Ultrastaging in Endometrial Cancer: Prognostic Significance and Artificial Intelligence (AI) Prediction Model of Additional Lymph Node Metastasis

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Directed by Professor Sang Wun Kim

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By Author

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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This study aimed to determine the rate of additional lymph node (LN) metastasis through ultrastaging in endometrial cancer (EC) patients who did not show LN metastasis on conventional Hematoxylin and Eosin (H&E) staining. The objectives also included developing an artificial intelligence (AI) model for predicting additional LN metastasis and comparing the prognosis between patients with and without additional LN metastasis on ultrastaging.

A total of 159 EC patients who underwent surgical staging with the sentinel lymph node (SLN) mapping algorithm using indocyanine green (ICG) between June 2014 and December 2017 at Yonsei Cancer Center were included in this study. A collection of 1001 paraffin blocks, comprising SLN (413 blocks) and non-SLN (588 blocks), was obtained from 138 patients who did not exhibit LN metastasis on H&E staining. Subsequently, serial sectioning with ultrastaging was performed. The final analysis included 109 patients with SLN samples.

In the analysis, LN metastasis detected through ultrastaging was identified in 14 out of 109 patients (12.8%) comprising 2 cases (1.8%) of macrometastasis (MAC); 11 cases (10.1%) of micrometastasis (MIC); and 1 case (0.9%) of isolated tumor cells (ITC). Notably, among 108 patients (excluding the ITC patient), the 3-year recurrence free survival showed significant differences based on the ultrastaging results: No metastasis (NM) at 98.9%; additional LN metastasis (MAC/MIC) at 69.2% ($p<0.001$). Similarly, the

3-year overall survival differed significantly between the groups: NM at 100%; additional LN metastasis (MAC/MIC) at 84.6% ($p<0.001$). The overall recurrence rate was 6.5%, with a significant difference between the groups (NM, 3.2%; additional LN metastasis (MAC/MIC), 30.8%; $p=0.004$). Analysis of the Cox proportional hazards model revealed that LN metastasis on ultrastaging was a significant prognostic factor for both recurrence free survival (hazard ratio 7.53, 95% confidence interval 1.49-39.99, $p=0.016$) and overall survival (hazard ratio 7.40, 95% confidence interval 1.21-51.97, $p=0.031$). Incorporating machine learning models to predict additional LN metastasis detection on ultrastaging, the LightGBM model demonstrated superior performance in the test set (sensitivity=1.000; specificity=0.778; F1 Value=0.750; ROC-AUC score=0.852). Histology (endometrioid grade 3 or non-endometrioid), histology (endometrioid grade 2), and age group(>60 years) were identified as significant features for predicting the detection of LN metastasis through ultrastaging.

In conclusion, the ultrastaging of LN in EC disclosed a notable incidence of metastasis, and the identification of LN metastasis on ultrastaging emerged as a prognostic factor. A machine learning model was developed to predict the detection of additional LN metastasis through ultrastaging. Subsequent large-scale research is necessary to validate the AI prediction model for identifying additional LN metastasis through ultrastaging in patients with EC.

Key Words: artificial intelligence; endometrial cancer; low-volume metastasis; sentinel lymph node; ultrastaging.

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I. INTRODUCTION

Endometrial cancer (EC) is the most common gynecological cancer in developed countries.[1-3] The incidence of EC has increased worldwide, including the Republic of Korea.[4,5] In 2023, it is estimated that there will be 3,813 new cases of endometrial cancer and 445 associated deaths in Republic of Korea.[6] While the prognosis is generally favorable when EC is detected early, the prognosis is poor for advanced or recurrent cases. Therefore, it is crucial to accurately diagnose and treat EC at an early stage. Total hysterectomy, bilateral salpingo-oophorectomy, and lymph node (LN) assessment are performed for surgical staging of EC, which is necessary for primary EC treatment and planning of proper adjuvant therapy.[3]

The determination of the disease stage through surgical staging is essential for the effective management of EC. The extent of disease progression revealed by the pathological findings of surgical staging influences both the prognosis and the choice of adjuvant therapy after surgery. Specifically, confirmation of LN metastasis is an important factor in determining the appropriate adjuvant treatments such as systemic chemotherapy or radiotherapy for patients with EC. LN metastasis can be definitively confirmed using intra-operative frozen sections or through a final pathological diagnosis.

In the past, lymphadenectomy was performed to confirm LN involvement and guide

treatment decisions. However, this procedure often led to complications such as lymphedema and decreased quality of life. Studies have investigated the impact of lymphadenectomy in surgical staging and its influence on patient prognosis, with the ASTEC trial demonstrating that lymphadenectomy does not improve outcomes in early-stage EC.[7] Another prospective study found that lymphadenectomy is significant for staging purposes but does not affect prognosis.[8] Based on these findings, alternative methods for accurate disease staging, such as sentinel lymph node (SLN) mapping, have been explored.

The concept of sentinel LN mapping was initially introduced in 1960 for parotid cancer, and since then, it has been applied to EC patients based on the use of SLN biopsy in 1996.[9,10] When cancer cells spread via the lymphatic pathway, they tend to accumulate in the LNs. SLN dissection involves identifying and removing the first LN that serves as the "sentinel" for cancer cells. Various substances such as methylene blue, technetium, and indocyanine green (ICG) can be used to locate the SLN.

Recently, SLN detection has been performed by injecting a fluorescent substance such as indocyanine green (ICG) during surgery and histological examination in patients with EC.[11] SLN mapping with ICG not only improves the diagnostic accuracy of LN metastasis but also prevents unnecessary LN dissection in EC.[12] Proper LN dissection can reduce mild-to-severe complications such as nerve injury, vessel injury, lymphedema, and infection. The FIRE trial confirmed the high accuracy of SLN mapping in low-risk EC, and subsequent prospective studies provided evidence supporting the application of SLN mapping in high-risk EC as well.[11-13]

Based on these results, our institution has been performing SLN biopsy during the staging procedure for endometrial cancer, and we have confirmed the safety and accuracy by applying a two-step SLN biopsy technique that allows the concurrent detection of aortic LN metastasis (one-step method, sensitivities 91.7%, negative predictive values 99.0%, false- negative rates 8.3%, accuracy rates 99.1%; two-step method, sensitivities 100.0%, negative predictive values 100.0%, false- negative rates 0%, accuracy rates

100.0%, respectively).[14,15] Additionally, we have compared the prognosis between SLN biopsy without ultrastaging and lymphadenectomy in endometrial cancer staging and found no significant difference.[16]

Ultrastaging typically involves performing H&E staining on grossly suspicious lesions and conducting further evaluation on negative LNs by creating serial sections with multiple slides stained with H&E or cytokeratin immunohistochemistry (IHC). Based on the size of the identified metastatic LNs, they are classified as macrometastasis (MAC, ≥ 2 mm), micrometastasis (MIC, 0.2-2 mm), or isolated tumor cells (ITC, ≤ 0.2 mm or ≤ 200 cells).

SLN mapping and ultrastaging in EC are recommended for LN assessment according to the National Comprehensive Cancer Network (NCCN) guidelines. The pathologic ultrastaging method, which analyzes pathologic slices more finely by "serial sectioning with the review of multiple H&E stained slides with or without cytokeratin IHC staining" according to the NCCN guidelines, is performed with SLN mapping. The European Society of Gynecological Oncology (ESGO) guidelines classify patients into risk groups and provide recommendations for LN dissection accordingly.[2] They recommend SLN mapping for low-intermediate risk and high-intermediate to high-risk cases of stage I or II EC. The recently introduced FIGO 2023 staging for endometrial cancer also incorporates SLN biopsy and staging based on ultrastaging.[17]

Ultrastaging is a "labor-intensive and time-consuming" method. In some cases of EC, metastatic LNs cannot be detected by classical pathological diagnosis and sometimes remain undetected.[18] EC histology, myometrial invasion, and lymphovascular invasion are considered risk factors for survival outcomes, but the prognostic impact of additional LN metastasis through ultrastaging is uncertain.[3,19,20] ITC may not be associated with survival outcomes.[20,21] In addition, few studies on the prognosis and postoperative adjuvant treatment of low-volume nodal metastasis of EC have been conducted.[18,22]

There was a study that applied a nomogram for predicting LN metastasis in patients with endometrial cancer using ultrastaging, but subsequent research on this topic has not

been conducted. Considering the safety of SLNs based on various research results for staging surgery in endometrial cancer and the guidelines developed from these studies, along with the changes in FIGO 2023 staging, predicting the necessity of ultrastaging is crucial.[17] It can provide additional confirmation of LN metastasis in appropriate patients, potentially influencing their treatment and prognosis.

Recently, artificial intelligence (AI) has been widely applied across various fields due to its advancements. In oncology, research on cancer diagnosis and prognosis is actively conducted. Machine learning, a subfield of AI, utilizes statistical and probabilistic mathematical knowledge and advanced technology to detect patterns in complex and extensive datasets. Researchers are generating models that can infer from these patterns. Applying these well-researched machine learning models to endometrial cancer could lead to the development of systematic classification models capable of categorizing diverse and complex patients appropriately. Such models could aid in diagnosis, treatment, and prognosis by providing inferences based on systematic classification.[23]

Based on this, an AI model will be effective in identifying patients with endometrial cancer who underwent SLN dissection and require additional ultrastaging. This model can be applied in clinical practice, potentially saving costs and time associated with ultrastaging procedures. By incorporating clinical data, pathological results, and ultrastaging data, a predictive model using machine learning will be capable of discovering additional LN metastasis through ultrastaging.

This study aimed to analyze and determine the number of patients initially negative for LN metastasis in routine H&E staining who subsequently showed additional LN metastasis through ultrastaging. Additionally, the study aimed to conduct a comparative analysis of the impact on survival outcomes between patients in whom additional LN metastasis was detected through ultrastaging and those without metastasis. Furthermore, the objective was to develop an artificial intelligence prediction model capable of identifying patients with a high probability of detecting additional LN metastasis through ultrastaging, utilizing clinical data such as pathological results.

II. MATERIALS AND METHODS

1. Study Design and Patients

This study was performed using electronic medical records (U-Severance 3.0; Severance Hospital Electronic Medical Record System). The study obtained approval from the Institutional Review Board of Severance Hospital (IRB No. 4-2018-0881). Informed consent was waived due to the retrospective nature of the study design.

Data on patients with EC who underwent SLN mapping with ICG injection using a robot or laparoscopy between June 2014 and December 2017 at Yonsei Cancer Center were screened. Patients who were diagnosed with EC, underwent surgical staging by gynecologic oncologists, including SLN mapping with ICG, and did not have metastatic pelvic/para-aortic LNs detected by routine H&E staining were included.

Patients who did not undergo SLN mapping during surgical staging, exhibited extensive metastasis during surgery, could not be injected with ICG due to contraindications (e.g., a hypersensitivity reaction or decreased liver function), or had a history of EC and had undergone a hysterectomy or LN dissection were excluded.

SLN tissue samples from selected patients were fixed with formalin, embedded in paraffin blocks, and archived in the Pathology Department. Furthermore, samples (1001 paraffin blocks) were collected and prepared for ultrastaging, with each LN not evenly resected.

2. Clinicopathological Data

The clinicopathological data of patients, including general characteristics such as diagnosis, stage, operation type, age, histology, grade and stage of malignancy, surgical management, adjuvant therapy, recurrence, and length of follow-up, were obtained. In addition, tumor size, invasion depth of the myometrium, and cell differentiation were investigated using postoperative pathological results. Demographic, histopathological, and surgical data were extracted from the electronic medical records. The demographic

data included age and body mass index (BMI). Histopathological data included International Federation of Gynecology and Obstetrics (FIGO) 2009 staging and FIGO grade; histological type (endometrioid or non-endometrioid); presence of lymphovascular space; myometrial, cervical stromal; Peritoneal cytology; serum CA 125; LN metastasis of preoperative MRI and PET-CT; pelvic and para-aortic LN dissection/sampling; and presence of pelvic and para-aortic LN metastases. Additionally, pathological markers used for diagnosis were extracted for AI prediction model analysis.

3. Staging Surgery using SLN Mapping with ICG

The surgical staging procedures included hysterectomy, bilateral salpingo-oophorectomy, and sentinel pelvic/para-aortic LN biopsy. SLN biopsy was performed using a one-step or two-step SLN mapping protocol, as described in a previous study.

In the one-step SLN mapping approach, in accordance with NCCN guidelines, indocyanine green (ICG) solution (1.25 mg/mL) was injected superficially (1–3 mm) and optionally deeply (1–2 cm) into the cervix at the 3 and 9 o'clock positions, respectively.

For the two-step SLN mapping method, 3 cc of ICG solution was injected into each cornu using a specific injection needle (0.7 mm needle tip, 330 mm working length, 5 mm diameter, 20 mm needle length, code no. 300-194-307, RZ Medizintechnik GmbH, Tuttlingen, Germany) inserted to a depth of 1 cm into the bilateral uterine cornus after tubal ligation. The lymphatics draining from the uterus were identified, and para-aortic SLNs were removed. After completing the para-aortic SLN biopsy, ICG was injected at the 3 and 9 o'clock positions of the ectocervix at superficial and deep cervix levels, 1 mL at each respective depth. All fluorescent SLNs were carefully dissected, and any suspicious LNs were surgically confirmed. Successful SLN mapping was defined as observing lymphatic drainage to at least one node on either side of the hemipelvis.

SLN mapping was performed for all patients included in the study. In specific cases, at the discretion of the clinicians and for validation purposes, lymphadenectomy was conducted following SLN mapping.

4. Ultrastaging of SLN

Ultrastaging of SLN was performed when routine H&E staining failed to detect LN metastasis. MAC was defined as metastatic tumors exceeding 2 mm in size. Low-volume metastasis encompassed ITC (≤0.2 mm in size and ≤200 cells) and MIC (>0.2–2 mm in size and >200 cells).

The ultrastaging process involved several steps, including slicing, staining, and slide examination. We utilized a modified ultrastaging method derived from the Gynecologic Oncology Reports of the Belgian Working Group for Gynecological Pathology and the pathologic ultrastaging algorithm for SLN mapping developed by the Memorial Sloan-Kettering Cancer Center [24,25].

Paraffin blocks were sliced into 1 to 3 levels at intervals of 200 μm. Each paraffin block was sectioned into three pairs of slices, each 3-μm thick, for H&E staining, IHC, and unstained slides. In cases where LN samples were small and multiple slicing was not feasible, a single-level slicing approach was adopted.

IHC staining was carried out using the anti-cytokeratin antibody AE1/AE3 (DAKO Company, Glostrup, Denmark) by a certified clinical laboratory technologist. Antigen retrieval was performed using the FLEX Target Retrieval Solution, High pH (K8004) (DAKO Company, Glostrup, Denmark).

A tissue sample slide was prepared, and the presence of LN metastasis was diagnosed by a gynecologic oncology pathologist based on the LN slides after special staining. To ensure consistency and accuracy in the pathological examination results, a qualified pathological specialist professor was selected for this analysis.

5. Postoperative Adjuvant Therapy

Adjuvant therapy, including radiotherapy, chemotherapy, and/or hormonal therapy, was administered according to the histopathological results and status of patients based on the EC treatment guidelines (NCCN and Korean Society of Gynecologic Oncology Guidelines) or consultative treatment recommendations.

6. Machine Learning Model

The machine learning model was constructed for predicting additional LN metastasis through ultrastaging in EC patients. The machine learning dataset was composed of the training set and test set. The training set consisted of data from patients who underwent ultrastaging through our research and were retrospectively analyzed. The test set was created with additional patient data for the purpose of evaluating machine learning performance. The test set included the data of randomly selected patients with endometrial cancer who had already undergone ultrastaging after SLN mapping through ICG injection, and the pathology results of the ultrastaging had been confirmed. Ultrastaging was performed to detect metastasis, serving as the ground truth for the study. The dataset was preprocessed to handle missing values and outliers before training the machine learning models.

Relevant features for prediction were selected from the clinical dataset. Various features were considered based on their potential impact on predicting metastasis. Feature selection techniques were employed to identify the most significant variables, ensuring the model's effectiveness and efficiency.

The training process incorporated ensemble learning techniques to enhance model performance due to their ability to alleviate overfitting and improve model robustness within the medical analysis context. The Random Forest, XGBoost, CatBoost, and LightGBM Classifiers—widely acknowledged in ensemble learning—were chosen for machine learning model training. Random Forest builds multiple decision trees in parallel and averages their predictions to achieve a more accurate and stable prediction. XGBoost sequentially builds trees, correcting errors from previous trees, and incorporates regularization techniques to control overfitting. CatBoost employs an efficient algorithm for handling categorical variables during tree construction. LightGBM grows trees leaf-wise, enhancing generalization performance by focusing on informative leaves.

All models were trained using a repeated 10-fold cross-validation approach on the training set. The repeated cross-validation aimed to robustly assess the models'

performance across various data splits.

The selected models were trained on features extracted from the training set. After training, model evaluations were conducted using standard classification metrics, including confusion matrices, sensitivity, specificity, precision, F1-score, and AUC-ROC score. F1-score was used as the primary metric for hyperparameter tuning to determine the optimal model configuration. The hyperparameter tuning process identified the following optimal configurations for each model.

- Random Forest: Tree max depth, 3; Number of trees, 10; Class weight, Balanced.
- XGBoost: Tree max depth, 3; Alpha, 2; Lambda, 2; Learning rate, 0.01; Weight for the positive label, the ratio of negatives to positives.
- CatBoost: Tree max depth, 3; Max iterations, 100; Learning rate, 0.01; Weight for positive label, the ratio of negatives to positives.
- LightGBM: Tree max depth, 3; Boosting type, gbdt; Number of leaves, 10; Learning rate, 0.01; Class weight, Balanced.

All models were implemented using the Python packages Scikit-Learn, XGBoost, CatBoost, and LightGBM.

7. Outcomes

The primary outcomes of the study were to determine the number of patients with additional LN metastasis detected through ultrastaging and to develop an artificial intelligence prediction model capable of identifying patients with a high likelihood of detecting additional LN metastasis through ultrastaging, utilizing clinical data including pathological results.

Secondary outcomes included comparing the recurrence rates and prognosis between patients with additional LN metastasis detected through ultrastaging and those without, measured through Recurrence Free Survival (RFS) and Overall Survival (OS) from staging surgery until disease recurrence or the last follow-up and identifying prognostic factors related to survival outcomes.

8. Statistical Analyses

Statistical analysis was performed using SPSS ver. 25.0 (IBM Corp., Armonk, NY) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Student's t-test, Mann–Whitney U test, or Kruskal-Wallis H test were used to compare numerical data. The $\chi 2$ or Fisher's exact tests were used to compare the proportions. Cox proportional hazard regression analysis was performed to investigate the prognostic factors for survival outcomes. In instances where perfect separation occurs, we employed a corrective measure using the Firth-type penalization method. The strength of the Firth-type penalty was set to 0.6 in order to mitigate this phenomenon. Survival curves obtained using the Kaplan–Meier method for the recurrence or survival of patients were analyzed using a log-rank test. Statistical significance was set at a p-value of <0.05.

III. RESULTS

1. Characteristics of the Patients

A total of 159 patients who underwent SLN mapping using ICG injection were analyzed. The flowchart of the study participants and recurrence rates is shown in Figure 1. A total of 1001 paraffin blocks from SLN and/or non-SLN (SLN, 413 blocks; non-SLN, 588 blocks) obtained from 138 patients presumed to be LN negative on H&E staining were examined. Those paraffin blocks were sliced into 6957 slides. A total of 2319 slides were stained IHC for ultrastaging of SLN and/or non-SLN.

Out of the total, 109 patients with SLN samples were included and analyzed for SLN and/or non-SLN samples according to the ultrastaging results: NM (n=95, 87.2%), MAC (n=2, 1.8%), MIC (n=11, 10.1%), and ITC (n=1, 0.9%).

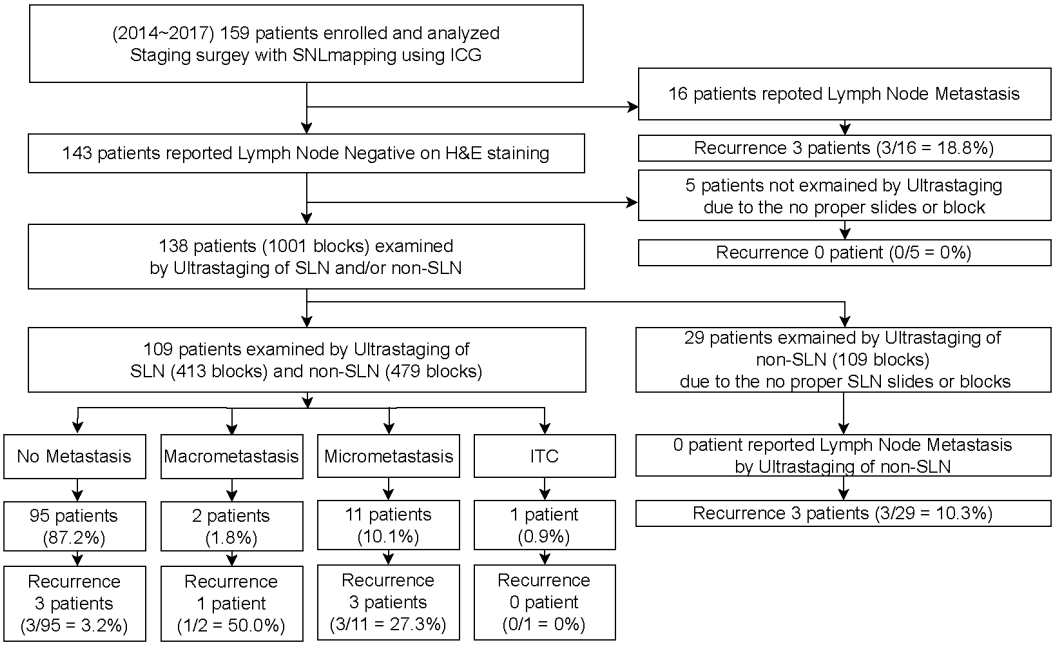

Figure 1. Flow Chart of the Study Participants and Examined Pathologic Blocks, and Ultrastaging Results and Recurrence Rate.

H&E, Hematoxylin and Eosin; ICG, Indocyanine Green; ITC, Isolated Tumor Cells; SLN, Sentinel Lymph Node.

The baseline characteristics of the patients are shown in Table 1. The median age and body mass index were 52.0 years and 24.6 kg/m2, respectively. Adjuvant treatment was administered after staging surgery. Vaginal brachytherapy was most commonly used in the NM group, and chemotherapy alone was most commonly used in the MIC group. Seventy-one (65.1%) patients did not receive postoperative adjuvant treatment (NM, n=66, 69.5%; MAC, n=0; MIC, n=5, 45.5%; ITC, n=0).

Table 1. Patient Characteristics based on Ultrastaging (n=109)

Characteristic	Total (n=109)	NM (n=95)	MAC (n=2)	MIC (n=11)	ITC (n=1)
Age (years), median (range)	52.0 (29-80)	51.0 (29-78)	52.5 (50-55)	59.0 (41-80)	65.0 (NA)
BMI (kg/m^2), median (range)	24.6 (16.5-41.7)	25.3 (16.5-41.7)	23.0 (22.2-23.7)	22.7 (17.2-28.8)	27.7 (NA)
CA 125 (U/ml) , median (range)	13.7 (4.3-647.5)	13.9 (4.3-182.2)	7.5 (6.8-8.2)	13.7 (7.0-647.5)	31.4 (NA)
FIGO 2009 Staging, N (%)					
Stage I	103 (94.5)	91 (95.8)	2 (100)	9 (81.8)	1 (100)
IA	93 (85.3)	83 (87.4)	1 (50.0)	8 (72.7)	1 (100)
IB	10 (9.2)	8 (8.4)	1 (50.0)	1 (9.1)	0
Stage II	4 (3.7)	3 (3.2)	0	1 (9.1)	0
Stage III	2 (1.8)	1 (1.1)	0	1 (9.1)	0
ESGO risk group, N (%)					
Low	78 (71.6)	72 (75.8)	0	6 (54.5)	0
Intermediate	8 (7.3)	7 (7.4)	1 (50.0)	0	0
High-Intermediate	10 (9.2)	7 (7.4)	1 (50.0)	1 (9.1)	1 (100)
High	13 (11.9)	9 (9.5)	0	4 (36.4)	0
Histology, N (%)					
Endometrioid	97 (89.0)	86 (90.5)	2 (100)	8 (72.7)	1 (1.0)
Endometroid FIGO Grade 1	62 (56.9)	56 (58.9)	0	5 (45.5)	1 (1.0)
Endometroid FIGO Grade 2	25 (22.9)	22 (23.2)	0	3 (27.3)	0
Endometroid FIGO Grade 3	10 (9.2)	8 (8.4)	2 (100)	0	0
Non-endometrioid	12 (11.0)	9 (9.5)	0	3 (27.3)	0
Serous	1 (0.9)	1 (1.1)	0	0	0
Clear cell	1 (0.9)	1 (1.1)	0	0	0
Mixed	2 (1.8)	2 (2.1)	0	0	0
Carinosarcoma	5 (4.6)	2 (2.1)	0	3 (27.3)	0
Mesonephric adenocarcinoma	3 (2.8)	3 (3.2)	0	0	0
Adjuvant therapy, N (%)					
BT alone	15 (13.8)	13 (13.7)	1 (50.0)	1 (9.1)	0
EBRT+BT	7 (6.4)	5 (5.3)	1 (50.0)	1 (9.1)	0
CT alone	13 (11.9)	8 (8.4)	0	4 (36.4)	1 (100)

BT+CT	1 (0.9)	1 (1.1)	0	0	0
EBRT+BT+CT	2 (1.8)	2 (2.1)	0	0	0
None	71 (65.1)	66 (69.5)	0	5 (45.5)	0
Myometrium invasion, N (%)					
None	45 (41.3)	43 (45.3)	0	2 (18.2)	0
<50%	52 (47.7)	43 (45.3)	1 (50.0)	7 (63.6)	1 (100)
≥50%	12 (11.0)	9 (9.5)	1 (50.0)	2 (18.2)	0
Cervical stromal invasion, N (%)					
Absent	104 (95.4)	91 (95.8)	2 (100)	10 (90.9)	1 (100)
Present	5 (4.6)	4 (4.2)	0	1 (9.1)	0
Lymphovascular invasion, N (%)					
Absent	86 (78.9)	77 (81.1)	2 (100)	7 (63.6)	0
Present	23 (21.1)	18 (18.9)	0	4 (36.4)	1 (100)
Peritoneal cytology, N (%)					
Negative	68 (62.4)	61 (64.2)	1 (50.0)	6 (54.5)	0
atypical cell	12 (11.0)	10 (90.9)	0	2 (18.2)	0
malignant cell	9 (8.3)	7 (7.4)	0	1 (9.1)	1 (100)
Not sampled	20 (18.3)	17 (17.9)	1 (50.0)	2 (18.2)	0
Tumor diameter, N (%)					
<2cm	47 (43.1)	43 (45.3)	1 (50.0)	3 (27.3)	0
≥2cm	62 (56.9)	52 (54.7)	1 (50.0)	8 (72.7)	1 (100)
Lymphadenectomy, N (%)					
Pelvic lymphadenectomy only	24 (22.0)	24 (25.3)	0	0	0
Pelvic lymphadenectomy and Para-aortic lymphadenectomy	85 (78.0)	71 (74.7)	2 (100)	11 (100)	1 (100)
Surgical approach, N (%)					
Laparoscopy	65 (59.6)	56 (58.9)	1 (50.0)	8 (72.7)	0
Robotic laparoscopy	44 (40.4)	39 (41.1)	1 (50.0)	3 (27.3)	1 (100)

BMI, Body Mass Index; BT, Brachytherapy; CT, Chemotherapy; EBRT, External Beam Radiation Therapy; FIGO, International Federation of Gynecology and Obstetrics; ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis; RT, Radiation Therapy.

2. Characteristics of Patients with Lymph Node Metastasis Detected through Ultrastaging

SLN and/or non-SLN metastasis was additionally detected in 14 patients through ultrastaging. The ITC patient (n=1) did not recur and was alive. The characteristics of patients with LN metastasis detected by ultrastaging are shown in Table 2. Four patients experienced recurrence among those with LN metastasis detected through ultrastaging (MAC, n=1; MIC, n=3).

Table 2. Characteristics of Patients with Lymph Node Metastasis Detected through Ultrastaging (n=14)

Patient	IHC	Age (yrs)	FIGO Stage	Histology	MI	TD (mm)	CSI	LVI	Cytology	Adjuvant therapy	Treatments of Recurrence	RFS	Site of Recurrence	OS	Status
1	MAC	50	IB	endometrioid (Grade 3)	≥50%	46	-	-	none	EBRT and BT	CT	14	Bone, muscle	39	death
2	MIC	48	IA	endometrioid (Grade 1)	None	26	-	-	malignant	CT alone		-	-	83	alive
3	MIC	80	IB	carcinosarcoma	≥50%	35	-	+	atypical	None†	Salvage RT	14	Lt.PALN	29	death
4	MIC	60	IA	carcinosarcoma	<50%	40	-	+	negative	CT alone	CT	11	Lung	29	death
5	ITC	65	IA	endometrioid (Grade 1)	<50%	27	-	+	malignant	CT alone		-	-	83	alive
6	MAC	55	IA	endometrioid (Grade 3)	<50%	17	-	-	negative	BT alone		-	-	87	alive
7	MIC	59	IA	endometrioid (Grade 2)	<50%	25	-	-	negative	None		-	-	85	alive
8	MIC	51	IA	endometrioid (Grade 1)	<50%	39	-	-	negative	None		-	-	94	alive
9	MIC	56	IA	endometrioid (Grade 1)	None	5	-	-	negative	None		-	-	95	alive
10	MIC	41	IA	endometrioid (Grade 1)	<50%	36	-	-	atypical	None		-	-	89	alive
11	MIC	64	IA	Endometrioid (Grade 1)	<50%	17	-	+	negative	BT alone		-	-	77	alive
12	MIC	70	II	endometrioid (Grade 2)	≥50%	74	+	+	negative	EBRT		-	-	100	alive
13	MIC	44	IIIA	endometrioid (Grade 2)	<50%	31	-	-	none	CT alone		-	-	71	alive
14	MIC	69	IA	carcinosarcoma	<50%	micro scopic	-	-	none	None	Lung wedge resection and CT	36	Lung	80	alive

BT, Brachytherapy; CSI, Cervical Stromal Invasion; CT, Chemotherapy; RFS; Recurrence Free Survival; EBRT, External Beam Radiation Therapy; IHC, Immunohistochemistry for ultrastaging; ITC, Isolated Tumor Cells; LVI, Lymphovascular Invasion; MAC, Macrometastasis; MI, Myometrial Invasion; MIC, Micrometastasis; NM, No Metastasis; OS, Overall Survival; PALN, para-aortic lymph node; RT, Radiation Therapy; TD, Tumor Diameter; †Patient's refusal.

3. Survival Outcomes

A. Survival Outcomes According to Ultrastaging

Table 3. Survival Outcomes According to Ultrastaging (n = 108, excluding ITC)

	Total (n=108)	NM (n=95)	MAC/MIC (n=13)	p-value
Length of Follow-up (months), median (range)	85.5 (29-111)	86.0 (47-111)	83.0 (29-100)	0.218
Recurrence Free Survival (Kaplan-Meier estimates), %				<0.001
at 3 year	95.4	98.9	69.2	
at 5 year	93.5	96.8	69.2	
Overall Survival (Kaplan-Meier estimates), %				<0.001
at 3 year	98.1	100	84.6	
at 5 year	95.4	97.9	76.9	
Recurrence, No. (%)	7 (6.5)	3 (3.2)	4 (30.8)	0.004
The sites of relapse, No. *(%) all recurrent sites in a patient were counted.*				
Lung	3 (2.8)	1 (1.1)	2 (15.4)	0.036
Liver	1 (0.9)	1 (1.1)	0	0.880
Bone	1 (0.9)	0	1 (7.7)	0.120
Peritoneum	1 (0.9)	1 (1.1)	0	0.880
Para-aortic LN	1 (0.9)	0	1 (7.7)	0.120
Deaths by cause, No. (%)	5 (4.6)	2 (2.1)	3 (23.1)	0.012
Unrelated morbidity	0	0	0	
Endometrial cancer	5 (4.6)	2 (2.1)	3 (23.1)	

ITC, Isolated Tumor Cells; LN, lymph node; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

Survival outcomes of 108 patients (excluding one patient of ITC) were analyzed. The total median length of follow-up was 85.5 (range, 29-111) months (NM, 86.0 (range, 47-111) months; additional LN metastasis (MAC/MIC), 83.0 (range, 29-100) months, p=0.218) (Table 3, Figure 2). The 3-year RFS differed significantly according to the ultrastaging results (Total, 95.4%; NM, 98.9%; additional LN metastasis (MAC/MIC), 69.2%; p<0.001). The 3-year OS was significantly different between the groups (Total, 98.1%; NM, 100%; additional LN metastasis (MAC/MIC), 84.6%; p<0.001).

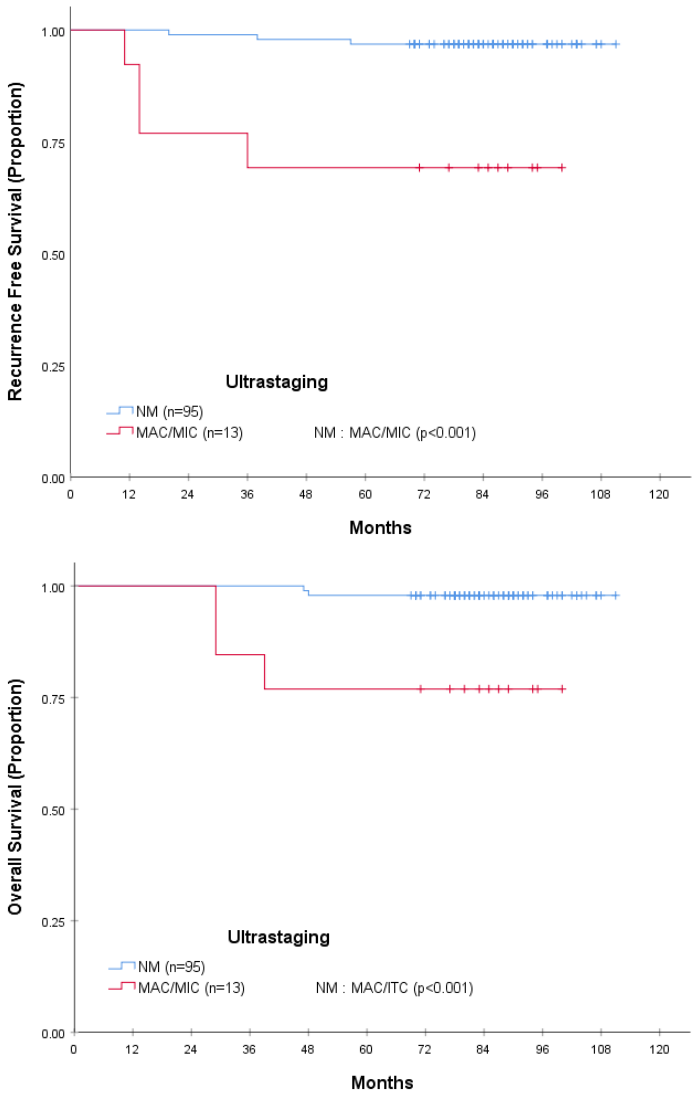

Figure 2. Survival Outcomes According to Ultrastaging (n = 108, excluding ITC).
ITC, Isolated Tumor cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

B. Survival Outcomes According to Pathologic Lymph Node Findings

The survival outcomes of 125 patients who underwent SLN mapping using ICG injection were analyzed based on pathologic LN findings as like LN metastasis detected by routine H&E staining before ultrastaging, NM or ITC detected through ultrastaging and MAC or MIC detected through ultrastaging (Table 4, Figure 3).

Table 4. Survival Outcomes According to Pathologic Lymph Node Findings (n=125)

	H&E (+) (n=16)	NM/ITC (n=96)	MAC/MIC (n=13)	p-value
Length of Follow-up (months), median (range)	74.0 (61-99)	86.0 (47-111)	83.0 (29-100)	0.028
Recurrence Free Survival (Kaplan-Meier estimates), %				<0.001
at 3 year	87.5	99.0	69.2	
at 5 year	81.3	96.9	69.2	
Overall Survival (Kaplan-Meier estimates), %				0.002
at 3 year	100	100	84.6	
at 5 year	100	97.9	76.9	
Endometrial cancer recurrence, No. (%)	3 (18.8)	3 (3.1)	4 (30.8)	0.001
The sites of relapse, No. *(%) all recurrent sites in a patient were counted.*				
Lung	0	1 (1.0)	2 (15.4)	0.035
Liver	0	1 (1.0)	0	1.000
Bone	0	0	1 (7.7)	0.104
Adrenal gland	1 (6.3)	0	0	0.232
Peritoneum	0	1 (1.0)	0	1.000
Pelvic LN	1 (6.3)	0	0	0.232
Para-aortic LN	0	0	1 (7.7)	0.104
Ascites	1 (6.3)	0	0	0.232
Deaths by cause, No. (%)	1 (6.3)	2 (2.1)	3 (23.1)	0.009
Unrelated morbidity	0	0	0	
Endometrial cancer	1 (6.3)	2 (2.1)	3 (23.1)	

ITC: Isolated Tumor Cells; LN, Lymph Node; MAC: Macrometastasis; MIC: Micrometastasis; NM: No Metastasis.

The 3-year RFS differed significantly according to the pathologic LN findings ($p<0.001$). The 3-year OS was significantly different between the groups ($p=0.002$). The total recurrence rate was a significant difference between the groups ($p=0.001$). Among the three groups, the highest recurrence rate was MAC/MIC detected through ultrastaging (30.8%). The recurrence rate of patients with LN metastasis detected by routine H&E staining before ultrastaging was 18.8%.

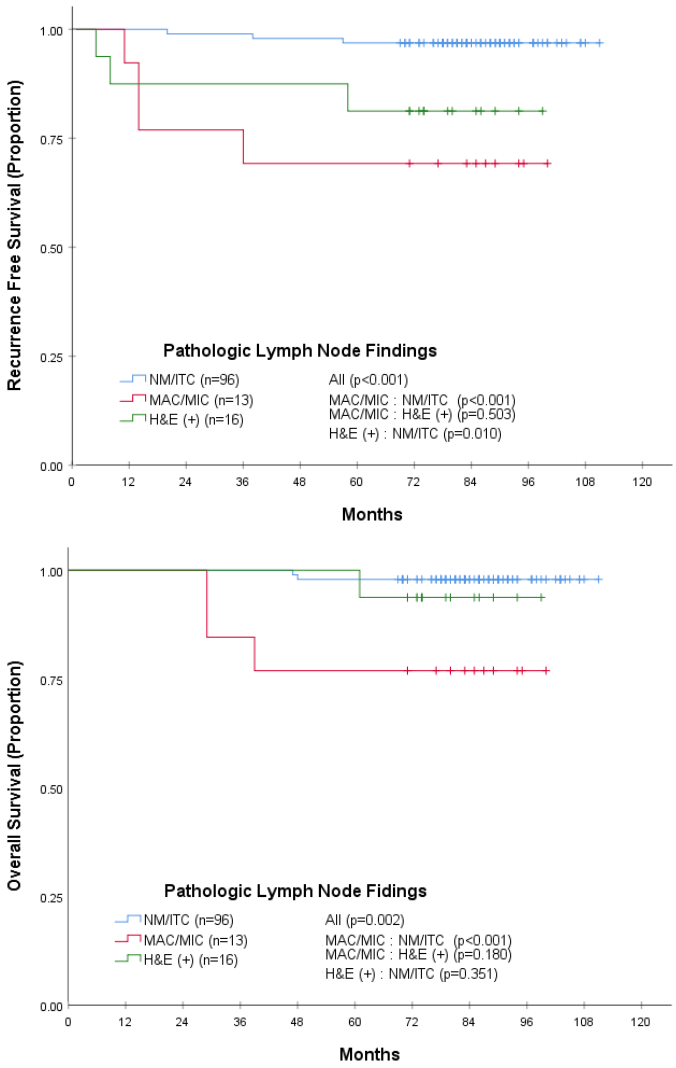

Figure 3. Survival Outcomes According to Pathologic Lymph Node Findings (n = 125). H&E, Hematoxylin and Eosin; ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

4. Metastasis Detected According to the Level of Examined Slides through Ultrastaging

The 1001 blocks of SLN and/or non-SLN of 138 patients were sliced into one to three levels and a total of 2319 slides were stained using IHC. In total, 27 paraffin blocks (SLN, 20; non-SLN, 7) were sliced into one level, 594 (SLN, 79; non-SLN, 515) were sliced into two levels, and 380 (SLN, 314; non-SLN, 66) were sliced into three levels.

Table 5. Pathologic Results of Additional Lymph Node Metastasis Detected through Ultrastaging

Patient	IHC	LN site	SLN/non-SLN	1 level	2 level	3 level
1	MAC	Rt. external	SLN	NM	MAC	NM
2	MIC	Lt. pelvic	SLN	NM	MIC	NM
3	MIC	Lt. internal	SLN	MIC	MIC	none
4	MIC	Rt. external	SLN	MIC	MIC	ITC
5	ITC	Rt. obturator	SLN	ITC	ITC	ITC
6	MAC	Rt. obturator	SLN	MIC	MIC	NM
		L3M	non-SLN	MAC	MAC	none
7	MIC	L3M	SLN	MIC	MIC	ITC
		Lt. parametrial	SLN	ITC	ITC	none
		Rt. internal	non-SLN	MIC	MIC	none
8	MIC	Rt. internal	SLN	MIC	ITC	ITC
		L34R	non-SLN	ITC	ITC	none
9	MIC	Lt. obturator	non-SLN	MIC	NM	none
10	MIC	Lt. obturator†	non-SLN	NM	NM	MIC
11	MIC	Lt. common	non-SLN	MIC	ITC	none
12	MIC	Lt. pelvic†	non-SLN	MIC	MIC	none
13	MIC	Rt. pelvic	non-SLN	MIC	MIC	none
14	MIC	Rt. pelvic	non-SLN	MIC	MIC	none

IHC, immunohistochemistry for ultrastaging; ITC, Isolated Tumor Cells; LN, lymph node; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis; SLN, Sentinel Lymph Node.† Lymph node dissection due to non-mapping.

There were 413 blocks of SLN for 109 patients and 588 blocks of non-SLN for 133 patients. There were 18 blocks of additional metastasis detected in SLN and/or non-SLN through ultrastaging among 14 patients (MAC, 2 blocks; MIC 13 blocks; ITC 7 blocks) (Table 5).

A total of 36 (1.6%) slides were additionally detected metastasis based on ultrastaging (MAC, 3 slides; MIC, 21 slides; and ITC, 12 slides). The metastasis detected in each sliced level is detailed as follows: the first level (n=10, 76.9%), the second level (n=2, 15.4%), and on the third-level slide, one patient (7.7%) showed metastasis. As we moved to

subsequent levels, the detection rate progressively decreased, highlighting the diminishing likelihood of finding additional metastases. Metastasis was detected on the third level slide in only one patient (#10) (Table 5, Table 6).

Table 6. Metastasis Detection in Sliced Levels through Ultrastaging (n=13, excluding ITC)

	MAC/MIC detected through ultrastaging
1st. level	10 (76.9%)
2nd. level	2 (15.4%)
3rd. level	1 (7.7%)

ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

5. Cox Proportional Hazards Model of Survival Outcomes

Table 7. Cox Proportional Hazard Model of Recurrence Free Survival (n=109)

Variables	Univariate analysis (RFS)			Multivariate analysis (RFS)		
	Hazard ratio	95% Confidence interval	*p*-value	Hazard ratio	95% Confidence interval	*p*-value
Age						
≤60	1.00	(Reference)		1.00	(Reference)	
>60	1.12	1.04-1.22	**0.002**	1.05	0.98-1.15	0.149
Ultrastaging						
NM or ITC	1.00	(Reference)		1.00	(Reference)	
MAC or MIC	11.51	2.84-50.46	**0.001**	7.53	1.49-39.99	**0.016**
Histology						
Endometrioid Grade 1	1.00	(Reference)		1.00	(Reference)	
Endometrioid Grade 2	0.93	0.01-14.93	0.962	0.38	0.00-7.25	0.531
Endometrioid Grade 3 or Non-endometrioid	13.50	2.93-118.83	**0.001**	6.25	1.00-61.24	**0.049**
Myometrial invasion						
None or <50%	1.00	(Reference)		1.00	(Reference)	
≥50%	7.24	1.65-29.28	**0.011**	2.44	0.43-13.90	0.302

RFS, Recurrence Free Survival; IHC, Immunohistochemistry; ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

Univariate analysis of the Cox proportional hazards model for RFS and OS showed that age, ultrastaging, histology and myometrial invasion were statistically significant. Multivariate analysis of the Cox proportional hazards model revealed that the prognostic factors for RFS were additionally detected LN metastasis through ultrastaging, and histology (endometrioid grade 3 or non-endometrioid) (additionally detected LN metastasis through ultrastaging, hazard ratio 7.53, 95% confidence interval 1.49-39.99, p=0.016; endometrioid grade 3 or non-endometrioid, hazard ratio 6.25, 95% confidence interval 1.00-61.24, p=0.049) (Table 7).

Multivariate analysis of the Cox proportional hazards model revealed that the prognostic factor for OS was additionally detected LN metastasis through ultrastaging (endometrioid grade 3 or non-endometrioid) (hazard ratio 7.40, 95% confidence interval 1.21-51.97, p=0.031) (Table 8).

Table 8. Cox Proportional Hazard Model of Overall Survival (n=109)

Variables	Univariate analysis (OS)			Multivariate analysis (OS)		
	Hazard ratio	95% Confidence interval	*p*-value	Hazard ratio	95% Confidence interval	*p*-value
Age						
≤60	1.00	(Reference)		1.00	(Reference)	
>60	1.12	1.02-1.23	**0.012**	1.04	0.96-1.13	0.383
Ultrastaging						
NM or ITC	1.00	(Reference)		1.00	(Reference)	
MAC or MIC	11.87	2.30-71.41	**0.004**	7.40	1.21-51.97	**0.031**
Histology						
Endometrioid Grade 1	1.00	(Reference)		1.00	(Reference)	
Endometrioid Grade 2	2.48	0.01-457.23	0.655	0.90	0.01-100.82	0.957
Endometrioid Grade 3 or Non-endometrioid	33.98	3.85-4462.27	**<0.001**	10.71	0.89-808.38	0.062
Myometrial invasion						
None or <50%	1.00	(Reference)		1.00	(Reference)	
≥50%	12.59	2.44-75.72	**0.004**	4.10	0.74-28.30	0.105

IHC, Immunohistochemistry; ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis; OS, Overall Survival.

6. Machine Learning Model for Prediction of Metastasis Detected of Ultrastaging

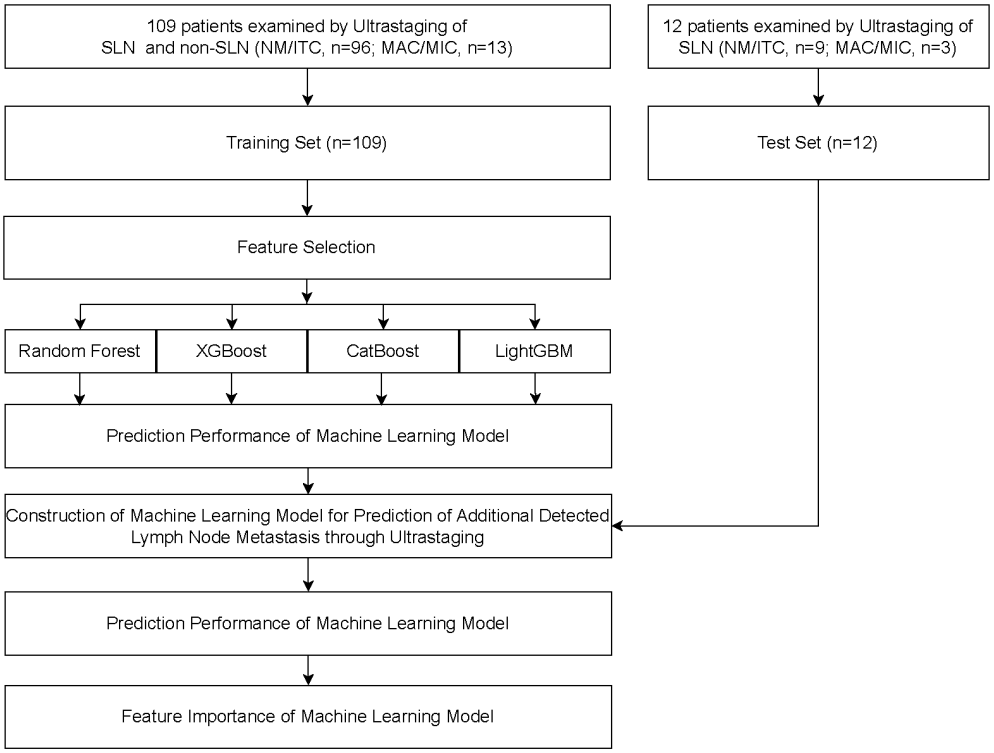

Figure 4. Flow Chart of the Construction of Machine Learning Model.
ITC, Isolated Tumor cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis; SLN, Sentinel Lymph Node.

A total of 109 patients with SLN who underwent ultrastaging after SLN mapping using ICG injection were included in the dataset of machine learning. The flowchart of the construction of the machine learning model is shown in Figure 4.

The training set consisted of 109 patients. The features selected for the machine learning model to predict additional LN metastasis detected during ultrastaging include age groups (≤60, >60 years), BMI (kg/m^2), serum CA 125 groups (≤35, >35 U/ml), histology (Endometrioid grade 1, Endometrioid grade 2, Endometrioid grade 3 or Non-endometrioid), myometrial invasion, lymphovascular invasion, cervical stromal invasion.

The data for all pathologic markers were extracted from the pathologic results of

patients. The p53, ER, PR, p16, PTEN, MMR deficiency (MLH1, MSH2, MSH6, PMS2), Vimentin, CD10, PAX2, Beta-catenin, and ARID1A were selected for feature selection. However, it was found that these markers were not suitable for constructing machine learning models and were therefore excluded.

The test set contained data from 12 patients who underwent ultrastaging after SNL mapping with ICG injection for surgical staging of endometrial cancer (Table 9).

Table 9. Characteristics of Patients in the Test Set of the Machine Learning Model (n=12)

Patient	SLN IHC	Age (years)	BMI (kg/m^2)	Histology	MI	CSI	LVI	CA 125 (U/ml)
Test 1	NM	62	30.82	carcinosarcoma	<50%	-	-	9.3
Test 2	NM	55	20.56	endometrioid (Grade 2)	<50%	-	-	46.5
Test 3	MIC	75	19.54	endometrioid (Grade 2)	≥50%	-	-	11.8
Test 4	NM	56	25.1	endometrioid (Grade 2)	≥50%	-	+	213
Test 5	NM	72	21.45	endometrioid (Grade 1)	≥50%	-	-	13.9
Test 6	NM	50	28.44	endometrioid (Grade 2)	≥50%	-	-	30.9
Test 7	MIC	70	23.34	endometrioid (Grade 2)	≥50%	-	+	309
Test 8	ITC	54	23.12	endometrioid (Grade 2)	<50%	-	+	13.8
Test 9	NM	61	23.75	endometrioid (Grade 2)	<50%	-	-	69.4
Test 10	NM	69	21.16	endometrioid (Grade 1)	<50%	-	-	17.9
Test 11	NM	34	22.2	endometrioid (Grade 1)	None	-	-	12
Test 12	MIC	55	20.13	endometrioid (Grade 3)	<50%	-	-	7.5

CSI, Cervical Stromal Invasion; IHC, immunohistochemistry for ultrastaging; ITC, Isolated Tumor Cells; LVI, Lymphovascular Invasion; MI, Myometrial Invasion; MIC, Micrometastasis; NM, No Metastasis; SLN, Sentinel Lymph Node.

A. Confusion Matrix of Machine Learning Model

Machine learning for classification was performed using Random Forest, XGBoost, CatBoost, and LightGBM.

The predictive performance of the machine learning model was assessed through the confusion matrix. To improve prediction accuracy, adjustments for imbalanced datasets were implemented using Random Forest, XGBoost, CatBoost, and LightGBM classifiers. The corresponding confusion matrices are presented in Table 10.

Table 10. Confusion Matrix of Machine Learning Model

A. Random Forest		Predicted NM/ITC	Predicted MAC/MIC
Training Set	Actual NM/ITC	78	18
	Actual MAC/MIC	8	5
Test Set	Actual NM/ITC	7	2
	Actual MAC/MIC	2	1

B. XGBoost		Predicted NM/ITC	Predicted MAC/MIC
Training Set	Actual NM/ITC	71	25
	Actual MAC/MIC	5	8
Test Set	Actual NM/ITC	5	4
	Actual MAC/MIC	1	2

C. CatBoost		Predicted NM/ITC	Predicted MAC/MIC
Training Set	Actual NM/ITC	75	21
	Actual MAC/MIC	6	7
Test Set	Actual NM/ITC	7	2
	Actual MAC/MIC	1	2

D. LightGBM		Predicted NM/ITC	Predicted MAC/MIC
Training Set	Actual NM/ITC	74	22
	Actual MAC/MIC	7	6
Test Set	Actual NM/ITC	7	2
	Actual MAC/MIC	0	3

ITC, Isolated Tumor Cells; MAC, Macrometastasis; MIC, Micrometastasis; NM, No Metastasis.

B. Prediction Performance of Machine Learning Model

Based on the confusion matrix, the prediction performance of the machine learning models was evaluated, as shown in Table 11. The model of the LightGBM classifier showed the highest performance on the test set (sensitivity=1.000; specificity=0.778; F1 Value=0.750; ROC-AUC score=0.852) (Table 11).

Table 11. Prediction Performance of Machine Learning Model

	Prediction Performance	Random Forest	XGBoost	CatBoost	LightGBM
Training Set	Sensitivity	0.385	0.615	0.538	0.462
	Specificity	0.812	0.740	0.781	0.771
	Precision	0.217	0.242	0.250	0.214
	F1 Value	0.278	0.348	0.341	0.293
	ROC-AUC Score	0.719	0.711	0.724	0.639
Test Set	Sensitivity	0.333	0.667	0.667	**1.000**
	Specificity	0.778	0.556	0.778	**0.778**
	Precision	0.333	0.333	0.500	**0.600**
	F1 Value	0.333	0.444	0.571	**0.750**
	ROC-AUC Score	0.519	0.685	0.741	**0.852**

C. Feature Importance of Machine Learning Model

The feature importance of the machine learning models is presented in Figure 5. All models consistently emphasized the feature importance related to histology. The LightGBM model, which demonstrated the highest prediction performance in the test set, highlighted the significance of histology (endometrioid grade 3 or non-endometrioid), histology (endometrioid grade 2), and age group (>60 years).

The other models highlighted the feature importance of histology (endometrioid grade 3 or non-endometrioid), histology (endometrioid grade 2), serum CA 125 group (>35 U/ml), lymphovascular invasion, and/or myometrial invasion ≥50%.

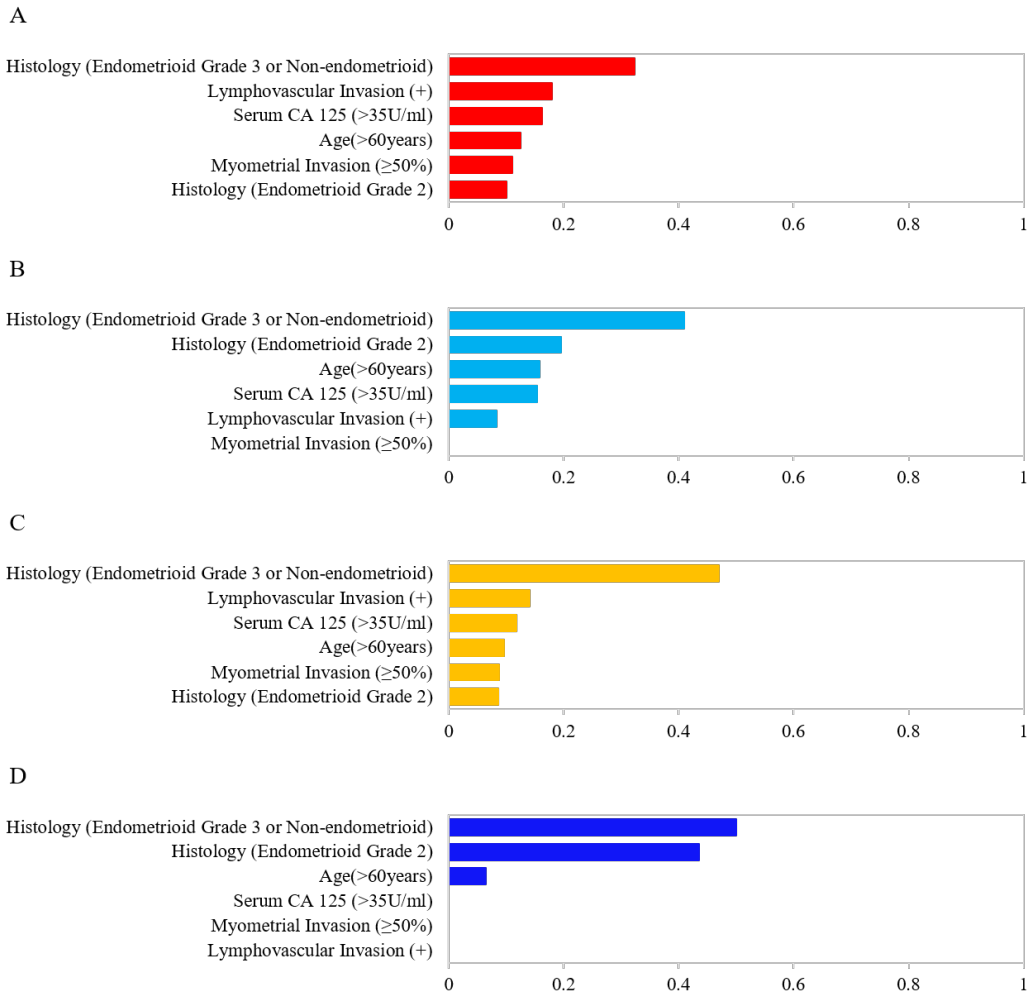

Figure 5. Feature Importance of Machine Learning Model.
A, Random Forest; B, XGBoost; C, CatBoost; D, LightGBM.

IV. DISCUSSION

In this study, ultrastaging after SLN mapping with ICG injection in endometrial cancer patients, who did not exhibit LN metastasis on H&E staining, enabled the detection of additional LN metastasis. The identification of additional LN metastasis through ultrastaging may be considered a prognostic factor for survival outcomes. The machine learning model, especially the LightGBM classifier, performed well, highlighting the importance of histology (endometrioid grade 3 or non-endometrioid), histology (endometrioid grade 2), and age group (>60 years) for additional LN metastasis prediction using ultrastaging.

Gracia et al. reported that the progression-free survival (PFS) and 5-year OS were significantly worse in patients with MAC than in those with low-volume metastasis and NM (PFS, p=0.018; OS, p<0.001).[26] Plante et al. reported that the 3-year PFS was significant among SLN metastases (p=0.0012). Additionally, 1 of 31 patients with ITCs experienced recurrence after adjuvant therapy.[20] Backes et al. reported that no patient experienced a recurrence of ITCs.[21] Goebel et al. reported that 21 of 155 (13.5%) patients had ITCs after initial non-metastatic SLN, 8 of 21 patients with ITCs (38.1%) underwent adjuvant therapy due to other high-risk factors, and the other patients with previously undetected ITCs showed no recurrence without adjuvant treatment.[27]

In our study, 14 out of 109 patients (12.8%) exhibited additional metastatic LNs after ultrastaging, with no metastatic LNs found on gross pathology. Similar to a previous study, an analysis of 108 patients, excluding ITC cases, revealed significant differences in 3-year RFS and OS based on the presence of additional metastatic LNs. The only patient (0.9%) detected with ITCs did not experience recurrence and remained alive after receiving adjuvant chemotherapy due to malignant peritoneal cytology.

EC recurrences often occur in the vagina, pelvic LNs, para-aortic LNs, peritoneum, and lungs but less often in the extra-abdominal nodes, intra-abdominal organs, musculoskeletal and soft tissues, and central nervous system (<1%).[28] In the LAP2 study of the Gynecologic Oncology Group, the recurrence sites included the lungs, vagina,

abdomen, pelvis, LNs, liver, and bones.[29] In the LACE trial, stage I EC after laparoscopic hysterectomy relapsed most commonly in the vaginal vault (3%) and <2% in the pelvis, abdomen, distant organs, or at multiple sites.[30]

Among 108 patients (excluding one patient with ITC after ultrastaging of SLN samples), there was a significant difference in the recurrence rate based on the detection of additional LN metastasis through ultrastaging (p=0.004). Recurrence sites included the lung, liver, bone, perineum, and paraaortic LN. The most prevalent recurrence site was the lung. While there is a limitation in the small sample size, the study exhibited a similar trend to previous research.

When examining survival outcomes based on pathologic LN findings (Table 4), the detection of additional LN metastasis (MAC/MIC) through ultrastaging appeared to indicate a trend toward poorer outcomes compared to those detected by routine H&E staining, although statistically significant differences were not observed. To confirm the existence of this trend in survival outcome differences and influencing factors more accurately, future large-scale studies would be necessary.

Previous studies have suggested prognostic factors such as age, stage, histology, LN status, tumor size, lymphovascular space invasion, and tumor involved the lower uterine segment in EC.[31-34] Ting et al. reported that the only prognostic factor for RFS in the multivariable Cox proportional hazards model was para-aortic LN metastasis (HR=7.60, p=0.03).[35] Tilman et al. showed that the prognostic factors of OS were stage, grade, lymphovascular space invasion, and histological subtype in multivariate analysis with Cox regression, but not the combination of MAC and MIC or ITCs.[36] Buda et al. reported that lymphovascular space invasion was a prognostic factor for recurrence but not the type of nodal metastasis.[37]

Unlike previous studies, our research identified additional LN metastasis (MAC/MIC) through ultrastaging as a prognostic factor for survival outcomes. This difference may be attributed to the fact that, unlike in other studies, the patient group in our study for Cox proportional hazard regression analysis excluded those with confirmed LN metastasis in

gross pathology. This exclusion could lead to different results in factors influencing survival outcomes compared to previous studies. Moreover, our study is limited by a small sample size, resulting in a low absolute number of recurrent and deceased cases. Due to the small absolute number, the Cox proportional hazard regression analysis applied the Firth-type penalization method to correct for perfect separation. However, additional analysis through larger-scale studies is necessary to validate these findings.

In endometrial cancer, the study predicting metastasis detected through ultrastaging was previously conducted by Koskas et al. In their research, they employed a nomogram integrating factors such as age, race, histology, tumor grade, and primary tumor extension (myometrial invasion or cervical stroma invasion) to predict metastasis detected through both conventional histopathology and ultrastaging. The nomogram demonstrated good discrimination for predicting MAC in conventional histopathology (AUC = 0.76) but showed lower accuracy for ultrastaging (AUC = 0.67).[38]

Recent studies have embraced advanced techniques in artificial intelligence to predict LN metastasis in various cancers.[23] Meng et al. applied logistic regression and random forest machine learning models to predict LN metastasis in patients with early cervical cancer.[39] Asami et al. constructed the prediction model of LN metastasis of endometrial cancer using logistic regression, supervised machine learning classifiers of support vector machines, and random forest.[40] Moreover, Li et al. reported the predicting model for LN metastasis in osteosarcoma using six machine learning models: logistic regression, gradient boosting machine, extreme gradient boosting, random forest, decision tree, and multilayer perceptron.[41]

The previous research has primarily focused on machine learning models related to gross LN metastasis in cancers. Moreover, no research on predictive models for ultrastaging in endometrial cancer has been performed since the use of the previous nomogram. Based on the current literature search, our study represents the first application of a machine learning model to predict additional LN metastasis through ultrastaging in endometrial cancer.

Among the Random Forest, XGBoost, CatBoost, and LightGBM classifiers, the LightGBM model exhibited the most superior performance on the test set. LightGBM showed better results on the test set compared to the training set, which is considered a significant model due to its analysis with fewer features than other models and superior performance on the test set. Additionally, all machine learning models consistently highlighted histology as a feature importance. The utilization of AI, incorporating these features, could assist in mitigating the time and labor-intensive nature of implementing ultrastaging. However, it is crucial to note that the study is based on a small dataset, and further validation through larger-scale studies is necessary. A comprehensive analysis was conducted to develop a predictive model for machine learning, utilizing various variables for feature selection. In particular, an investigation into the extracted pathological markers used for diagnosis revealed potential issues. Notably, not all patients in the training set underwent the same examinations. The limited data in the test set posed challenges in discerning relationships among common markers, contributing to overfitting results. Additionally, the presence of missing values being perceived as having feature importance rendered the variables unsuitable for inclusion in the model training process. Therefore, addressing the limitations of the current dataset and conducting additional research are necessary for the enhanced development of a model based on pathological diagnostic markers, including molecular markers.

The strengths of this study are as follows. First, we analyzed and confirmed the prognostic factors for survival outcomes and investigated the long-term impact of ultrastaging in patients with endometrial cancer. Second, we analyzed the impact of ultrastaging, including all SLN and non-SLN, on patients with EC. Third, a machine learning model was constructed for the first time to predict additional LN metastasis detected through ultrastaging in EC.

The limitations of this study are as follows. Firstly, the sample size of this study was small, given the restriction imposed by the single-institution design. Secondly, ultrastaging was conducted on previously selected patients and paraffin blocks that were

embedded earlier, introducing a potential source of bias. In our study, SLN tissue samples were formalin-fixed and fully embedded in paraffin blocks but were not evenly resected for each LN. Thirdly, LN dissection to validate LN metastasis was not performed in all patients. Finally, adjuvant therapy was not restricted, which may have introduced bias. Further prospective studies are needed to investigate the effects of ultrastaging without these biases.

V. CONCLUSION

In conclusion, our study highlighted the significant impact of ultrastaging following SLN mapping with ICG injection in EC patients. This approach not only detected additional LN metastasis more accurately but also may be considered a prognostic factor for survival outcomes. The integration of machine learning, specifically the LightGBM model, enhances our ability to predict additional LN metastasis effectively. Histology (endometrioid grade 3 or non-endometrioid), histology (endometrioid grade 2), and age group (>60 years) were identified as feature importance in predicting metastasis through ultrastaging.

These findings emphasize the necessity for further research on a large scale to validate our predictive model in EC patients. This study lays the foundation for a more precise and personalized approach to patient care, potentially influencing future guidelines for EC treatment strategies.

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ABSTRACT (IN KOREAN)

자궁내막암에서의 초병기화: 추가 림프절 전이의 예후인자로서의 중요성 및 인공지능 예측 모델

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조 상 현

본 연구는 전통적인 헤마톡실린과 에오신염색에서 림프절 전이가 나타나지 않은 자궁내막암 환자에서 초병기화를 통한 추가적인 림프절 전이 비율을 확인하고자 하였다. 또한, 초병기화에서 추가 림프절 전이가 있는 환자와 없는 환자 간의 예후를 비교하고, 추가 림프절 전이를 예측하는 인공 지능 모델 개발을 목표로 하였다.

2014년 6월부터 2017년 12월까지 연세암병원에서 인도시아닌그린 주사를 이용한 감시림프절 매핑으로 병기 결정 수술을 받은 총 159명의 자궁내막암 환자가 연구에 포함되었다. 헤마톡실린과 에오신염색에서 림프절 전이가 나타나지 않은 138명의 환자로부터 얻은 총 1001개의 파라핀 블록(감시림프절 413개, 비감시림프절 588개)을 연구하였다. 이후 연속절편을 통한 초병기화를 수행하였다. 최종 분석에는 감시림프절 검체가 있는 109명의 환자가 포함되었다.

109명 중 14명 (12.8%)의 추가 림프절 전이가 대량 전이증 (1.8%), 미세 전이증(10.1%), 독립 종양 세포(0.9%)의 경우로 확인되었다. 특히, 독립 종양 세포 환자를 제외한 108명의 환자 중에서 3년 생존율은 초병기화 결과에 따라 유의한 차이가 있었다 (전이 없음, 98.9%; 추가 림프절 전이 (대량 전이증/미세 전이증) 69.2% ($p<0.001$)). 3년 전체 생존율도 군간에 유의한 차이가 있었다 (전이 없음, 100%; 추가 림프절 전이 (대량 전이증/미세 전이증), 84.6% ($p<0.001$)). 전체 재발률은 6.5%로, 군간에 유의한 차이가

있었다 (전이 없음, 3.2%; 추가 림프절 전이 (대량 전이증/미세 전이증), 30.8%; p=0.004). 콕스 비례위험 모형 분석에서 초병기화의 림프절 전이는 재발 없는 생존율 (위험 비율 7.53, 95% 신뢰 구간 1.49-39.99, p=0.016) 및 전체 생존율 (위험 비율 7.40, 95% 신뢰 구간 1.21-51.97, p=0.031)에 대한 유의한 예후 요인으로 나타났다. 기계 학습 모델을 이용하여 초병기 결정을 통한 추가적인 림프 전이 검출을 예측하는 경우, LightGBM 모델이 테스트 세트에서 우수한 성능을 나타냈다 (민감도=1.000; 특이도=1.778; F1 값=0.750; ROC-AUC 점수=0.852). 자궁내막양 3등급 또는 비내막양 조직, 자궁내막양 2등급 조직, 연령군(60세 초과)이 초병기 결정에 의한 추가 림프절 전이 검출을 예측하는 중요한 특성으로 나타났다.

결론적으로, 자궁내막암에서의 림프절 초병기화로 추가 림프절 전이가 검출되었고, 초병기화를 통한 림프절 전이 검출은 예후 요인으로 확인되었다. 초병기화를 통한 추가 림프절 전이 검출을 예측하기 위한 기계학습 모델이 개발되었다. 자궁내막암 환자의 초병기 결정을 통한 추가 림프절 전이 검출을 위한 인공지능 예측 모델을 검증하기 위해 추가적인 대규모 연구가 필요하다.

핵심되는 말: 감시림프절; 인공지능; 자궁내막암; 저용량 전이; 초병기화.