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SARS-CoV-2 Virus in the Peritoneum during Emergency Laparoscopy in Pandemic Covid19: Results of a Prospective, Multicenter, Interventional, Cohort Study: The Laptranscov Trial

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1. Abstract

1.1. Objective: This study aimed to determine the presence of SARS-CoV-2 virus in the pneumoperitoneum and/or peritoneal fluid during emergency laparoscopy performed in patients infected by SARS-CoV-2 virus during the first waves of COVID-19 pandemic.

1.2. Summary background data: All recommendations edited by the French and foreign Surgical Societies to prevent subsequent infection by SARS-CoV-2 during surgical procedures were edited with a lack of evidence, and mainly focused on avoidance of leaks of pneumoperitoneum, and/or surgical smoke and complementary measures to minimize the risk of aerosol production and emissions in a CO2 closed system to protect all healthcare providers from SARS-CoV-2 infection.

1.3. Methods: From April 2020 to December 2021, we made a prospective, multicenter, interventional trial called LAPTRANSCOV and all patients infected by SARS-CoV-2 and needing an emergency laparoscopy were included in the study. During the operation, liquefied samples of pneumoperitoneum or free intra-abdominal effusion were collected at different steps of the procedure and sent to the laboratory to perform 2 different types of RT-PCR for SARS-CoV-2 detection.

1.4. Results: Data from 12 patients that represented 54 samples were analyzed to detect SARS-CoV-2. Only one sample tested positive in a patient operated on for acute peritonitis secondary to perforated acute appendicitis.

1.5. Conclusion: In our experience, the presence of SARS-CoV-2 virus in the pneumoperitoneum of patients with COVID-19 disease was conditioned upon the presence of hollow organ perforation. Our results were reassuring concerning the spread risk of SARS-CoV-2 during laparoscopy without intra peritoneal hollow organ perforation.

2. Highlight

-SARS-CoV-2 virus was not detected in pneumoperitoneum during emergency laparoscopy

-Exception was one sample in a case with intra peritoneal hollow organ perforation.

-reassuring data for healthcare providers for SARS-CoV-2 diffusion during laparoscopy

3. Introduction

During the COVID-19 pandemic, all national health authorities worldwide imposed quarantine measures to slow down the spread of the disease. Despite of these measures, the disease rapidly spread all over the world. In this setting, the French health authorities recommended: the deferral of all non-urgent surgical procedures, the spread for endoscopic approach, pursuit of surgical oncology treatments, and protection of all healthcare providers [1-3].

All recommendations edited by the French and foreign Surgical Societies to prevent subsequent infection by SARS-CoV-2 during surgical procedures were edited with a lack of evidence, and mainly focused on avoidance of leaks of pneumoperitoneum, and/or surgical smoke created by energy devices during laparoscopic procedures as well as complementary measures to minimize the risk of aerosol production and emissions in a CO2 closed system [1,2]. These recommendations were based on previous observations in which aerosols (droplets) produced during surgical procedures may harbor various pathogenic micro-organisms which represents a risk for the spread of infections [4–8].

Our study was based on the fact that laparoscopy may carry a risk of transmission of the SARS-CoV-2 to the healthcare providers through aerosols produced in the pneumoperitoneum of patients infected with the SARS-CoV-2. This study aimed to determine the presence of SARS-CoV-2 virus in the pneumoperitoneum and/or peritoneal fluid during emergency laparoscopy performed in patients infected by SARS-CoV-2 virus during the first waves of COVID-19 pandemic.

4. Patients and Methods

4.1. Patients

A prospective, multicentric, and interventional trial was conducted in 4 large third-level regional hospitals located at the administrative region of Île de France. After local and national institutional board review approval, all consecutive patients with confirmed or highly suspected infection with COVID-19 (by oropharyngeal RT-PCR swab or thoracic CT scan) and admitted between April 2020 and December 2021 to the emergency unit for immediate or urgent surgical intervention by laparoscopy were invited to participate in the study. Ethics approval was obtained from Comité de Protection des Personnes Ile-de-France VII ID-RCB n°: 2020-A01063-36 on April 21rst 2020 and registered in Clinical Trial Gov ID NCT04361396.

All patients or family members with tutor legal by default signed a written informed consent. In case of patients uncapable to sign the informed consent and absence of family member or tutor legal, patients were included to the study as per protocol. Patients underwent standard preoperative evaluation that included a RT-PCR nasopharyngeal swab test at admission at the emergency unit or by the anesthesia team before induction at the operative theater.

Demographic characteristics, anthropometric measurements, medical history, medication use including immunosuppressor medication were prospectively collected at the time of surgery. Perioperative data concerning date and time of surgery, medical condition for which surgery was indicated, method of creation of pneumoperitoneum, intraoperative findings, type of energy device used, and surgical procedure performed were also prospectively collected. Inclusion criteria were: 18-year-old patients or older highlysuspected or tested positive for COVID-19 at RT-PCR during the last 10 days before admission or a thoracic CT scan at admission showing pathognomonic signs of COVID-19 for whom an emergency laparoscopy was indicated for a medical abdominal or gynecological emergency. Clinical suspicion was defined by the presence of at least one of the following symptoms: cough which has been evolving over the last 15 days, recent fever having excluded other etiologies, anosmia without obstructive rhinitis, and contact with a patient with COVID-19 infection over the last 21 days. Non-inclusion criteria was: patient already participating in another interventional research project. Exclusion criteria were: patients testing negative for COVID-19 at a RT-PCR nasopharyngeal swab, withdrawal of consent from patients, family member or tutor legal by default; or refusal to sign the consent.

Primary outcome was the semi quantitative detection (evaluation of the threshold value) of the SARS-CoV-2 virus by RT-PCR in a liquefied sample of pneumoperitoneum at the end of an emergency laparoscopy at exsufflation (T4).

Secondary outcomes included the semi quantitative detection of the virus in 4 different surgical steps during emergency laparoscopic procedure: (i) at the very beginning of emergency laparoscopy just after creation of pneumoperitoneum (T1); (ii) detection of the virus within intra-abdominal effusion if present (T2), or within peritoneal lavage at the end of the procedure (T4) if intra-abdominal effusion was absent; (iii) detection of the virus in the pneumoperitoneum during surgical dissection of tissues and anatomical plans of the abdomen and pelvis (T3),and (iv) detection of the virus in the bile at the end of the procedure if cholecystectomy was performed (T5). All samples were immediately sent for processing to the molecular biology laboratory at the promoting center.

5. Methods

Sampling Techniques at the operative theater:

The nasopharyngeal swab for detection of the virus (RT-PCR) was spilled in a Universal Transport Medium or Transport Medium

Amies.

o The liquefied samples of pneumoperitoneum (T1, T3 samples) were collected in a neutral sterile reservoir system without additive (5 mL) as follow : 5 mL of sterile saline solution was previously collected in the reservoir system (Figure 1), the aspiration tube connected to the reservoir system was introduced through one of the trocars, aspiration of 60 mL of pneumoperitoneum, manual shaking of the reservoir, and the reservoir content was then collected in another sterile bottle for transport to the laboratory.

o The T2 sample was collected in a neutral sterile reservoir without additive (5 mL): 5 mL of free intra-abdominal effusion was sampled using a suction probe connected to a 60-mL syringe, the reservoir content was then collected in another sterile bottle for transport to the laboratory.

o In case of absence of free intra-abdominal effusion at exploratory laparoscopy, sampling of peritoneal lavage at the end of surgical procedure just before exsufflation (T4) was collected in a neutral sterile reservoir without additive (5 mL): a suction probe connected to a 60-mL syringe filled with 10ml of sterile normal saline was introduced through one of the trocars, 5mL of peritoneal lavage was obtained, The syringe content was then collected in another sterile bottle for transport to the laboratory.

o The bile was sampled at the end of the laparoscopic procedure (T5), if cholecystectomy was performed, and collected in a neutral sterile reservoir without additive (5 mL): specimen extraction within a sterile plastic bag, antisepsis of the external gallbladder wall with iodine solution, aspiration through punction of the gallbladder using a 10-mL syringe with a 21G needle, the syringe content was then collected in another sterile bottle for transport to the laboratory.

Molecular detection of SARS-COV-2 virus in the samples in the laboratory:

In the molecular biology laboratory, 2 different types of RT-PCR were performed systematically to increase the reliability of RT-PCR testing: (i)the RT-PCR test using the primer and probe sequences of DNA according to the diagnostic test developed by the National Reference Center (CNR) for respiratory viruses at the Institut Pasteur, Paris. In this method, amplification of 2 groups of separate molecular targets of the SARS-CoV-2 genome, known as IP2 and IP4, located at the RdRp gene were used [9]; (ii) the RT-PCR test from Bosphore ® Novel Coronavirus (2019-nCoV) (later renamed SARS-CoV-2 by WHO) detection kit v2 which targets the E-gene and the orf1ab gene specific to SARS-CoV-2 and includes an internal control [10].

The remaining of the separate samples were stocked at the molecular biology laboratory at ≤ 70° C (600-µL-Aliquotes) for eventual further testing or re-use.

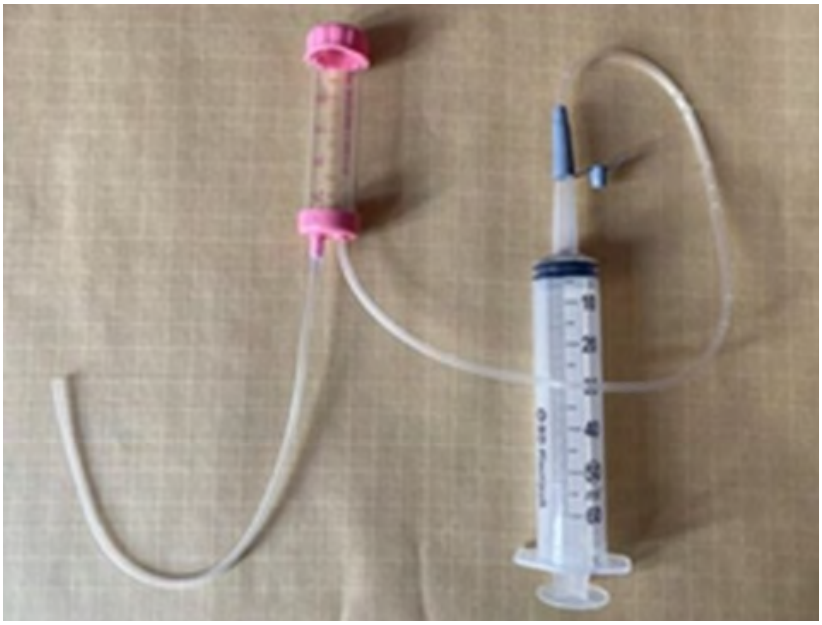

Figure 1: neutral sterile reservoir system without additive for liquefied pneumoperitoneum sampling.

5.1. Statistical Analysis

Data are presented as mean (SD), median (IQR) or n (%), unless otherwise specified.

6. Results

A total of 17 patients were included over a period of 18 months from June 16th 2020 to December 16th 2021. Five patients were withdrawn from the final analysis (negative RT-PCR from nasopharyngeal swab (n=3), and RT-PCR from nasopharyngeal swab (n=2) performed more than 10 days before the date of inclusion). Data from 12 patients were analyzed for evaluation of primary and secondary outcomes.

The number of inclusions per center was as followed: Center 01

– Pontoise (General Surgery) =5 (42%); Center 02 – Pontoise (Gynecology) = 1 (8,3%); Center 03 – St-Denis = 4 (33%); Center 04 – Argenteuil = 0; Center 05 – Créteil = 2 (17%). All patients concluded the study protocol, and none has withdrawn the informed consent.

Patients' demographics data and comorbidities were summarized in Table 1.

Concerning the operative data, the mean operative time of all laparoscopic emergency procedures was 94 min [65; 128]. The performed surgical procedures were: appendectomy for acute appendicitis (n=5, 42%), cholecystectomy for acute cholecystitis (n=4, 33%), ovarian cystectomy for ovarian torsion (n=1, 7.14%), a laparoscopic suture of a iatrogenic posterior bladder wound wall (n=1, 7.14%), and an exploratory laparoscopy in a patient with unknown severe epigastric pain after normal upper endoscopy with history of Roux-en-Y gastric bypass (n=1, 7.14%).

We found per operatively 11 (92 %) patients with peritoneal effusion that were: serous, purulent, cloudy, biliary, sero-hematic, and urine in 5, 2, 1, 1, 1, 1 cases, respectively. In one case, a purulent effusion was an acute peritonitis due to intra peritoneal hollow organ perforation (acute perforated appendicitis). Surgeons used only electric devices (mono-or bipolar cautery). No death was observed.

Concerning the sampling achievement of pneumoperitoneum, the T1 sampling was performed in all 12 patients and no presence of SARS-COV-2 virus was detected. The T2, T3, T4, and T5 sampling were performed in 11, 10, 11 and 4 patients, respectively. All T2, T3, T5 samples were negative for SARS-CoV-2 at RT-PCR, and 1 patient with peritonitis secondary to acute perforated appendicitis was positive for SARS-CoV-2 RT-PCR at T4 sampling.

Table 1: Patients' demographics data and comorbidities

	Mean ± SD or N/total (%)	IC 95 %
Age	50.5 ± 15.7	[42.7 ; 58.1]
Gender		
Female	8/12 (66.6 %)	[28.2 ; 83.7]
Male	4/12 (33.4 %)	[16.3 ; 71.8]
Height	166 ± 9.99	[161 ; 172]
Weight (kg)	81.6 ± 16	[73.5 ; 89.8]
BMI		
Normal	4/12 (33.3 %)	[8.05 ; 65.4]
Overweight	3/12 (25 %)	[3.61 ; 56.8]
Moderate Obesity	2/12 (16.7 %)	[0.617 ; 47.1]
Sevee Obesity	1/12 (8.33 %)	[0.595 ; 36.1]
Morbid Obesity	2/12 (16.7 %)	[0.617 ; 47.1]
Tobacco		
No	12/12 (100 %)	[68.6 ; 99.5]
Alcohol		
No	11/12 (91.7 %)	[58.7 ; 99.5]
Yes	1/12 (9.3 %)	[0.513 ; 41.3]
High Blood Pressure		
No	7/12 (58.3 %)	[28.2 ; 83.7]
Yes	5/12 (41.7 %)	[16.3 ; 71.8]
Complicated diabetes mellitus		
No	11/12 (91.7 %)	[68.6 ; 99.5]
Yes	1/12 (9.3 %)	[0.513 ; 31.4]
Immuno-suppressive treatment		
No	11/12 (91.7 %)	[68.6 ; 99.5]
Yes	1/12 (9.3 %)	[0.513 ; 31.4]

7. Discussion

Our prospective, multicenter, and interventional trial showed that SARS-CoV-2 virus had not been detected in pneumoperitoneum during emergency laparoscopy excepted in one case with intra peritoneal hollow organ perforation. Our results were reassuring about the induced pneumoperitoneum for emergency laparoscopy concerning SARS-CoV-2 spread risk. Sole hollow organ perforation may lead to SARS-CoV-2 virus dissemination in pneumoperitoneum.

The World Health Organization (WHO) declared on the 5th May 2023 the end to COVID-19 as a global health emergency. However, the COVID-19 disease is still a global threat. As of mid-June 2023, over 768 million confirmed cases and over 6.9 million deaths have been reported globally since the declaration of pandemic by WHO on March 12, 2020 [11].

The COVID-19 pandemic dramatically affected the management of many surgical pathologies with a significant shift toward

non-operative management (NOM) or surgery cancelling/delaying at the early stage of the outbreak due to concern of virus transmission by laparoscopy through aerosols production from pneumoperitoneum [12,13].

This shift was also explained by the increased mortality (23.8%) and pulmonary complications (51.2%) observed in patients infected by SARS-CoV-2 undergoing surgery [14].

In the emergency setting, international surgical societies recommended to prioritize NOM whenever applicable to reduce the risk of SARS-CoV-2 infection in surgical patients [15–18].

After major efforts deployed overtime by the scientific community and healthcare authorities to improve knowledge of the SARS-CoV-2 infection, several strategies were adopted to reduce the risk of infection mainly related to improved availability of personal protective equipment and wider population-based screening. However, the safety of laparoscopic surgery in infected patients with SARS-CoV-2 virus remains unclear so far, as there is still no rock-solid evidence concerning the presence of the virus SARS-CoV-2 in the peritoneal cavity. Evidence for the presence of SARS-CoV-2 virus is mixed and mainly came from case reports and small case series at the early stage of the outbreak [19,20].

The largest series of 34 pregnant women undergoing C-section published by Jakimiuk et al reported negativity for SARS-CoV-2 of peritoneal fluid samples (21). Other smaller case series reported the same negative findings [22–24].

One interesting point is the fact that all samples in all studies were taken at once at the very beginning of the surgery (open or laparoscopy) without any precaution to avoid contamination during sampling or without any consideration that open surgery may represent a state of the abdominal cavity prone to contamination from aerosols eliminated during intubation of the patient, for example. One exception to the rule was the report from Safari et al which examine for tissue involvement of SARS-CoV-2 virus in the wall of small bowel, appendix, gallbladder, bile, liver, urine, and visceral and subcutaneous fat. However, those sampling could have not answered our question if SARS-CoV-2 involves the peritoneal cavity [24]

More recently, Tartaglia et al, reported a case series of 18 patients infected with SARS-CoV-2 which underwent emergency surgery. They found 2 cases with positive peritoneal swabs in their series. What was remarkable was the fact that those cases were associated with ischemic colitis and adhesive small bowel occlusion which may be associated with bacterial or viral translocation through mucosa disruption and micro perforation of the bowel. Those events may explain the peritoneal swab positivity in these cases, even though rectal swabs were negative. On the other hand, a contradictory observation was the negativity of peritoneal swabs in those patients with perforated bowel [25].

To our knowledge, our study is the only prospective trial to examine the presence of the SARS-CoV-2 virus in all steps of the laparoscopic procedure from creation of pneumoperitoneum through exploration and tissue dissection with energy devices up to exsufflation. We also took all precautions to avoid contamination of the samplings and adopted a more standardized sampling method other than peritoneal swab. The spontaneous presence of SARS-CoV-2 virus over the peritoneum or within the surgical smoke produced during emergency laparoscopy performed in patients with COVID-19 disease was not observed. The only positive case we found was associated to appendicular perforation which may finally explain the presence of the SARS-CoV-2 virus in the peritoneal effusion. We failed to get samples from rectal swabs which might have directly related the positivity of the peritoneal effusion to visceral perforation.

The major limit of our study is the small number of included patients. Many external factors negatively influenced the rate of inclusion, mainly: organization in one of the recruiting centers; national and international recommendations prioritizing NOM, surgery deferral, and human resources and/or availability.

Romero-Velez, et al, in a systematic review of the literature, reported 357 samples for detection of SARS-Cov-2 virus from 295 patients reported in 36 studies (50% case reports and 50% case series). They observed 21 positive samples (5.8%). They also found that patients with severe COVID-19 disease were more prone to have positive samples from peritoneal cavity (37.5% vs 3.8%, p< 0,001) [26].

8. Conclusion

In our experience, the presence of SARS-CoV-2 virus in the pneumoperitoneum of patients with COVID-19 disease was conditioned upon the presence of intra peritoneal hollow organ perforation. Maintenance of a closed CO2 system as much as possible during the whole laparoscopic procedure and avoidance of leakage of pneumoperitoneum should be a state-of-the-art approach in patients with COVID-19 disease undergoing emergency laparoscopic surgery at least until ruling out visceral perforation. Maintenance of those precautions in severe COVID-19 cases undergoing emergency laparoscopy was also recommended even in the absence of visceral perforation. All protective measures need to be employed during laparoscopic surgery performed in patients with COVID-19 disease to protect healthcare providers. Larger prospective, well-designed controlled studies are still needed to better identify which factors influence the presence of SARS-CoV-2 virus in the peritoneal cavity.

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