Indocyanine green is superior to blue colorimetric method for identifying sentinel lymph nodes during laparoscopic surgery for uterine malignancies: a pilot study

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Summary

Aim: The objective of this study was to compare indocyanine green (ICG) and blue colorimetric methods for detection of sentinel lymph nodes (SLN) during laparoscopic surgery for uterine malignancies. Materials and Methods: Over a two years and 3 months period, laparoscopic surgery was performed on 16 cases of uterine malignancies using near infrared (NIR) fluorescence imaging of ICG tracer uptake to direct SLN biopsy (NIR-FI-SLNB). ICG was injected into the uterine cervix prior to surgery. For comparison of detection efficacy, blue dye used for traditional colorimetric method was injected concurrently into 14 of these 16 cases. After pneumoperitoneum, we opened the retroperitoneum to laparoscopically identify the SLN. After biopsy of the SLN, a systemic pelvic lymph node dissection was performed. Results: Using ICG, we were able to identify SLN in 15/16 cases (93.7%), and achieved bilateral pelvic mapping in 12/16 (75%). With the blue dye, we were also able to identify SLN in only 64.3% of the 14 cases so tested, and bilateral pelvic mapping in only 14.3%. There were no intraoperative complications during any of the procedures. For conducting a laparoscopic bilateral SLN biopsy, we found that use of ICG was significantly superior to the blue colorimetric method (14.3% vs. 75%, p = 0.0009). Conclusions: These pilot data provide suggestive evidence that the laparoscopic NIR fluorescence imaging for SLN biopsy provides superior efficacy, compared to the traditional blue colorimetric method, without additional complications.

Key words: Indocyanine green; Sentinel lymph node; Near-infrared fluorescence imaging; Blue dye.

Introduction

Sentinel lymph node (SLN) biopsy is now widely used for several gynecologic malignancies, as it has been shown to be both effective and safe for selecting patients who would benefit from lymphadenectomy, for example those women diagnosed with in vulvar and breast cancers [1]. However, due to the confounding bilateral multifarious drainage pattern of the uterus and cervix, the SLN procedure is more challenging in cervical and endometrial cancer patients than in breast or vulvar cancer patients [2].

Use of the SLN procedure in endometrial and cervical cancers have recently been investigated using traditional blue dyes or radiocolloids, or a combination of both, with variable results [3,4,5]. However, exposure to ionizing radiation, and the need for a specialized nuclear medicine unit, have limited the use of the radioactive tracer technique. More recently, the intraoperative near-infrared (NIR) fluorescence imaging method, using indocyanine green (ICG), has emerged as an effective technique for SLN detection during laparotomy in cervical cancer patients [6]. Due to the limited number of reports of NIR imaging being used for laparoscopic cervical surgery prior to 2012, we began our own prospective comparative assessment of the laparoscopic SLN detection rates for the ICG and blue colorimetric methods.

Materials and Methods

The use of patient data in this study was reviewed and pre-approved by Osaka University’s Ethical Committee for Human Subjects (Ethics Ref: 11077) on December 15, 2011. Written informed consent was obtained from all patients. All patients (n = 16) who had planned SLN mapping using fluorescence imaging between May 2012 and August 2014 were identified and prospectively entered into the study database.

Indocyanine green (ICG, 25-mg vial) was purchased from Daiichi Sankyo (Tokyo, Japan). A 2.5 mg/ml ICG stock solution was made by adding 10 ml of sterile water to the vial. About 500 μM stand-alone working solution for ICG injection was made by mixing 8 ml of the stock with 42 ml of sterile water. The dose of 500 μM ICG was chosen based on previous optimal dose studies [7,8]. Usually, prior to initiating laparoscopic entry, the cervix is divided submucosally into 0-, 3-, 6-, and 9-o’clock positions and a total of from 2 to 4 ml of ICG working solution is injected vaginally. Patent blue dye was injected concurrently in 14 of the 16 cases, using the same method as described for ICG.

During the study period, beginning May 2012, we performed 16 laparoscopic surgeries using NIR-FI-SLNB, visualizing lymph node ICG uptake via an InfraRed Imaging
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| Table 1. — Clinicopathologic demographics of the study cohort |
|---------------------------------------------------------------|
| Age, mean (years) (range) | 47.1 (30-67) |
| Body mass index, mean (kg/m²) (range) | 21.1 (16.9-24.6) |
| Preoperative diagnosis | Endometrial cancer |
| Grade 1 | 2 |
| Grade 2 | 1 |
| Cervical cancer | Squamous cell carcinoma (SCC) | 10 |
| Adenocarcinoma | 3 |
| FIGO stage | Endometrial cancer |
| 1A | 3 |
| Cervical cancer | IA2 | 2 |
| IB1 | 11 |

Table 2. — Sentinel lymph node detection rate

| SLN mapping time (min), mean (range) | 26.8 (17-40) |
| SLN identified per patient, mean (range) | 4.4 (1-12) |
| Dye used | ICG and blue dye | 14 |
| | ICG only | 2 |
| Overall case SLN detection rate | 15/16 (93.7%) |
| Mapping by pelvis, ICG | None | 1/16 (6.3%) |
| | Unilateral | 3/16 (18.7%) |
| | Bilateral | 12/16 (75%) |

Table 3. — Sentinel lymph node mapping - according to dye used

| Mapping, by patient | Patent blue, n = 14 | ICG, n = 16 | p |
|---------------------|---------------------|-------------|---|
| No mapping | 5/14 (35.7%) | 1/16 (6.3%) | |
| Mapped | 9/14 (64.3%) | 15/16 (93.7%) | 0.1337 |

| Mapped, by hemi-pelvis | |
|------------------------|---|
| No mapping | 5/14 (35.7%) | 1/16 (6.3%) |
| Unilateral | 7/14 (50%) | 3/16 (18.7%) |
| Bilateral | 2/14 (14.3%) | 12/16 (75%) | 0.0009 |

System (Olympus corporation, Tokyo, Japan). The cervix was injected with tracer after the patient was prepped and draped, but prior to the insertion of any uterine manipulator. After creating the pneumoperitoneum, we opened the retroperitoneum and identified the SLN (Figures 1 and 2). For safety and validation, in all 16 cases a systemic pelvic lymph node dissection was performed after the SLN biopsy.

Patient demographics, surgical data, and final pathology results were collected and examined. After conducting definitive histology, all 16 cases were staged according to the 2009 International Federation of Gynecology and Obstetrics Classification system. All lymph nodes were handled in a standardized manner, without ultrastaging. The patient’s median age, BMI, estimated blood loss (EBL), time to find the SLN, number of SLN identified, and anatomic location of the SLN were recorded. The time to perform the SLN mapping was defined as the time from the injection of tracer until the surgeon felt that an SLN was, or was not, identified. Overall detection and bilateral detection rates to find the SLN were calculated. Fisher’s exact test was used to compare the SLN detection and mapping rates for the dyes used.

Results

Sixteen patients with endometrial or cervical cancer were prospectively included into this study. Their mean age was 47.1 (range 30-67) years and mean BMI was 21.1 (range 16.9-24.6) kg/m². The majority of the patients were diagnosed as having cervical cancer (Table 1).

Operating time spent specifically on SLN mapping was available from patient records for 12/16 (75%) patients. The mean surgical time to complete the SLN mapping was 26.8 (range 17-40) minutes. The mean number of SLNs removed per patient was 4.4 (range 1-12). During the 16 procedures there were no adverse intraoperative complications.

NIR-FI-SLN mapping was used in all 16 cases, although blue dye was used concurrently in only 14/16 cases. SLNs were most frequently identified in the obturator region (62.3%), followed by the internal iliac (19.7%) and external iliac (18%) regions.

Using ICG, we were able to identify the SLN in 93.7% (15/16) of the cases, with bilateral pelvic mapping in 75% (12/16) (Table 2). With blue dye, they were able to identify the SLN in only 64.3% of 14 cases, with bilateral pelvic mapping in 14.3% (Table 3). When comparing mapping rates between the two methods, the mapping rate was higher with ICG than with the blue dye (64.3 vs. 93.7%, p = 0.1337). In bilateral hemi-pelvic mapping, ICG was significantly superior to blue dye (14.3 vs. 75%, p = 0.0009; Table 3).

All SLN detected by blue dye were also detected by NIR-FI-SLN mapping, but the converse was not true. In addition, a single confirmed metastatic-positive lymph node was identified in one (6.3%) of the 16 patients using ICG however this was not detectable by the blue colorimetric method.

Discussion

Despite many published positive results [9], SLN biopsy for cervical and endometrial cancer is not yet widely practiced. This may be due to the challenging methodology and
Table 4. — Previous reports of SLNB comparing ICG and blue dye

| Author   | n   | Diagnosis type of surgery | Tracer type | Tracer dye | Tracer volume | ICG concentration mg/ml | Bilateral detection rate | Sensitivity | NPV | FN |
|----------|-----|---------------------------|-------------|------------|---------------|--------------------------|--------------------------|-------------|-----|----|
| Holloway | 35  | Em                        | Robot       | ICG vs. blue | ISB           | 1.25                     | 100 vs. 100               | 97 vs. 77   | NA | NA | NA |
| Sinno    | 71  | Em                        | Robot       | ICG vs. blue | ISB           | 1.25                     | 92.7 vs. 77              | 78.9 vs. 42.4 | 100 | NA | NA |
| How      | 100 | Em                        | Robot       | ICG vs. blue | patent blue or methylene blue | 0.4 vs. 0.4 | 87 vs. 70 | 65 vs. 43 | NA | NA | NA |
| Buda     | 43  | Cx, Em                    | open laparo | ICG vs. blue | methylene blue | 4 vs. 4 | 100 vs. 84 | 88 vs. 71 | NA | NA | NA |
| Tanaka   | 119 | Cx                        | open laparo | ICG vs. IDC  | v RI          | 5 vs. 20.2               | 61.6 vs. 85.8             | 80 vs. 70.3 | 2.0 | NA | NA |
| Eriksson | 472 | Em                        | Robot       | ICG vs. blue | ISB           | 1.25                     | 95 vs. 85 vs. 81          | 54 vs. 54   | NA | NA | NA |

Abreviations: Em, endometrial cancer; Cx, cervical cancer; ICG, Indocyanine green; IDC, Indigo carmine; laparo, laparoscopy; ISB, Isosulfan blue; bil, bilateral; NPV, Negative predictive value; FN, false negative, NA, not applicable.

sometimes false-negatives obtained with traditional SLN identification techniques, which use injections of radiola-beled isotopes, such as Technetium, in conjunction with blue dyes, typically methylene blue or Lymphozurin). Although the isotopes need to be injected several hours before the surgery, to enable tracer uptake and transit-time to the SLN, the use of radiolabeled isotopes requires additional coordination with the Nuclear Medicine Department, and includes painful cervical injections. Hand-held or laparoscopic Geiger counters are used intraoperatively to detect isotope uptake by the SLNs, with the blue-dye acting as a visible guide to the SLN’s exact location.

Considerable experience and skill are required in the identification of SLN’s using this technology. Some suggest that a learning curve of 30 patients is necessary to achieve a level of competency associated with an acceptable false negative rate [10]. We hypothesized that these challenges could be overcome using the higher sensitivity of NIR imaging of fluorescent dyes. In addition, we used the novel optical filtering arrangement of the Olympus InfraRed Imaging System for visualizing lymph node drainage field. This would allow simultaneous real-time detection of bright-field color images and fluorescence images from multiple dyes (ICG and blue dye) using a single camera, with potential additive detection sensitivity. Thus, we introduced the ICG method into our surgical practice as potentially both a more effective and more convenient method than traditional isotope/blue dye techniques. We then compared their SLN detection rate using the ICG method with that of the blue colorimetric method.

The present results showed that NIR-FI-SLNB was significantly superior to the blue colorimetric method in bilateral SLN detection rate (75% vs. 14.3%, p = 0.0009). The bilateral detection rate of NIF-FI-SLNB (75%) was directly comparable to reports using radioisotope (RI) [9].

In this study, two findings were of major importance: first, all SLNs detected by blue dye were also detected by NIF-FI-SLNB, but the converse was not true; second, a single metastatic-positive lymph node was identified in one of the 16 patients, and critically, this positive SLN was detected only by the NIF-FI-SLNB method.

There are two reasons to explain the difference in results between the blue dye and NIF-FI-SLNB methods. Firstly, we could recognize the lymph vessels and nodes more clearly using the NIR optics system, as compared with visible light detection of the blue dye, even when the
camera’s surgical field was smudged by blood. Secondly, ICG fluorescent imaging was more prominently visualized through the visceral and retroperitoneal fat, as compared with the blue dye. Fluorescence imaging was able to identify lymphatics through up to approximately 3 mm of undissected tissue, assisting the surgeon to identify the lymphatic channel before it was interrupted, facilitating the ease of dissection [11].

From 2010 to 2017, use of ICG for SLN biopsy for gynecologic cancers has now been reported in 28 case series. Among these reports, there were four series using only laparotomy, five using only laparoscopy, six included cases of both laparoscopy and laparotomy, and 13 of the series used a robotic surgeon [12-17]. Six series compared the SLN detection rate of blue dye vs. ICG. Among them, four series used robots and two series reported both laparotomy and laparoscopy cases. To our knowledge, our study is unique in that it compares ICG and blue dye results using only laparoscopy.

In all 28 reports, ICG was found to be superior to blue dye for detection of SLN (Table 4). Tanaka et al. compared ICG’s detection rate to three different tracers (RI, indocyanine green, indigo carmine). They also reported that patients who underwent a laparoscopic procedure had a higher SLN detection rate (100% vs. 77%, \( p < 0.01 \)) and lower false negative (FN) rate (0% vs. 7.4%, \( p < 0.01 \)) than those who underwent laparotomy [16].

In order to better directly compare the actual detection rates of various SLN tracers, we believe it is mandatory to determine the surgical procedure being used for the comparison (laparotomy or laparoscopy).

A key limitation of this study is the small number of patients that could be safely used, as at the time this was still largely an experimental procedure for this type of uterine gynecological cancer. During the pre-sent study we gained such confidence that ICG was significantly superior to use of blue dye. We abandoned the blue colorimetric method altogether and currently use only the ICG-NIR method.

In conclusion, fluorescence imaging of SLN’s with endoscopic ICG NIR imagers is both a more effective and more convenient method than traditional blue colorimetric method. During SLN biopsy, the concomitant use of a blue dye might be no longer necessary.

Conflict of Interest

The authors have no conflicts of interest.

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