International Journal of Surgery 80 (2020) 74–78

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.elsevier.com/locate/ijsu

Effectiveness and safety of tumor site marking with near-infrared fluorescent clips in colorectal laparoscopic surgery: A case series study

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ARTICLE INFO

Keywords:
Colorectal cancer
Fluorescent clip
Tattoo marking
Intraoperative endoscopy

ABSTRACT

Background: In colorectal laparoscopic surgery, accuracy of tumor marking has been an important but not fully resolved issue. The tattoo marking technique or intraoperative endoscopy have been used but they either carry the risk of accidental intestinal puncture or require either longer operation times, a skilled endoscopist and/or intraoperative colon insufflation. We supposed that tumor site marking with the near-infrared fluorescent clips, ZEOCLIP FS clips (Zeon Medical Co., Ltd., Tokyo, Japan) might overcome disadvantages of both tattoo marking and intraoperative endoscopy-based tumor localization methods. This is the first report on the case series using near-infrared fluorescent marking clip. We summarize the early results in 30 patients, who underwent colorectal laparoscopic surgery; we focus particularly on effectiveness and safety of the method.

Materials and methods: Thirty consecutive patients, who underwent laparoscopic surgery for colorectal cancer after previous endoscopic ZEOCLIP FS placement were enrolled from May 2019 till October 2019. The primary endpoint was the rate of intraoperative clip detection and the secondary endpoints were: the rate of adverse effects, percentage of slipped clips and usefulness of plain abdominal radiography to preoperatively confirm the clip retention. Locations of fluorescent clips were identified with a full-color fluorescence laparoscope. All operations and clip placements were performed by the same senior surgeon with sufficient experience in both procedures.

Results: Fluorescent clips could be detected in 94.1% of tumor lesions. Three (2.1%) clips dropped before surgery. Plain abdominal radiography was sufficient to assess clip retention in all cases. **No** adverse effects related to either clip placement or clip detection were observed.

Conclusion: The ZEOCLIP FS could be easily detected from the serosal side of the intestinal tract when placed 1–2 days before surgery. Fluorescent clip-guided laparoscopy may be considered a safe and effective method for localization of colorectal tumor sites.

The Research Registry UIN: researchregistry5400.

1. Introduction

Accuracy of tumor marking has been a prerequisite of successful outcome in colorectal laparoscopic surgery but the current methods still need to be improved to reach the ideal. Under pneumoperitoneum, the intestinal tract cannot be directly and tacitly examined even if "palpation" of the serosal side of the colon using laparoscopic devices is to some extent possible. Therefore, some submucosal (SM) or muscularis propria (MP) lesions may remain intraoperatively undetected. The tattoo marking technique has been commonly applied [1,2] but carries risks of accidental intestinal puncture, peritoneal scattering or injury to other abdominal organs (Fig. 1) [3–7]. Intraoperative endoscopy has also been utilized for identification of tumor sites in colorectal surgery; however, because of longer operation times, intraoperative colon insufflation and the need of a skilled endoscopist, it has not become a standard tumor marking procedure [8].

Near-infrared fluorescent (NIRF) radiation penetrates soft tissues [9,10] and the NIRF signals can be detected as translucent light through

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https://doi.org/10.1016/j.ijsu.2020.06.014
Received 9 March 2020; Received in revised form 13 May 2020; Accepted 2 June 2020
Available online 27 June 2020

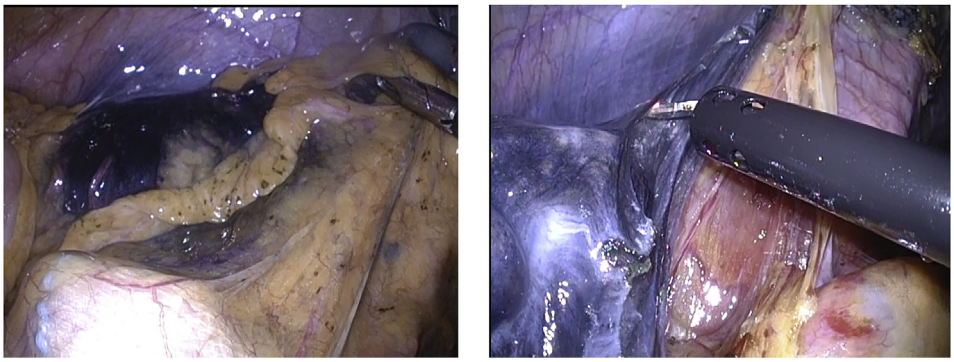

Fig. 1. Tattoo marking method carries risks of accidental intestinal puncture potentially leading to peritoneal scattering or puncture(s) to other abdominal organs.

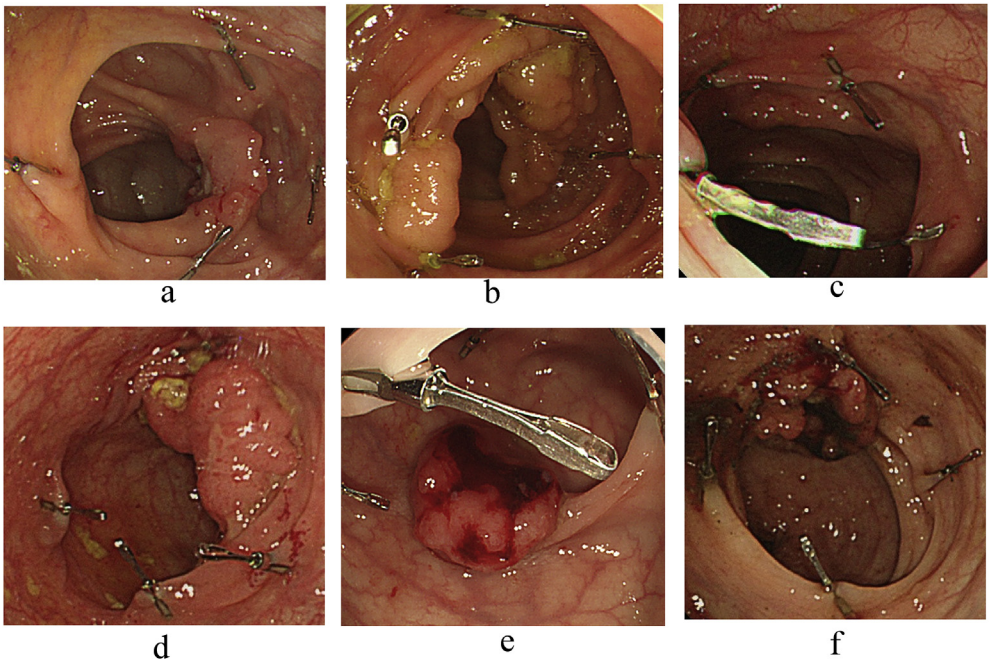

Fig. 2. Four clips intraluminally placed every 90° around the corresponding lesion in all colon/rectum regions (a, b, c, d, e, f).
a: Fluorescent clip in the cecum
b: Fluorescent clip in the ascending colon
c: Fluorescent clip in the transverse colon
d: Fluorescent clip in the descending colon
e: Fluorescent clip in the sigmoid colon.
f: Fluorescent clip in the rectum.

the wall of hollow organs when both fluorescence and excitation sources have the NIR wavelength and are observed with an NIRF camera [11]. Since 2016, we had cooperated in the development of NIRF clips for tumor site marking in laparoscopic surgery and evaluated potential clinical use of their prototypes in animal models (porcine stomach and large intestine) [12]. In cooperation with Zeon Medical Co., Ltd., Tokyo, Japan, disposable NIRF clips, ZEOCLIP FS clips, with peak excitation and fluorescence wavelengths of 760 and 790 mms were manufactured and approved for clinical use in 2019 (Reg. No. 13B1X001111000020). The ZEOCLIP FS clips have fluorophore resin-filled tips, which emit NIRF signals when excited. If placed within the lumen of the intestinal tract, these signals can be detected from the serosal side and thus clips can be localized.

We hypothesized that tumor site marking with the ZEOCLIP FS clips might overcome disadvantages of both tattoo and intraoperative endoscopy-based tumor localization methods. The fluorescent clips should be intraoperatively detected as sources of translucent light through the intestinal wall on the serosal side of the colon after their previous intraluminal placement. Previously, we could successfully localize a tumor lesion during laparoscopic sigmoidectomy in a colon cancer patient [13]. In the present study, we summarize our experience of tumor site marking with fluorescent ZEOCLIP FS clips and examine effectiveness and safety of this method based on data in 30 patients. This is the first report on the case series using near-infrared fluorescent marking clip.

2. Materials and Methods

The study was a prospective single-center case series study performed accordingly with the PROCESS Guidelines [14] and was registered in the Research Registry under the UIN: researchregistry5400 [15]. Thirty consecutive patients who underwent laparoscopic surgery for colorectal cancer since May 2019 till October 2019 were enrolled after approval of the Institutional Ethics Committee (No. 13-B-344). From all patients, their written informed consent for participation in the study was obtained. All patients were treated and followed at the same tertiary academic institution-affiliated hospital. In all patients, both clip placement and endoscopic surgery were performed by the same surgeon (S.N.), a senior colon and rectal surgery specialist with longer than 10 years of experience of more than 300 cases in both colonoscopy and colorectal surgery.

All patients underwent bowel preparation before colonoscopy. The fluorescent ZEOCLIP FS clips were placed during colonoscopy performed 1–3 days before surgery. Following colonoscopy, the NPO status was managed until surgery. The perioperative optimization included: discontinuation of anticoagulation/antiplatelet therapy with preoperative heparin-bridging (4 patients: post-percutaneous coronary intervention, 2 patients; arteriosclerosis obliterans, 1 patient; atrial fibrillation, 1 patient) or stress-dose steroid administration (2 patients: chronic interstitial pneumonia, 1 patient; bronchial asthma, 1 patient). In each case, 4 clips were attached to the intestinal mucosa

intraluminally close to the distal extent of the lesion, 4 clips for each lesion, each clip in 90° distance from the other (Fig. 2) [13]. If the clips were deployed 2–3 days before the operation, a plain abdominal X-ray was taken to confirm position and number of the attached clips. If surgery was performed the next day after clip placement, the clip positions and numbers were examined on the resected surgical specimens without preoperative radiographic evaluation. Thus, the rate of clip retention the next day after placement could be confirmed.

In all patients, the method of clip detection was same as the procedure presented in our previously reported case [13]. Namely, the full-color fluorescence laparoscope, VisionSense (Medtronic, Co., Minneapolis, U.S.A.), which allows adjustments of both excitation light and the fluorescence display thresholds, was used to identify the clips. Depending on locations of the tumor sites in the colon/rectum, observations with the fluorescence camera were performed before detachment and/or mobilization of the respective part of the colon (tumors located in the cecum to the sigmoid colon) or after dissection of the perirectal tissues to improve access of the laparoscope (rectal lesions). Following detection of tumor sites via translucent signals emitted from the fluorescent clips, the corresponding parts of the intestine were excised, and in all cases clip retention was confirmed on the resected specimens.

The primary endpoint of the study was the rate of intraoperative clip detection and their usefulness for tumor site marking. The secondary endpoints were: the rate of adverse effects, percentage of slipped clips and confirmation of clip retention with preoperative plain abdominal radiography.

3. Results

All enrolled 30 patients underwent laparoscopic colorectal surgery. The patients' demographics with comorbidities, tumor localization and tumor progression are shown in Table 1.

Preoperative colonoscopy was performed on the day preceding the surgery in 13 patients, 2 days before surgery in 16 patients and 3 days before in one patient (Table 1). No bleeding, perforation or other complications were experienced at the time of clip placement. The clip locations could be confirmed with the fluorescence laparoscope in 28 out of 30 patients and in 32 out of 34 (94.1%) lesions, respectively (Fig. 3). The imaging was precise enough so the distal end of a tumor could be determined. There were no adverse events observed during surgery. We did not experience nay cases where the clips were dislodged and caught in the linear stapler at the time of intestinal dissection or interfered with the stapling of the left colon or rectum.

Only 3 out of total 137 clips slipped off (2.1%), in 2 cases. In one case, attachment of 1 clip was insufficient during placement, therefore instead of 4 clips, an additional clip was deployed (total 5 clips); the weakly attached clip slipped off before surgery. In another case, colonoscopy with tumor site marking was performed 3 days before surgery and even though all 4 clips were present on the plain abdominal X-ray taken the next day, 2 clips could not be identified during the operation. The plain abdominal radiography was performed in 16 cases and in all those 16 cases deployed clips were identified and their number confirmed.

There were 6 patients with colonic diverticulosis, 2 patients with T4 tumor progression and 11 patients with thick fatty tissue deposits around the colon; in all such patients, those potentially interfering issues did not affect detection of the ZEOCLIP FS clips. Furthermore, clip detection failure occurred in patients who were neither morbidly obese nor in whom the clips were buried under a heavy omentum or splenic flexure.

Table 1
Demographics of study patients.

Parameter		
Sex (M:F)		18:12
Median age (range), years		72 (2–89)
BMI (range)		23.5 (16.7–31.2)
Smoking (number of patients)		4
Comorbidities: ∗ (number of cases)	post-PCI status	2
	ASO	1
	atrial fibrillation	1
	bronchial asthma	1
	interstitial pneumonia	1
Colorectal tumor lesion (C:A:T:D:S:R) ∗∗		(4:3:5:2:13:7)
Stage (I:IIa:IIb:IIc:IIIa:IIIb:IIIc:IV)		(12:8:1:1:1:3:2:2)
Preoperative colonoscopy (day1:2:3)		(13:16:1)

∗ PCI (percutaneous coronary intervention), ASO (arteriosclerosis obliterans).
∗ ∗ C (cecum), A (ascending colon), T (transverse colon), D (descending colon), S (sigmoid colon), R (rectum).

4. Discussion

In the present study, we sought to evaluate the effectiveness of colorectal tumor marking with fluorescent ZEOCLIP FS clips and found that the clips could be detected in 94.1% of lesions, allowing for accurate localization of the tumor sites in all 30 enrolled patients. Clip identification through the translucent intestinal wall using fluorescence laparoscopy was successful regardless of the tumor location within the colon and upper rectum, BMI, stage of the disease or size of the tumor, presence of diverticula and/or fatty tissue deposits or adhesions around the colon, indicating relatively high reliability of the method.

Clip retention seemed to be inversely proportional to the clip attachment period: the more days passed after the endoscopic clip placement the higher was the risk of slipping. Two clips attached 3 days before surgery dropped in 1 patient, in whom endoscopic placement had been performed a day earlier than in other patients. The clip gripping force might have decreased with time or clip deployment had been insufficient due to procedural or device failure. Indeed, the manufacturer's preliminary data with fluorescent clip prototypes indicated that clip attachment weakens within a week after deployment though fluorescence may be detected longer (data not shown). This may be caused by structural differences between the tips of fluorescent and conventional clips (the former being covered with an additional resin containing fluorescent dye). The weaker clip attachment might also be caused by stretching of the colonic mucosa during colonoscopy or during surgery but in our series the causes were likely to be different. Influence of bowel distension may be reduced or eliminated by additional suctioning performed upon clip deployment.

In all patients, in whom endoscopic clip placement had been performed 2–3 days before surgery, plain abdominal radiographs taken 1 day after clip attachment confirmed retention of all clips, and the clips remained in place in all patients, in whom endoscopic placement was performed 1 day before surgery (confirmed also on the resected surgical specimens). It seemed advisable to deploy the ZEOCLIP FS clips 1–2 days before surgery to obtain almost complete retention. In cases when clips were placed 2 days before surgery, plain abdominal radiography proved to be sufficient in assessing potential clip slippage and useful in selecting the surgical procedure.

With almost complete retention rate among all patients, tumor locations could not be identified in 2 cases. Clip attachment failure might be the main reason of the unsuccessful intraoperative clip detection in 1 patient, in whom the ZEOCLIP FS clips were deployed 3 days before surgery. On examination of the surgical specimen, 2 out of 4 clips were lost and the fluorescence intensity of the remaining clips was decreased to the extent that possibly rendered the clips undetectable through the intestinal mucosa. These two factors, namely, clip dropout and potential loss in fluorescence intensity, might again suggest that the highest possibility of clip detection can be expected when the fluorescent clips are endoscopically placed not earlier than 2 days before surgery.

In the second case, the clips were not intraoperatively identified because the optimal conditions for fluorescence excitation could not be obtained due to tumor localization and access difficulty. In this patient,

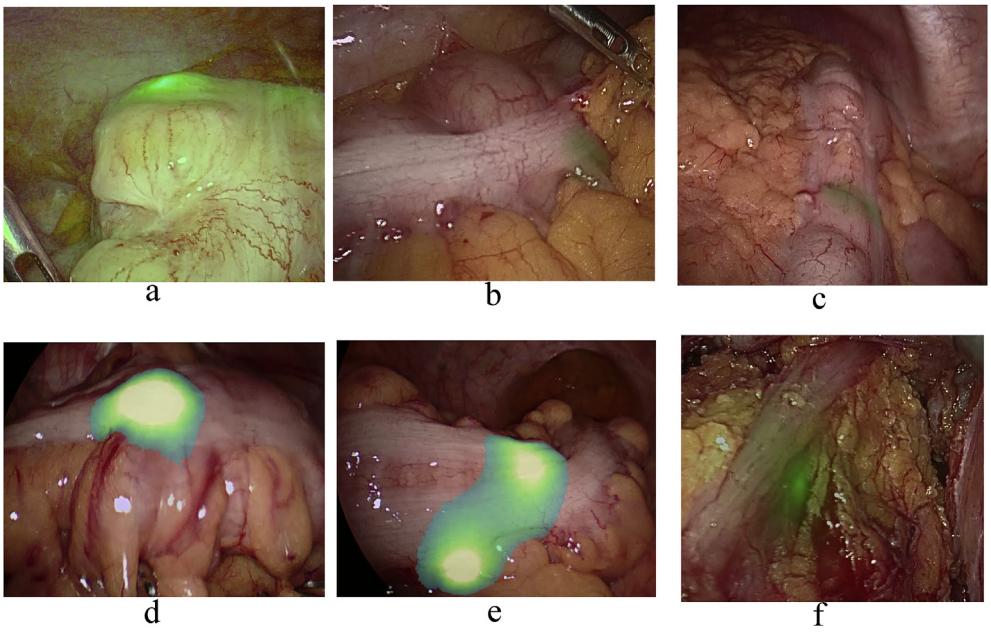

Fig. 3. Intraoperative observation using a full-color fluorescent laparoscope (VisionSense) from the serosal side. Fluorescent clips could be observed in all colon lesions (a, b, c, d, e, f).
a: Fluorescent clip in the cecum
b: Fluorescent clip in the ascending colon
c: Fluorescent clip in the transverse colon
d: Fluorescent clip in the descending colon
e: Fluorescent clip in the sigmoid colon
f: Fluorescent clip in the rectum. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

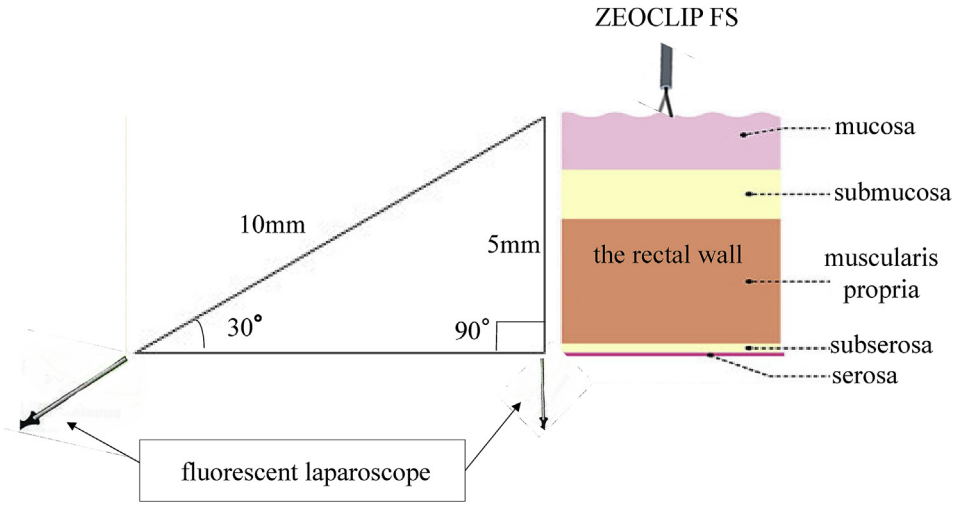

Fig. 4. It is estimated that an angle of 30° or more is necessary to excite fluorescent signal from the clip, assuming that the thickness of the intestine is 5 mm and that near-infrared rays can pass through 10 mm-thick tissue.

the clips were placed 2 days before surgery for a rectal tumor and remained in place on the plain abdominal X-ray taken the following day. Since the rectum could not be sufficiently mobilized during the operation, we could position the laparoscope only in the tangential angle to the intestinal mucosa, and the excitatory light was insufficient to produce fluorescent light. Fluorescence intensity may be affected by direction of the excited light. The near-infrared light can pass through soft tissues up to 10 mm [16–18] and to obtain the strongest intensity signals, soft tissue penetration distance should be as short as possible. The thickness of the rectal wall is about 5 mm and is thicker than colon wall [19,20]. Assuming the rectal wall thickness is 5 mm, the vertical placement of the laparoscope produces sufficient fluorescent excitation light angle to detect the clips. However, if the laparoscope excitatory light axis becomes close to the 30° angle to the intestinal tract, the fluorescent signal transmission distance rises to 10 mm (Fig. 4). Exceeding that distance renders excitation and detection of the fluorescent signals almost impossible, therefore for tumors affecting the lower rectum or in cases with extensive adhesions, prior mobilization of the intestine is necessary for the appropriate laparoscope placement. Our results showed the sufficient clip retention, and it may be allowed to try the mobilization before fluorescent observation. Future technological improvements in fluorescence laparoscopy that would allow adjustments of the excitatory light axis may be necessary to overcome this problem.

Patient's safety remains one of the most important aspects of tumor making techniques and attempts to reduce complications related to currently applied tumor site marking techniques have been undertaken. Recently, there have been reports on using indocyanine green (ICG) solutions instead of black ink for marking [21,22]; however, even with the ICG solutions the possibility of accidental intestinal puncture could not be completely reduced since the procedure does not much differ from the tattoo marking technique. Cases of peritoneal scattering with the ICG marker have been also reported [22]. Avoiding or eliminating these complications might be the main potential advantage of tumor site marking with the ZEOCLIP FS clips. Furthermore, the intraoperative fluorescent clip-guided tumor marking is a simple and inexpensive method (the required cost was around $100 per 1 fluorescent clip), concerning the potential costs of abovementioned complications or necessity of a skilled endoscopist during the operation.

There are 3 main limitations of the study. First, all operative

procedures were carried out by one surgeon, who was also one of the assessors of the technique and who himself had coined the concept of the fluorescent clip-guided tumor site marking; such a setting might have been a possible source of the optimism bias. Second, the study group did not include obese patients (BMI > 35 kg/m^2) and thus potential interference of obesity on intraoperative clip detection could not be examined. Third, the study included only colorectal laparoscopy cases and utility of the technique in robotic laparoscopic surgery was not evaluated.

5. Conclusions

The present study outlines and evaluates the novel method of pre-operative colorectal tumor marking with fluorescent clips that can be intraoperatively detected during laparoscopic surgery. Intraluminal placement of the ZEOCLIP FS clips combined with fluorescent clip-guided laparoscopy is a safe, feasible and accurate method for colon and upper rectum tumors. The highest efficacy of clip detection was obtained when the endoscopic clip placement was performed 1–2 days before surgery. The reliability of the fluorescent clip-guided tumor marking should be further addressed in prospective and multi-center studies with a larger series and more diverse patient groups (ex. lower rectum tumors, obese patients and in laparoscopic robotic surgery), in studies designed accordingly with the IDEAL Collaboration recommendations [23].

Funding received

None declared.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Data statement

The data that support the findings of this study are available from the corresponding author, [S.N], upon request.

CRediT authorship contribution statement

Satoshi Narihiro: Data curation, Formal analysis, Project administration, Writing - original draft, Writing - review & editing. **Masashi Yoshida:** Conceptualization, Methodology. **Hironori Ohdaira:** Investigation, Visualization. **Takayuki Sato:** Conceptualization, Methodology. **Daisuke Suto:** Investigation. **Sojun Hoshimoto:** Investigation. **Norihiko Suzuki:** Investigation. **Rui Marukuchi:** Investigation. **Teppei Kamada:** Investigation. **Hideyuki Takeuchi:** Investigation. **Yutaka Suzuki:** Supervision.

Declaration of competing interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijsu.2020.06.014.

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