Loop-Mediated Isothermal Amplification Screening for COVID-19 in Asymptomatic Preoperative Orthopedic Patients in a General Hospital in Kanagawa, Japan

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Abstract

The global coronavirus disease 2019 (COVID-19) pandemic has caused several million infections and hundreds of thousands of deaths. A large number of healthcare workers have died as a result of infection with this virus. Therefore, elective surgery was markedly reduced or stopped in our hospital’s orthopedic department. The detection of asymptomatic COVID-19-positive patients became key to reducing the infection risk to physicians and staff to allow orthopedic surgery to be performed. A total of 21 patients were scheduled to undergo orthopedic surgery, including elective surgery, in Shonantobu General Hospital, Chigasaki City, Kanagawa, Japan. All 21 patients gave permission to undergo loop-mediated isothermal amplification (LAMP) screening the day before surgery. None of the 21 patients we tested was positive for COVID-19. All patients remained asymptomatic during the two to four weeks of postoperative follow-up. No physicians or medical staff developed COVID-19 symptoms. This was a very small study in a city with a relatively low incidence of COVID-19. We found that LAMP screening was accurate, in terms of its negative predictive value. Larger studies are needed.

Introduction

The first case of coronavirus disease 2019 (COVID-19) occurred in Wuhan, China, and was reported in December 2019. On January 16, 2020, the first reports of COVID-19 were officially announced by Japan’s Ministry of Health, Labour, and Welfare. The COVID-19 pandemic is the largest health care crisis of this century. A large number of health care workers have died following infection with this virus. As a consequence, elective surgery was markedly reduced or stopped in the orthopedic department of the hospital, despite its importance in preserving the quality of life and activities of daily living. A number of lines of evidence indicate that transmission of the virus by asymptomatic patients is possible and that this may have hastened the spread of COVID-19 [1-2]. Therefore, the management of asymptomatic patients to reduce the infection risk to physicians and medical staff is important to allow orthopedic surgery to be performed safely. Some guidelines for orthopedic procedures have been reported [3-5].
However, evidence-based clinical practices have not been fully developed.

Shonantobu General Hospital is located in Chigasaki City, Kanagawa Prefecture, Japan. Kanagawa Prefecture has the third-highest number of infected patients in Japan. The number of COVID-19-infected patients in Chigasaki City has been relatively small as compared to other cities in Kanagawa Prefecture. A strategy for the management of orthopedic surgery, including elective surgery, during the pandemic, was needed to maintain orthopedic surgery practice during and after this pandemic.

Loop-mediated isothermal amplification (LAMP) with simple visual detection of amplification provides rapid analysis in field applications [6]. LAMP was first established as a rapid and reliable method for amplifying a small amount of target sequence at a single reaction temperature, eliminating the requirement for sophisticated thermal cycling equipment. Previous studies have reported that the LAMP method is useful to detect COVID-19 [7]. We hypothesized that the LAMP method would be useful for screening for COVID-19 in asymptomatic patients undergoing orthopedic surgery.

**Materials And Methods**

**Patients and methods**

Syonantobu Hospital is located in Chigasaki City in the south-central Kanagawa Prefecture (Figure 1).

**FIGURE 1: Map of Kanagawa Prefecture**

The number of COVID patients counted by Public Health Center in six cities, including Yokohama, Kawasaki, Sagamihara, Yokosuka, Chigasaki, and Fujisawa City, and by the public health and welfare office, which examined four cities, including Kamakura, Atsugi, Hiratsuka, and Odawara. We divided these into 10 areas.
The number of COVID patients were counted by Public Health Centers at six cities: Yokohama, Kawasaki, Sagamihara, Yokusuka, Chigasaki, and Fujisawa City and by public health and welfare offices at four cities, including Kamakura, Atsugi, Hiratsuka, and Odawara. We estimated COVID-19 patient numbers in 10 areas of Kanagawa Prefecture from February 12 to May 24, 2020, using information from the Kanagawa Prefecture official home page [8]. We also calculated the approximate incidence of COVID cases in populations in each area.

This study was approved by the Shonantobu Institutional Review Board (approval number 2020-005). Informed consent was obtained from all individual participants. A total of 21 patients (age 21-90 y) without symptoms of fever, shortness of breath, or desaturation were included. Patients underwent LAMP screening using a commercial kit (Loopamp®, Eiken Chemical Co., Ltd, Tokyo, Japan) for COVID-19 detection one day before surgery. Nasopharyngeal samples were used to detect COVID-19. Total viral ribonucleic acid (RNA) was extracted from the specimens using the RNA extraction kit (QIAamp Viral RNA Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Isolated RNA reacted with enzyme mix (avian myeloblastosis virus (AMV) reverse transcriptase and Bst DNA polymerase) and primer mix at 62.5°C for 35 min according to the manufacture’s protocol. Turbidity measurement was carried out at an optical density of 650 nm, and the reaction was considered positive when the turbidity values were >0.1.

Results
Epidemiology
In Japan, the weekly rate of infection gradually increased from February 2-9 to April 5-11. Prime Minister Shinzo Abe declared a state of emergency in seven urban areas, including Kanagawa Prefecture, on April 7 extending until May 25. After the declaration of the state of emergency, the number of newly infected patients gradually decreased in Kanagawa Prefecture (Figures 2A-2B).
Most cases in Kanagawa Prefecture were concentrated in Yokohama and Kawasaki Cities (Table 1; 530 and 269 patients, respectively). Twenty-seven cases were observed in Chigasaki City; this rate was lower than that in the other nine areas (Table 1).

FIGURE 2: Sequential changes in the number of COVID-19 patients in Kanagawa Prefecture and 10 areas
A. COVID-19 patients in Kanagawa Prefecture, B. COVID-19 patients in 10 areas
| City            | Population  | COVID patients | COVID cases/billion people |
|----------------|-------------|----------------|---------------------------|
| Yokohama       | 3,763,771   | 530            | 1.41                      |
| Kawasaki       | 1,535,415   | 269            | 1.75                      |
| Sagamihara     | 722,252     | 78             | 1.08                      |
| Yokosuka       | 390,549     | 51             | 1.31                      |
| Fujisawa       | 435,121     | 55             | 1.26                      |
| Chigasaki      | 290,411     | 27             | 0.93                      |
| Atsugi         | 856,148     | 109            | 1.27                      |
| Kamakura       | 304,150     | 62             | 2.04                      |
| Hiratsuka area | 583,073     | 59             | 1.01                      |
| Odawara        | 319,641     | 83             | 2.60                      |

**TABLE 1: COVID patients in Kanagawa Prefecture cities**

1. Chigasaki Public Health Center calculated the patients' numbers in Chigasaki and Samukawacho.

2. Atsugi public health and welfare office calculated the patients’ numbers in Atsugi, Ebina, Zama, Aikawa, Kiyokawa, Yamoto, and Ayase. Therefore, the number of patients and the population in the Atsugi area included these cities and towns.

3. Kamakura public health and welfare office calculated the patients’ numbers in Kamakura, Zushi, Miura, and Hayama. Therefore, the number of patients and population in Kamakura included these cities and towns.

4. The Hiratsuka public health and welfare office calculated the patient’s number in Hiratsuka, HadanoIsehara, Oiso, and NinomiyaYamachi. Therefore, the number of patients and the population in Hiratsuka included these cities and towns.

5. The Odawara public health and welfare office calculated the patient numbers in Odawara City, Minamiashigara, Nakaimachi, Oimachi, Matsudamachi, Yamakitamachi, Oimachi, Matsudamachi, Yamakitamachi, Kaiseimachi, Hakone, Yamakitamachi, and Manazuru. Therefore, the number of patients and the population in the Odawara area included these cities and towns.

**Incidence of COVID-19 in patients scheduled to undergo orthopedic surgery**

None of the 21 patients we tested was positive for COVID-19. All underwent orthopedic surgery. Nine patients requiring fracture repair and one patient with a spinal tumor underwent emergency surgery. Eleven cases, including one each with osteoarthritis, ligament tear, and meniscus tear, underwent elective surgery following negative COVID-19 screening. The clinical characteristics of the patients are listed in Table 2.
All patients remained asymptomatic after orthopedic surgery during the two to four weeks follow-up. No physicians or medical staff developed symptoms of COVID-19.

**Discussion**

The global COVID-19 pandemic is caused by the SARS-CoV-2 virus and has so caused several
million infections, with hundreds of thousands of deaths around the world. Previous studies have reported on emergency orthopedic surgery during a pandemic. A recent study reported that the occurrence of COVID-19 pneumonia in patients with a fracture can result in severe adverse outcomes and increase mortality [9]. Surgery was performed following pneumonia and COVID-19 testing under pandemic conditions and significantly improved outcomes. Upper extremity numbness was resolved in patients with a spinal tumor [10]. In contrast, the widespread recommendation to postpone elective operations in the US during the pandemic has produced a large population of patients with hip and knee osteoarthritis who are unable to receive their recommended surgical treatment [11]. In our cases, we performed both emergency and elective surgery following COVID-19 screening with LAMP during a declared state of emergency in Japan. No complications were observed after surgery in any patient. During the study period, 1324 COVID-19 cases were reported in Kanagawa Prefecture. However, COVID-19 cases in Chigasaki City, where our hospital is located, were lower than in other cities. Our experience with LAMP screening may provide a strategy to begin orthopedic surgery, including elective surgery, in asymptomatic patients with orthopedic problems in a post-pandemic future.

A previous study found the colorimetric LAMP assay provided 100% agreement with quantitative reverse transcription-polymerase chain reaction (RT-qPCR) results across a range of Cq values and rapidly detects COVID-19 [7]. Here, we used the LAMP method as a rapid detection tool to test asymptomatic patients with orthopedic problems. A LAMP assay screening may be important to ensure the safety of the medical staff and other patients.

Some studies have reported that concerns have been raised about the poor sensitivity of nucleic acid-based tests. The sensitivity of PCR was this might be as low as 59% [12]. On the day of symptom onset, the median false-negative rate was 38% [13]. Therefore, further investigations with larger sample sizes are needed to clarify the usefulness of the LAMP method to identify a COVID-19 infection in patients requiring orthopedic surgery in the post-pandemic future.

**Conclusions**

A total of 21 patients gave permission to undergo LAMP screening the day before orthopedic surgery. None of the patients we tested was positive for COVID-19. All patients remained asymptomatic during the two to four weeks of postoperative follow-up. No physicians or medical staff developed COVID-19 symptoms. LAMP screening may provide a strategy to begin orthopedic surgery, including elective surgery, in asymptomatic patients with orthopedic problems in a post-pandemic future.

**Additional Information**

**Disclosures**

**Human subjects:** Consent was obtained by all participants in this study. Shonantobu Institutional Review Board issued approval 2020-005. This study was approved by the Shonantobu Institutional Review Board (approval number: 2020-005). Informed consent was obtained from all individual participants. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.
References

1. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, Wang M: Presumed asymptomatic carrier transmission of COVID-19. JAMA. 2020, 323:1406-1407. 10.1001/jama.2020.2565

2. Li C, Ji F, Wang L, et al.: Asymptomatic and human-to-human transmission of SARS-CoV-2 in a 2-family cluster, Xuzhou, China. Emerg Infect Dis. 2020, 26:1626-1628. 10.3201/eid2607.200718

3. Hirschmann MT, Hart A, Henckel J, Sadoghi P, Seil R, Mouton C: COVID-19 coronavirus: recommended personal protective equipment for the orthopaedic and trauma surgeon. Knee Surg Sports Traumatol Arthrosc. 2020, 28:1690-1698. 10.1007/s00167-020-06022-4

4. Mouton C, Hirschmann MT, Ollivier M, Seil R, Menetrey J: COVID-19 - ESSKA guidelines and recommendations for resuming elective surgery. J Exp Orthop. 2020, 7:28. 10.1186/s40634-020-00248-4

5. Sarac NJ, Sarac BA, Schoenbrunner AR, et al.: A review of state guidelines for elective orthopaedic procedures during the COVID-19 outbreak. J Bone Joint Surg Am. 2020, 102:942-945. 10.2106/JBJS.20.00510

6. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T: Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000, 28:E63. 10.1093/nar/28.12.e63

7. Baek YH, Um J, Antigua KJC, et al.: Development of a reverse transcription-loop-mediated isothermal amplification as a rapid early-detection method for novel SARS-CoV-2. Emerg Microbes Infect. 2020, 9:998-1007. 10.1080/22221751.2020.1756698

8. Kanagawa Prefecture official homepage [In Japanese]. (2020). Accessed: May 25, 2020: https://www.pref.kanagawa.jp/docs/ga4/bukanshi/occurrence_03.html

9. Mi B, Chen L, Xiong Y, Xue H, Zhou W, Liu G: Characteristics and early prognosis of COVID-19 infection in fracture patients. J Bone Joint Surg Am. 2020, 102:750-758. 10.2106/JBJS.20.00510

10. Kessler RA, Zimering J, Gilligan J, et al.: Neurosurgical management of brain and spine tumors in the COVID-19 era: an institutional experience from the epicenter of the pandemic. J Neurooncol. 2020, 148:211-219. 10.1007/s11060-020-05525-7

11. Brown TS, Bedard NA, Rojas EO, et al.: The effect of the COVID-19 pandemic on electively scheduled hip and knee arthroplasty patients in the United States. J Arthroplasty. 2020, 55:49-55. 10.1016/j.arth.2020.04.052

12. Ai T, Yang Z, Hou H, et al.: Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020, 296:10.1148/radiol.2020200642

13. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J: Variation in false-negative rate of reverse transcriptase polymerase chain Reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med. 2020, 20:1495. 10.7326/M20-1495