating theater, which could be present in the room for a limited period of time during the surgical procedure, in contrast with the rooms analyzed elsewhere occupied constantly with COVID-19 patients. It might be also related to the variability of virus-laden aerosol exhalation rate, which would change between infected persons (26), as well as at different time points during the infection (27). It could also be related to variability in methodological aspects to capture the aerosols and subsequent analysis such as the techniques used for RNA extraction.

In relation to the filters derived from laparoscopy, although previous studies carried out with the same methodology had led to detection (13), no genetic material could be detected in any of the laparoscopic filters available from the dates that positive air samples were detected. Therefore, it was not possible to compare the results obtained through the different types of filters. However, it is important to take into account the low number of laparoscopic filters that were available. A total of 75 operations were carried out in the emergency operating room during the sampling period. Of these, 19% were laparoscopies, 17% open surgeries, and 48% were classified as other operations. In the scheduled operating room, 23 operations were performed, from which 4% were laparoscopic operations, 44% were open surgery, 17% involved a combination of laparoscopy and open surgery and 35% were defined as other operations (Tables S1 and S2 in the supplementary material).

According to hospital records, 12 patients in the emergency operating room had a positive diagnosis of SARS-CoV-2 infection in the past, either with a positive PCR result ($n = 8$) or a positive antibodies test ($n = 4$). None of the patients in the scheduled operating room had evidence of having been previously infected with SARS-CoV-2 through a COVID-19 test. Moreover, none of the patients operated on the days with positive detection of SARS-CoV-2 in air samples had been previously diagnosed with COVID-19. In addition, none of the patients who were operated presented a positive PCR test 24 h prior to the operation. Only one patient had a positive IgG and IgM result on the same day of the operation in the emergency operating room, with the IgM result indicating a recent infection with the virus or a recent vaccination.

TABLE 2 Number of interventions and type of intervention conducted in the scheduled and adjacent operating theatres on the dates (lag 0 and 1) of SARS-CoV-2 detection

	Scheduled monitored operation room		Adjacent operating room	
Date of SARS-CoV-2 RNA detection	No. of interventions (and type) on date detected (lag0)	No. of interventions (and type) on previous date (lag1)	No. of interventions (and type) on date detected (lag0)	No. of interventions (and type) on previous date (lag1)
8 July 2021	5 (others)[a]	1 (open surgery)[b]	4 (open surgery)	3 (others)
16 July 2021	0	2 (open surgery)	0	0
20 July 2021	0	1 (open surgery)	1 (laparoscopy)[c]	0

[a]Others refers to surgery not requiring opening the abdominal cavity.
[b]Open surgery refers to surgery requiring opening the abdominal cavity.
[c]Laparoscopy refers to surgery conducted with laparoscopic methods.

Despite the clinical evidence available of the COVID-19 diagnosis of the patients, in addition to the tests conducted prior to the operation, it cannot be excluded the hypothesis that the source of the viral load detected in the air might not be attributed to the patients. On the one hand, the fact of not having a diagnosis of COVID-19 might not rule out having had the disease in the past as some patients who might have had COVID-19 during the first wave, where detection resources were limited, might have not received a diagnosis. Moreover, if the patient was asymptomatic during the infection, a diagnosis might have not been received due to the unawareness of it. Likewise, a COVID-19 test of the patients might be negative if the test is conducted early on the incubation period, or at the end of the illness, where the viral load is low and might be non-detectable, especially in the case of the antigen tests (28, 29). The fact that the patients might have been infected previously with the virus, but are unaware, hence not recorded could be relevant. This is because previous studies have identified RNA and proteins of SARS-CoV-2 in multiple organs beyond the respiratory system, including blood, heart, vessels, intestines, brain, male genitals, and kidneys (30–33). Nonetheless, some other studies have not detected viral load in blood samples (34). In addition, it is known that the excretion of virus by feces occurs (35, 36), even after the oropharyngeal samples become negative (37). Therefore, it cannot be discarded the presence of SARS-CoV-2 in those patients that tested negative on an oropharyngeal test or have had no record of the illness. Because the virus might be embedded in human tissue of infected patients (32, 33) and there is likelihood of it being aerosolized during the surgical procedure, the opening of the abdominal cavity of asymptomatic patients with a previous negative PCR test might be a plausible source of the detected levels. Laparoscopy surgery could be another possibility of transmission of the virus to the indoor air, although considered very unlikely (13, 38, 39). Likewise, aerosol-generating procedures, such as induction of anesthesia and intubation (40) might be another possible source of SARS-CoV-2 RNA airborne.

On the other hand, on the days where SARS-CoV-2 RNA was detected in aerosol samples, no operation of this type (i.e., open surgery) was carried out in neither of the two operating rooms monitored (Table 2 and Table 3). It is worth noting that in 4 out of 5 days where viral load was detected, open surgery was conducted the previous

TABLE 3 Number of interventions and type of intervention conducted in the emergency and adjacent operating theaters on the dates (lag 0 and 1) of SARS-CoV-2 detection

	Emergency monitored operation room		Adjacent operating room	
Date of SARS-CoV-2 RNA detection	No. of interventions (and type) on date detected (lag0)	No. of interventions (and type) on previous date (lag1)	No. of interventions (and type) on date detected (lag0)	No. of interventions (and type) on previous date (lag1)
8 July	4 (3 others[a]; 1 laparoscopy[b])	4 (3 others[c]; 1 open surgery)	1 (others)	1 (others)
15 July	3 (2 others; 1 laparoscopy)	4 (2 others; 2 laparoscopy)	2 (laparoscopy; others)	2 (laparoscopy)

[a]Others refers to surgery not requiring opening the abdominal cavity.
[b]Laparoscopy refers to surgery conducted with laparoscopic methods.
[c]Open surgery refers to surgery requiring opening the abdominal cavity.

day. However, it seems unlikely that the detection of the virus genetic material could be attributable to open surgery procedures conducted the previous day. Firstly, it was not detected on the filters collected representative of the previous 24 h. Secondly, the strict ventilation protocols applicable to the operating theaters would ensure the removal of any pathogen released on the previous day.

The possibility of adjacent operating theaters being the source of SARS-CoV-2 RNA airborne was also investigated. Information on the number and type of surgical procedures conducted in adjacent operating rooms was gathered, alongside the COVID-19 status of the patients that were intervened in those rooms (Table 2 and Table 3). Abdominal operations were performed in adjacent operating rooms on 2 days out of 5 where SARS-CoV-2 RNA was detected in aerosol samples collected in the two monitoring operating rooms. Particularly, one of those patients had been previously infected by SARS-CoV-2, but the diagnosis was 10 months before the intervention. The scheduled operating room and the adjacent operating room share some parts of the ventilation system. These are the ducts that connect the outside air intake. This ventilation system contains a F7 grade filter, the treatment unit, and the outward duct from the treatment unit, which contains a F9 grade filter after the unit and at the beginning of the discharge duct. Afterwards, the discharge duct splits in two, and each operating room contains its own supply terminal unit fitted with a H13 HEPA filter. Although a 50% recirculation of the air is allowed, the recommendation at the hospital is not to recirculate and to source all the air in the operating rooms coming from outside. If some of the air originating from the adjacent room was recirculated, and the two filters after the treatment unit (F9 and H13) were inefficient, the open surgery conducted in the adjacent operating room could be a source of the viral load into the monitored operating theater. However, this instance would be very unlikely for the following reasons. Firstly, it would explain only one case but not the others. Secondly, there is one date where no interventions were performed in any of the two operating rooms. Thirdly, none of the patients operated with open surgery tested COVID-19 positive on the date of the intervention. Fourthly, in the event of recirculation, the air goes through two highly efficient filters. Finally, the hospital recommendation is not to recirculate the air, and the ventilation system of the hospital follows a strict maintenance program.

Other source of SARS-CoV-2 virions airborne could be related to the health personnel, either those in charge of the operations, supporting health care staff, or the personnel who perform cleaning or maintenance tasks on the selected operating rooms. An incorrect use of masks (41, 42) by an infected worker could have led to detection of the SARS-CoV-2 RNA in the filters. Alternatively, the relaxation of preventive measures, when there were no operations in progress, might be another source of the viral RNA detected in the operating theatres. This would be consistent with detecting SARS-CoV-2 RNA on 2 days were no operations were performed in the scheduled operating room, one of them on a Sunday, where the scheduled (but not the emergency) activity in the operating theaters is nil. Although the hospital recommendation is to maintain the operating theaters hermetically closed even outside of the operating periods, the monitored scheduled operating theater might be incorrectly used as a shortcut to arrive to the emergency room, when not in use, especially in the evenings and weekends, where less personnel is on the premises. The frequency of passage through the scheduled operating room, as a shortcut, on days not in use for interventions would be consistent with detecting SARS-CoV-2 on the samples collected on dates where no operations took place, if health care personnel on duty was asymptomatically infected with COVID-19. Although a meta-analysis reported that approximately 40% of the population infected with COVID-19, where asymptomatic (43), vaccine trials suggest that two doses of COVID-19 vaccines provide at least 90% efficacy against symptomatic disease (44, 45). In addition, previous studies have reported that approximately 2% of health care workers vaccinated were identified to be asymptomatic via routine universal surveillance testing (46). Likewise, COVID-19 outbreaks among vaccinated health

care workers have been reported (47, 48). The sampling period was concurrent to the fifth wave of COVID-19 in Spain, and the accumulated incidence (PCR+ in the previous 14 days) in the Department of Health of Castellón was in the range of 225.33–617.62 per 100,000 population. It is, thus, likely that some health care personnel would have been infected with the virus, despite being vaccinated. At the time of the study, most of the health care workers in the operating theaterstheaters had received the full course of vaccination, which would be consistent with being asymptomatic if infected. In the University General Hospital of Castellón, approximately 180 health care workers work each day in the operating theaters. Therefore, according to Novazzi and colleagues (46), around four of those workers could be potentially infected with the virus and be asymptomatic at the time of the sampling.

The source of airborne virions attributed to health care personnel is also consistent with the fact that detection occurred in the two operating rooms on the same day (8 July), which in addition was the date that the samplers were installed and up to eight health staff members were instructed on the operation of the samplers. The other dates where viral RNA was detected on the filters are close in time (15, 16, and 20 July), which suggests the possibility of asymptomatic health care worker(s) exhaling the virus inadvertently into the operating theaters in the course of their professional duties.

The fact that 2 of the 5 days where SARS-CoV-2 RNA was detected in aerosol samples were collected on days where no operation was performed, lead to interrogating the ventilation system of the monitored operating rooms. These health care environments require very high ventilation standards in the air supply and the local microenvironment to ensure sufficient fresh air, whereas limiting the presence of pathogens in the air and surfaces (operating area and instrumental stations), minimizing the concentration of anesthetic gases and other toxic substances, and maintaining comfortable environmental conditions. The ventilation system of operating theaters is regulated by national legislation (four royal decrees), seven national standards, and one international standard (Supplementary Material). The supplied air into the operating theaters requires a minimum airflow of 2,400 m^3/h when equipped with an air-mix diffusion system (with a minimum of 20 movements/h), and it is triple filtered, as indicated above. Up to 50% recirculation would be possible, but the recommendation in the hospital is not to do so. Though positive pressure must be guaranteed at all times, a reduced service mode of the ventilation system is activated when the operating theaters are not in use. In this mode, the flow of air supplied and extracted in the operating room is reduced to achieve energy savings, so comfort conditions are not maintained, but positive pressure is. The levels of CO_2 recorded by the sensors (Tables S3 and S4 in the supplemental material) are not statistically different on days where operations took place than on days where no operations were conducted ($P > 0.05$). This indicates that the ventilation system is appropriately supplying fresh air on days where CO_2 sources were present (i.e., health care and patients breathing) and on days were operating rooms were not in use, and hence no CO_2 source should be present. Likewise, no difference in CO_2 levels is observed between days where positive SARS-CoV-2 RNA was detected and days where no virus genetic material was detectable ($P > 0.05$), indicating that the detection of SARS-CoV-2 RNA traces might not be attributable to a faulty ventilation system.

Therefore, a possible explanation of the detection of SARS-CoV-2 traces in aerosol collected on dates where no operation took place could be a combination of the following three reasons. Firstly, the ventilation system was set to reduced mode, hence supplying and extracting less fresh air on dates where the operation rooms are not used. Secondly, there was an unauthorized transit use of the scheduled operating room as a shortcut between hospital premises. Thirdly, considering the reported incidence of asymptomatic infections among vaccinated health care personal it is a possibility that some of the health care workers could be unawarely infected with the virus, and hence exhaling inadvertently SARS-CoV-2 virions into the air.

In addition to the aforementioned explanations, there could be other sources of airborne SARS-CoV-2 RNA, such as by anesthetic induction or intubation and by aerosolizing the virus embedded in human tissues during open surgery from patients that

might have been recently infected with the virus, despite presenting a negative COVID-19 test result at the time of the surgery.

Strengths and limitations. This study is evaluating the presence and abundance of SARS-CoV-2 viral load in aerosol collected from operating rooms, which collected daily samples for 3 weeks, both in operating rooms with scheduled operations and with emergency operations. As far as the authors are aware, this is the first study to evaluate the presence of SARS-CoV-2 in aerosol samples in operating theaters. It also combines environmental information with health information on medical procedures and operations that have been performed in the sampled and adjacent operating rooms.

This study also presents some limitations. There is no PCR or lateral flow test information of the health workers who were working in the monitoring spaces to be able to confirm if the emission source could be associated with the health care workers. Patients who have had previous COVID-19 asymptomatically, it is unknown how long ago they have had the disease and the possibility that the virus could still be in their body, e.g., in the intestinal cavity, despite testing negative in recent oropharyngeal PCR tests. Another limitation of our study is that our technique detects genetic load but does not report on the viability of the virus. However, the fact that the virus is detected in the environment indicates that at some previous point in time, this virus was viable, and capable of producing infection, although the concentrations reported in the present study are very low.

Conclusions. The results of the current study show that SARS-CoV-2 RNA was detected in 11% of aerosol samples collected in operating rooms, despite with low concentrations. These results highlight the importance of reinforcing preventive measures against COVID-19 in operating rooms. It should also be emphasized the appropriate use of surgical mask to avoid virus transmission from asymptomatic health care workers. The benefits of conducting routine surveillance testing to health care professionals, especially during waves of infection, should be considered. Information campaigns to reinforce the message among health care workers that vaccines protect from the severity of the illness, but not from being infected and spreading the virus, would be beneficial. These information campaigns should also target the importance of using the appropriate circulation pathways within the health care premises, especially in those areas that have heighten demands to minimize environmental pathogens, such as operating theaters and other clean rooms. Given that health care workers are among the population groups with the highest incidence of COVID-19, strengthening health care preventative measures would be beneficial not only for patients, a critical vulnerable group of population, but also for ensuring the health and working conditions of the health care workforce.

MATERIALS AND METHODS

Sampling location. The sampling campaign was carried out from 7 to 29 July 2021 inside two operating rooms of the University General Hospital of Castellón. One of them is an emergency operating theater (footprint area of 38 m^2) and the other is an operating theater where scheduled operations are performed (footprint area of 42 m^2) (Figs.S1 and S2 in the supplementary material).

Air sampling. In each operating theater, an air sampler (flow 38L/min) (Comde Derenda) was installed during the sampling campaign. Samples were collected for 24 h onto 47-mm quartz filters (Merck) and were transported just at the end of each sampling period to the lab. They were extracted and analyzed upon arrival. Samples collected over the weekend were stored at −20°C until analysis. Field blanks were used to control for any cross-contamination of samples during handling and processing.

Samples were collected by instructed health professionals working in the selected operating rooms. All personnel involved in instrument setup, sample collection, and sample processing wore personal protective equipment.

In addition, a sensor (KKMoon) was installed in each operating room to record CO_2 levels, temperature and relative humidity during each sampling.

Collection of laparoscopy filters. When the operations performed in the selected operating rooms led to the availability of laparoscopy filters, these were collected by health personnel and delivered to the research staff alongside the corresponding daily aerosol samples. The sampling of laparoscopy filters followed the same procedure described in Llueca et al. (13). Laparoscopy filters were stored at −80°C until analysis.

RNA extraction. Genetic material was extracted from filters to detect the presence and quantify the abundance of SARS-CoV-2 genetic material in the aerosol collected.

For RNA extraction, quartz filters were placed in a 5 mL tube and spiked with 500 infective units of mengovirus vMC0 (CECT 100000) (MgV), a nonenveloped virus used as a process control to monitor the amount of virus particles lost during sample processing, as well as to evaluate the efficiency of RNA extraction, according to ISO 10705-3: 2003, ISO15216-1: 2017 (49).

In addition, 900 μL of lysis buffer (Nucleospin RNA Vvirus Kit; Macherey-Nagel) and 1,000 mg of glass beads 4 mm in diameter, followed by 20 s of vortex. Then, 5 mL tubes are holey before placed in a 50 mL tube to collect the supernatant after centrifugation for 12 min at 8500 rpm. From the initial 900 μL of lysis buffer, approximately 800 μL of solution is collected, which is processed for viral RNA extraction using the Nucleospin RNA virus Kit, following the recommended protocol. Extracted nucleic acid was eluted in a volume of 70 μL and stored at −80°C until analysis.

Laparoscopic filters were extracted following the protocol described in Llueca et al. (13).

SARS-CoV-2 analysis. SARS-CoV-2 RNA detection by RT-qPCR was performed as previously described (13, 50). Briefly, presence of SARS-CoV-2, was determined using the 2019-nCoV RUO Kit (Integrated DNA Technologies) by specific amplification and FAM-probe detection of N1 and N2 regions of the nucleocapsid genes, validated by the US Centre for Disease Control prevention and the envelope gene E, validated by the Charité Berlin Institute of Virology (Berlín, Germany) (51). Mengovirus (vMC0 CECT 100000, Spanish type culture collection, Valencia, Spain), was spiked into the filters to be used as internal standard and was determined using specific primers and FAM-probe described in ISO 15216-1:2017 (52). Information about the primers and probes can be found in the supplementary information.

Reverse transcription PCR (RT-PCR) was performed using the One Step PrimeScript RT-PCR Kit (Perfect Real Time, TaKaRa) following the manufacturer's instructions and carried out in a StepOnePlus Real-Time PCR Systems (Applied Biosystems). Briefly, a first step of retrotranscription at 45°C for 10 s, followed by 40 cycles of PCR amplification and fluorescence recording (consisting of a denaturing step at 95°C for 5 s, and annealing/elongation at 55°C for 34 s), was set up (CDC-006-00019, Revision 06).

Calibration curves for MgV were performed using extracted MgV genomic RNA. In the case of SARS-Co-V-2, calibration curves for were performed using the 2019-nCoV_N Positive plasmid control for gene targets N1 and N2, and the i2019-nCoV_E Positive plasmid control for the E gene (Integrated DNA Technologies). All calibration curves for MgV and SARS-Co-V-2 were done in triplicate and with serial 10-fold dilutions.

Negative and positive controls were included in each PCR batch to verify the specificity of amplicons. Nuclease-free water was used as a negative control and control plasmids containing the complete nucleocapside and envelope genes (nCoV-N and nCoV-E plasmids controls) were used as positive controls.

Cycle threshold (Ct) values were used to calculate genomic copies (gc)/L in the original sample. Ct values lower than 40 were considered positive for SARS-CoV-2, as proposed previously (35). For each sampling day on which SARS-CoV-2 was detected, the average of gc/μL was calculated from the signal measured in each of the duplicate RT-qPCR wells containing the undiluted RNA sample for each of the selected gene targets. The concentration of SARS-CoV-2 in the air (gc/m^3) was calculated considering the number of genomic copies in the final extract and the volume of sampled air.

Health data. Information on the interventions performed in the selected operating rooms was obtained from the clinical-assistance information system for hospital centers of the Valencian Community (known as Orion). Number, duration, and type of the interventions performed on each sampling day was extracted from the system. The operations were classified into three categories: laparoscopic, open surgery (when intervention required the opening of the abdominal cavity), and others (included those that did not involve cavity opening). Information on whether patients had previously been infected with SARS-CoV-2 and the date was also registered. In addition, the same information was extracted from the operating theaters contiguous to those where the sampling campaign was performed. Ethical approval was obtained from the Institutional Review Board of the University General Hospital of Castellón.

Data description. Characteristics of interventions, such as date, number of interventions per day, number of interventions per day and type, duration of all interventions per day, number of patients with previous COVID-19 diagnosis, date of diagnosis and type of diagnostic test in the sampled emergency and programmed operating rooms are displayed in Tables S1 and S2 in the supplemental material, respectively. The concentrations of SARS-CoV-2 genetic material (gc/m^3) measured in the emergency and programmed operating rooms are displayed in Tables S1 and S2, respectively.

Concentrations of CO_2 (ppm), temperature (T, °C) and relative humidity (RH, %) in the emergency and in the programmed operating theaters are displayed in Tables S3 and S4, respectively. The Mann–Whitney U test was used to assess if CO_2 levels were different on days where genetic material of the virus SARS-CoV-2 was detected compared with days with no detection.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1 MB.

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