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# Tracer injection sites and combinations for sentinel lymph node detection in patients with endometrial cancer

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## HIGHLIGHTS

- We analyzed the most suitable combination of tracer type and injection sites to detect SLNs in 100 cases of endometrial cancer.
- SLNs detected by cervical RI injection were consistent with physiological lymphatic flow from uterine body in the pelvic cavity.

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## ABSTRACT

*Objective.* The aim of the present study was to clarify the most effective combination of injected tracer types and injection sites in order to detect sentinel lymph nodes (SLNs) in early endometrial cancer.

*Patients and methods.* The study included 100 consecutive patients with endometrial cancer treated at Tohoku University Hospital between June 2001 and December 2012. The procedure for SLN identification entailed either radioisotope (RI) injection into the endometrium during hysteroscopy (55 cases) or direct RI injection into the uterine cervix (45 cases). A combination of blue dye injected into the uterine cervix or uterine body intraoperatively in addition to preoperative RI injection occurred in 69 of 100 cases. All detected SLNs were recorded according to the individual tracer and the resultant staging from this method was compared to the final pathology of lymph node metastases including para-aortic nodes.

*Results.* SLN detection rate was highest (96%) by cervical RI injection; however, no SLNs were detected in para-aortic area. Para-aortic SLNs were detected only by hysteroscopic RI injection (56%). All cases with pelvic lymph node metastases were detected by pelvic SLN biopsy. Isolated positive para-aortic lymph nodes were detected in 3 patients. Bilateral SLN detection rate was high (96%; 26 of 27 cases) by cervical RI injection combined with dye.

*Conclusion.* RI injection into the uterine cervix is highly sensitive in detection of SLN metastasis in early stage endometrial cancer. It is a useful and safe modality when combined with blue dye injection into the uterine body.


## Introduction

The feasibility of sentinel lymph node (SLN) mapping for staging of gynecological malignancies has been explored in vulvar cancer [1,2], cervical cancer [3–6], and endometrial cancer [7–9]. In cervical and vulvar cancers, the appropriate tracer injection site for SLN detection is established. However, optimal injection sites for SLN detection in endometrial cancer are unclear. We previously reported $^{99m}$Tc-phytate injection into the subendometrium utilizing hysteroscopy and its detection rates; sensitivity, specificity, and negative predictive value were 82%, 100% and 100%, respectively, in a small series [7]. Other detection methods, including injection of isosulfan blue dye into the subserosal myometrium, or radioisotope (RI) or blue dye injection into the uterine cervix, have also been reported [10].

Regarding tracer injection utilizing hysteroscopy, there are reports [7,10] showing low SLN detection rates, despite the fact that testing the lymphatic flow of the uterus seems an appropriate way to detect cancer spread from the endometrium. It is difficult to identify SLNs in the para-aortic lymphatic basin by tracer injection into the uterine cervix [8,9]. However, it has been reported that cervical injection is technically easier and that the detection rate of SLNs is high; it is unclear, though, that these lymphatic pathways are physiologic. Recently, it was reported in a multicenter feasibility study that a dye-RI combination injected into the uterine cervix is feasible in stage I or II endometrial cancer and that a SLN could be detected in the pelvic area by the cervical injection method [9]. Furthermore, the number of cases in which para-aortic lymphadenectomy was

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performed was low (12%). It is not clear how many cases of true para-aortic SLN metastasis were missed.

Until the present study, there are no reports to our knowledge comparing SLN detection rates by analyzing the lymphatic flow from the uterine cervix and the uterine body. It is important to make clear whether SLN detection in the pelvic cavity is possible with any of the aforementioned methods and whether SLN detection or biopsy in the para-aortic lymphatic basin can be omitted with positive SLN identification in the pelvic cavity for the staging of endometrial cancer.

We sought to clarify the best combination of tracer injection (tracer types and injection sites) by analyzing SLN detection rates with each method (tracer) and by comparing the results of ultrastaging via SLN detection in the pelvic cavity to the postoperative pathologic presence of lymph node metastases including those in the para-aortic area.

## Materials and methods

### *Patients*

Consecutive patients between June 2001 and December 2012 with endometrial cancer were included in the study. All patients signed an informed consent and were treated at Tohoku University Hospital. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan (2001-099). Patients underwent laparotomy (total abdominal hysterectomy, bilateral salpingo-oophorectomy, total pelvic lymphadenectomy and para-aortic lymphadenectomy to the level of the renal veins) with SLN biopsy.

### *Eligibility criteria*

The eligibility criteria for SLN biopsy are as described previously [7], and are summarized as follows: endometrial carcinoma confirmed by biopsy, preoperative stages I and II (according to the 1988 International Federation of Gynecology and Obstetrics (FIGO) classification) [11], and adequate surgical staging (2009 FIGO classification) [12]. Excluded were patients who had evidence of lymph node metastases or other distant metastases on MRI and/or CT at their preoperative evaluation. Among those who underwent radioactive tracer injecting method by hysteroscopy, excluded were patients who could not undergo hysteroscopy.

### *SLN detection procedure*

The procedures for SLN identification are as described previously [3,7] and summarized as follows. On the day before surgery, preoperative lymphoscintigraphy was performed by injection of 99mTc-labeled phytate (DRL, Tokyo, Japan) into the endometrium around the tumor or into 5 sites (fundus, anterior and posterior wall, right side and left side wall) during hysteroscopy, or direct injection into the uterine cervix at the 3, 6, 9, and 12 o'clock positions. At the time of surgery, a gamma-detecting probe (Navigator GPS, RMD; Watertown, MA) was used to locate radioactive lymph nodes. In cases in which dual modality SLN detection was performed, patent blue (patent blue violet; Sigma, St. Louis, MO) was injected into the uterine cervix or the uterine body directly. In the uterine body injection cases, dye was injected at 5 sites into subserosal endometrium. In the cervical injection cases, dye was injected at the same site as RI injection site. Blue dye was utilized only for pelvic SLN detection in the present study.

### *Histopathological examination*

Frozen section analysis of detected SLNs was performed intraoperatively. Each SLN was examined utilizing step sectioning at 2 mm intervals parallel to the short axis of the node and examined with hematoxylin and eosin (H&E) staining. The remaining tissue was formalin-fixed and embedded in paraffin for routine histological analysis.

All surgically removed lymph nodes, including SLNs, were examined histopathologically using routine hematoxylin and eosin (H&E) staining. At least one section from each lymph node was taken at the maximal diameter and reviewed by two independent pathologists. When SLNs were diagnosed as negative for metastasis by routine H&E staining, immunohistochemistry (IHC) staining with an anti-cytokeratin antibody (AE1/AE3, DAKO, Japan) to detect cytokeratin was performed as follows. Paraffin-embedded tissue blocks were cut into 3 μm thick slides at 100-200 μm intervals in SLNs. IHC analysis was performed using an anti-cytokeratin antibody, AE1/AE3, at a 1: 500 dilution and the standard avidin-biotin complex technique (Ventana ES autostainer; Ventana Medical Systems, Tucson, AZ).

Micrometastases were defined as metastases measuring between 0.2 mm and 2 mm. A lymph node load measuring less than 0.2 mm was designated as isolated tumor cells (ITCs).

## Results

### *Patient characteristics*

One hundred patients were considered eligible and enrolled in this study. Ninety-nine patients underwent pelvic lymphadenectomy and para-aortic lymphadenectomy; only one patient was found to have peritoneal carcinomatosis and underwent no systematic lymphadenectomy. Fifty-five patients were in the hysteroscopic RI injection group. Twenty-four of 55 patients underwent direct blue dye injection into the uterine body and 1 patient underwent injection into the uterine cervix. Thus, a total of 25 patients underwent the combined (RI and blue dye) method in the hysteroscopic RI injection group. Forty-five patients were in the cervical RI injection group. Twenty-seven of these 45 patients underwent direct blue dye injection into the uterine body and 17 patients underwent injection into the uterine cervix. Thus, a total of 44 patients underwent the combined (RI and blue dye) method in the cervical RI injection group. We reanalyzed SLN sites of all 100 cases according to the individual (single) tracer. In other words, 55 patients underwent hysteroscopic RI injection, 45 underwent cervical RI injection, 51 underwent direct blue dye injection into the uterine body and 18 underwent direct blue dye injection into the uterine cervix. The clinicopathological characteristics of patients in these four groups are summarized in Table 1.

### *Detection rates and sites of SLNs*

Results of SLN detection are summarized in Tables 2 and 3. The detection rate was 78% (43/55) by hysteroscopic RI injection, 96% (43/45) by cervical RI injection, 63% (32/51) by direct dye injection into the uterine body and 89% (16/18) by direct dye injection into the cervix. SLNs were detected at the para-aortic lymph node area by only hysteroscopic RI injection (31/55, 56%). A SLN in the suprainguinal area was detected in only one of the patients by hysteroscopic RI injection. A SLN in the sacral area was detected in two patients by cervical RI injection (one had dye injection into the uterine cervix and one into the uterine body). Most patients had SLNs in the obturator or external iliac lymph node areas limited to the pelvic cavity.

### *Lymph node metastasis*

Characters of patients with metastasis confirmed by final pathology are summarized in Table 4. Frozen section analysis of detected SLNs was performed intraoperatively in 40 patients. No lymph node metastases were detected intraoperatively in 6 hysteroscopic injection RI cases, but in 6 of the 34 cervical RI injection cases SLN metastases could be detected. Six of 8 cases with lymph node metastasis by final pathology