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## Research Article

# A Pilot Study for the Validation of Sentinel Lymph Node Biopsy with Indocyanine Green Fluorescence Method in Early Endometrial Cancer at Fundación Jiménez Díaz University Hospital

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### ABSTRACT

**Background:** Sentinel Lymph Node Biopsy is a technique developed to predict lymphatic involvement in patients with early endometrial cancer, decreasing the morbimortality associated with routine systematic lymphadenectomy and improving quality of life.

**Main Objective:** To determine the detection rate and negative predictive value of the Sentinel Lymph Node Biopsy by Immunofluorescence in patients with early endometrial cancer.

**Methods:** A descriptive observational study in patients with early endometrial cancer (FIGO stage I-II) for all histological types and grades, who underwent the Sentinel Lymph Node by immunofluorescence Technique, between June 2019 and March 2020 at the Fundación Jiménez Díaz University Hospital.

We used indocyanine green powder for injection, with a concentration of 25 milligrams (mg). We proceeded to dissolve it in 10 cubic centimeters (cc) of distilled water to. After which, we injected 2 cc of the prepared solution into the cervix at the 3 and 9 o'clock positions at a depth of 1 centimeter.

**Results:** Eighteen patients were included, analysing a total of 26 sentinel nodes: 24 pelvic and 2 paraortic; and a total of 273 lymph nodes (sentinel and non-sentinel nodes): 83 right pelvic, 86 left pelvic and 104 paraortic. All nodes were negative for metastasis. Global and bilateral detection rates were 77.78% and 50% respectively. The Negative Predictive Value and sensitivity were 100%. No significant difference in morbimortality was found between performing only Sentinel Lymph Node technique or systematic lymphadenectomy, but the association with quality of life was significant, with better results for those who only underwent the sentinel lymph node technique versus systematic lymphadenectomy (0% vs 77%).

**Conclusion:** The global and bilateral detection rates of the Sentinel Lymph Node Technique by immunofluorescence were 77.78% and 50% respectively, obtaining a Negative Predictive Value and Sensitivity of 100%. Sentinel Node Biopsy is a valid technique to predict lymphatic affectation in early endometrial cancer, with lower morbimortality than systematic lymphadenectomy.

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## Introduction and Theoretical Framework

### I Introduction

Excluding breast cancer, endometrial cancer is currently the most common gynaecological cancer in developed countries; and ranks second in mortality, after ovarian cancer [1]. It is the gynaecological tumor that has the best prognosis, with an overall survival of 5 years for 74-91% in the case of most patients. It mainly affects postmenopausal women, the most frequent manifestation being the appearance of abnormal uterine bleeding from very early-stages, which explains the high cure rate [2].

The treatment of these patients is essentially surgical, with certain differences depending on the FIGO stage and risk of lymphatic involvement. Currently, endometrial cancer is divided into four risk groups: low, intermediate, high-intermediate, and high; Therefore, the hysterectomy (HT) and the double adnexectomy (DA), that are performed, a systematic aortopelvic lymphadenectomy will be added in high-risk cases [3]. The goal of lymphadenectomy is to group the patients and select those who will benefit from adjuvant treatment more accurately. The indication is usually made based on the histological characteristics of the tumor, pre-operative imaging tests (Magnetic Resonance, MRI; and/or pelvic ultrasound) or finally based on the pathological study of the surgical specimen (intraoperative or delayed) [1].

While it seems clear that systematic lymphadenectomy has no impact on survival in low-risk tumors and that it could have a therapeutic role in high-risk tumors; its performance in those cases of intermediate and intermediate-high risk is still the fruit of debate. This group of tumors presents a risk of lymphatic involvement of around 15% and the benefit/risk ratio of the operation is not clear [1, 4, 5]. To not perform the lymphadenectomy could lead to the undertreatment of cases with lymphatic involvement that could have been pre-operatively under staged; while the procedure in all patients would increase the likelihood of complications and a decrease in quality of life, especially related to the appearance of lymphedema in the lower limbs; in women who may not obtain any benefit from such a procedure [5, 6].

Given this controversy, the idea of introducing the Sentinel Lymph Node (SLN) technique in endometrial cancer is becoming more popular. It is a technique capable of predicting the presence of lymphatic metastases without the need of a systematic lymphadenectomy [7]. There are two main reasons for doing this procedure: to reduce the morbidity and mortality associated with systematic lymphadenectomy and to increase the capacity to detect micro metastases by applying ultra-staging techniques and immunohistochemistry on said lymph node; since applying them throughout the ganglion chain, it is not only less efficient, could delay the start of the adjuvant treatment [5, 8].

More and more studies are trying to validate this technique, some of them achieving a detection rate (DR) of 86% and a negative predictive value (NPV) of 99.6% [9]. However, most of these published studies have evaluated SLN only in high-risk cases, where there is already evidence of the benefits of systematic lymphadenectomy [3].

In addition, despite its good prognosis, up to 15-20% of patients with endometrial cancer have recurrences, so an attempt has been made to define other prognostic factors that allow us to identify these patients [10]. Traditionally, following the Bokhamm's model, endometrial cancer has been divided into two types: Type I, associated with the estrogen exposure and with mutations in *PTEN*, *KRAS*; and Type II, or non-endometrioid, with a worse prognosis than the previous one and is associated with mutations in *Her2* and *TP53*. However, some tumors present characteristics that overlap in both groups, which is why the search for other histopathological markers continues to make the treatment more specific to each individual [11].

A new tumor classification has recently emerged based on the molecular profile that divides Endometrial Cancer into four groups: Ultra muted (*POLE*), Unstable Microsatellites (MSI), Low copy number and High copy number [12]. Each of them associated with a prognostic value, which could lead to a reconsideration of the classic classification of risk groups [13]. This could mean that tumors that would usually be considered to be low risk were in fact, really risky, which again raises the question of which patients would really benefit from a lymph node study.

### II Theoretical Framework of the Sentinel Ganglion in Endometrial Cancer

The Sentinel Ganglion is the one with the highest probability of having metastasis, therefore, theoretically, its biopsy would allow us to know the state of the rest of the lymphatic chain [14]. This technique was described for the first time in 'Endometrial Cancer' in 1996 by Dr. Thomas Burke and its use has been widely used in the field of gynaecological oncology, in breast cancer and more recently, in vulvar cancer [15, 16]. When considering the technique in Endometrial Cancer, it should be taken into account that the uterus is a central organ with bilateral lymphatic drainage, so that endometrial tumors can develop metastases in both hemipelvises. That is why the technique used for the SLN biopsy must be able to detect it bilaterally, which is not always achieved [7, 16].

Traditionally, markers such as Technetium99 or Methylene Blue have been used to detect Endometrial Cancer, but these methods are not as popular nowadays due to their complex management as well as their low bilateral detection rate (43% and 71%, respectively [16]). As an alternative, Indocyanine Green (ICG) arises, an immunofluorescent marker is already widely used in other fields of medicine and doesn't present many contraindications: hypersensitivity to its active ingredient or to iodine, as well as suffering from hyperfunctioning alterations thyroid; and in very rare cases (1 <10,000 cases), hypersensitivity reactions have been recorded, frequent in patients with kidney failure [6, 17]. In addition, the ICG does not require a prior admission of the patients or the coordination with the nuclear medicine team and different studies have already demonstrated, not only because it's better than traditional methods, but also its superior bilateral detection rate (84% ICG vs 73.9% Methylene blue + Technetium99) [16]. In addition, the ICG involves easy handling and has a lower cost and better ergonomics for detection and doesn't seem to be affected by obesity which has a limiting factor with other markers [16, 18].

## Hypothesis and Objectives

### I Hypothesis

Selective Sentinel Lymph Node Biopsy (SLNB) by means of immunofluorescence with cervical injection of ICG in initial Endometrial Cancer is a valid lymphatic assessment technique, reflecting the true tumor status of the rest of the lymph nodes in that region without the need of systematic aorto-pelvic lymphadenectomy.

### II Objectives

#### i Main Objective

To determine the DR and NPV of the SLNB in initial Endometrial Cancer, in patients undergoing surgical treatment.

#### ii Secondary Objectives

To determine the morbidity and mortality associated with SLNB compared to that associated with systematic lymphadenectomy. Determine the quality of life of patients who have had only SLNB compared to those who have had a systematic lymphadenectomy

## Materials and Methods

### I Design

Descriptive, observational and ambispective study approved by the Ethics Committee of the Jiménez Díaz Foundation University Hospital (HUFJD), of all the cases who were in the initial stages of Endometrial Cancer that have been treated between June 2019 and March 2020. Following the following inclusion and exclusion criteria:

#### i Inclusion Criteria

- Women over 18 years of age.
- Diagnosed with Endometrial Cancer in the initial FIGO stage (stage I-II) in the pre-operative stage of any type and histological grade.
- Intervened between January 2019 and March 2020 and subjected to systematic lymphadenectomy and/or sentinel node biopsy.

#### ii Exclusion Criteria

- Undergoing a previous hysterectomy for the diagnosis of Endometrial Cancer.
- Presence of pathological lymphadenopathy in the clinical and/or radiological pre-operative study (it would be considered pre-operative FIGO stage III).
- High surgical risk.
- Not candidates for systemic treatment for medical or personal reasons.
- Synchronous cancer diagnosis.
- Undergoing previous pelvic lymphadenectomies for other causes.
- Previously subjected to pelvic radiation for any reason.

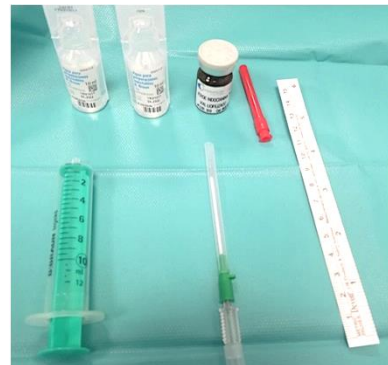
Patients are included after signing the informed consent, to whom an identification code is assigned in a chronological order according to their date of diagnosis (FJDEND1, FJDEND2 etc.); and later a database is created using Microsoft Excel. The variables collected are in (Table 1).

### II Pre-Operative Staging

Prior to the indication for surgery, our patients undergo extension studies: Computed Tomography (CT), pelvic MRI and/or gynaecological ultrasound; to determine the stage they are at. In addition, endometrial biopsies are analysed to determine pathological characteristics, in order to determine risk groups. We did not perform intraoperative studies of the surgical specimen to determine myometrial infiltration, because of the high correlation of the imaging tests with the final pathological anatomy.

### III Preparation of the Marker

We use ICG in powder form, with a concentration of 25 milligrams (mg). We proceed to dissolve it in 10 cubic centimeters (cc) of Distilled Water to avoid precipitation of the marker, obtaining a concentration of 2.5 mg (Figure 1).



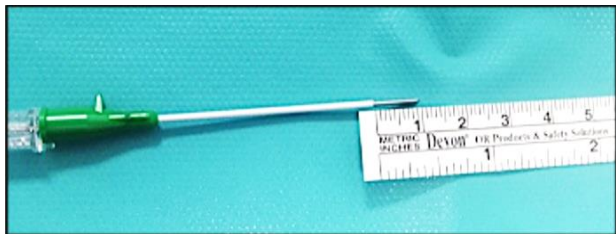
**Figure 1:** Intraoperative Indocyanine Green marker dissolution preparation. Standardization of the technique with Abbocath No. 12G.

### IV Sentinel Lymph Node Selective Biopsy Procedure

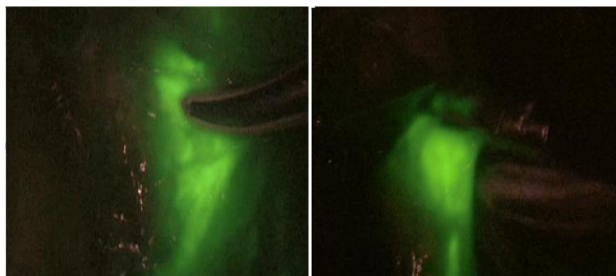
All patients underwent HT plus DA and SLNB with STORZ laparoscope model OPAL1® -NIR/ICG. For this, once located in the abdominal cavity, and before proceeding to place the uterine manipulator, 2 cc of the prepared solution is injected into the uterine cervix at time positions 3 and 9 to a depth of 1 cm (Figure 2). The time that elapsed from the time of the injection of the ICG to the time of the detection of the SLN in infrared mode with 0-degree optics is then measured and the pelvic lymph nodes that showed fluorescence are identified according to lymphatic drainage and subsequently removed (Figure 3). Ideally 1 SLN is detected in each hemipelvis, but if a second SLN is detected in some of the hemipelvis, both will be removed, and there may be more than one SLN per hemipelvis. In exceptional cases, a para-aortic SLN is identified, and it is also removed.

All the SLNs are sent to Anatomic Pathology, indicating their location, for deferred study. Subsequently, in patients that appear to have a systematic lymphadenectomy, it was performed; while in those in which it was not indicated, HT with DA was performed directly. This procedure

was performed by an expert surgeon, in all cases by the same surgical team.



**Figure 2:** Cervical injection at 3 and 9 o'clock positions to a depth of 1 cm.



**Figure 3:** Sentinel lymph node biopsy by Indocyanine Green and infrared light (ICG/NIR).

## V Patient Follow-up

Patients are followed up one month after surgery, before starting the adjuvant treatment, if needed; where they are asked about symptoms related to surgery and specific regarding:

- i. Appearance of lower limb lymphedema: inflammation or sensation of swelling/heaviness of lower limbs or genital area, pain in lower limbs, problems when walking long distances, interference with their activities.
- ii. Loss of sensibility of lower limbs.
- iii. Urinary disorders: incontinence, itching, pain or frequent urination.
- iv. Faecal alterations: faecal or gas incontinence, constipation or pain.

Physical examination and pelvic ultrasound are performed on all patients. Follow-up is continued at the sixth month after surgery and thereafter every six months until 5 years are completed, asking about the symptoms at each consultation and performing a gynaecological examination and pelvic ultrasound on all patients.

## VI Histopathological Analysis

The SLN are sent to Anatomic Pathology independently from the rest of the non-sentinel lymph nodes and indicating their location in each one of them. Their analysis is performed by conventional techniques and then, if negative, they are submitted to ultra-staging techniques to increase the detection capacity of Micrometastasis (defined as metastases measuring between 0.2 and 2mm) and Isolated Tumor Cells (ITC) (metastases smaller than 0.2mm). The rest of the non-sentinel lymph nodes are studied only by conventional techniques (haematoxylin-eosin staining, H&E).

The protocol used for their study is as follows, following the recommendations of The British Association of Gynaecological Pathologist:

### i Macroscopic Analysis

- a. The periganglionic fat is removed and the lymph node is isolated.
- b. Once measured and weighed, each ganglion is cut perpendicular to the major axis. In those of 2 to 4mm, it will result in two sections, while those larger than 4 mm are cut in sections 2-3mm thick, will generally result in four sections.
- c. Each of the tissue sections of the lymph node is fixed in formalin for 24 hours and then in kerosene [14].

### ii Microscopic Analysis

First, a single section of each block is analysed by H&E, with minimal cutting to avoid tissue waste. If this section is negative, we proceed with the following steps:

- a. Each of the blocks is cut at 200-micron depth intervals until the tissue is exhausted, thus generally obtaining 10 additional sections, of which in turn will be obtained from 4 consecutive levels.
- b. From each set of 4 sections, two are selected: one for staining with H&E and another for immunohistochemical study with cytokeratins AE1-AE3. The rest of the sections are reserved for possible technical errors, to extend the study etc.

All SLN samples are reviewed by a pathologist with expertise in gynaecological tumors and always by the same team of pathologists.

## VII Statistical Analysis

### i Description of Variables

- a. Negative SLN: lymph node detected by fluorescence, and which does not show metastasis in the pathology study.
- b. True Negative (TN): negative SLN, demonstrating absence of metastasis in the rest of the lymph nodes after systematic aorta pelvic lymphadenectomy.
- c. False Negative (FN): negative SLN, demonstrating the presence of metastases in the remaining nodes after aorta pelvic lymphadenectomy.
- d. Positive SLN: lymph node detected by fluorescence and shows metastasis on pathological examination.

In the presence of lymph node metastasis, the terms true positive and false positive do not apply, since the positive lymph node is already considered a metastatic lymph node stage, regardless of the presence of metastases in the remaining nodes.

- a. Negative predictive value (NPV): probability that there is no lymphatic involvement, in case the sentinel node is negative. It is the proportion of NPV among all the negatives.
- b. Sensitivity: the ability of the technique to detect disease, the probability that the SLN will be positive when the patient has lymphatic involvement. The higher the number of FN, the lower the sensitivity of the test.

**ii Statistical Programme**

The SPSS Version 25 Chicago programme is used for the descriptive analysis of the variables and to determine the TD, NPV and sensitivity of the technique. The qualitative variables will be described by means of frequencies and percentages. Quantitative variables will be described by means of mean, standard deviation, median, minimum value and maximum value. To assess the significant association between morbidity and mortality, a Chi-Square test or Fisher's exact test was performed.

**Results**

During the study period, 18 patients with early-stage Endometrial Cancer recommended for surgical treatment underwent SNLB by immunofluorescence with IGC at HUFJD. The demographic and clinicopathological characteristics of the patients are shown in (Table 1). The mean age of diagnosis of the patients participating in the study was

64 years (range 48-80). A total of 44.44% (8/18) were a normal weight (Body Mass Index, BMI, < 25 kg/m<sup>2</sup>); 22.22% (4/18) were overweight (BMI 25-30 kg/m<sup>2</sup>) and 33.33% (6/18) were obese (BMI >30 kg/m<sup>2</sup>). One patient (5.6%) was diagnosed with Lynch syndrome due to loss of MSH6 expression. Regarding staging, 50% (9/18) of the patients presented pre-operative FIGO stage IA; and the other 50% (9/18), FIGO stage IB. A statistically significant association was found between FIGO stage and surgical procedure (systematic lymphadenectomy and BSGC vs. BSGC) (p 0.015). In addition, 44.4% (8/18) had discordance between pre-operative and post-operative FIGO stage (Figure 4). 100% (18/18) of patients underwent BSGC via laparoscopy and 55.55% (10/18) of patients also underwent systematic lymphadenectomy. The mean duration HT+DA+SLNB was 115 minutes (range 100-150) and for HT + DA + SLNB + Systematic lymphadenectomy, 243 minutes (range 205-300). The mean time taken for SLN identification was 16.3 minutes (range 14-20).

**Table 1:** Pre- and post-operative characteristics of patients with early-stage Endometrial Cancer.

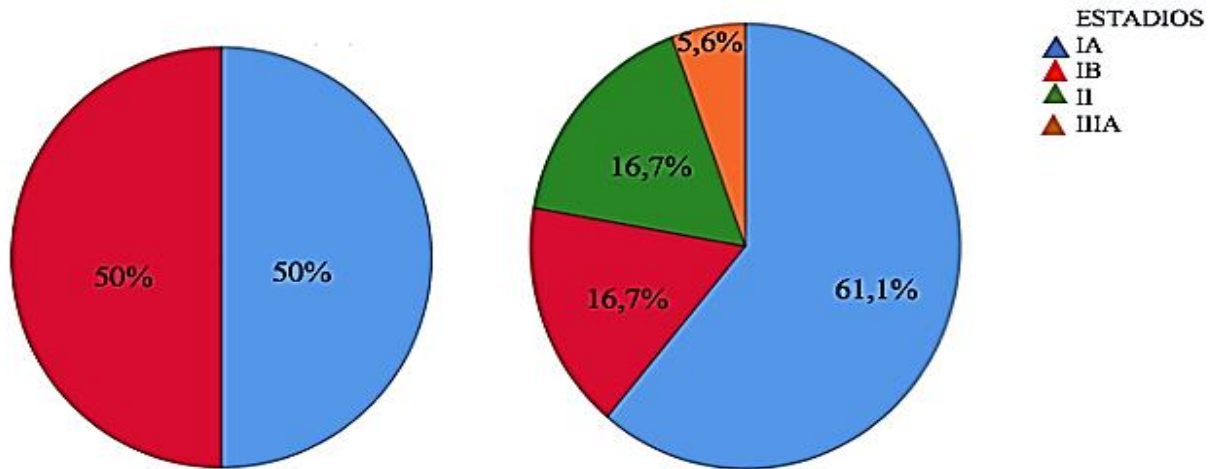
	Patients that underwent HT+DA+BSGC (N= 8)	Patients that underwent HT+DA+BSGC+ LNF (N=10)	p
<b>QUALITATIVE VARIABLES</b>			
<b>ASSOCIATED RF</b>	%	%	p
None	50	70	>0,05
HTA	12,5	30	
DM	12,5	0	
DM y HTA	25	0	
SOP	0	0	
<b>TOBACCO USAGE</b>	12,5	20	>0,05
<b>BMI (kg/m<sup>2</sup>)</b>			>0,05
< 25	37,5	50	
25-29,9	25	20	
>30	37,5	30	
<b>HEREDITARY SD</b>	12,5	0	>0,05
THS	12,5	0	>0,05
<b>TREATMENTS WITH TMX</b>	0	0	>0,05
<b>GLOBAL DETECTION SN</b>	62,5	90	p>0,05
<b>BILATERAL DETECTION SN</b>	80	66,7	>0,05
<b>LOCATION RIGHT SN</b>			>0,05
Common Iliac Artery Bifurcation	80	57,1	
External Iliac Artery	20	28,6	
Obturator Fossa	0	14,3	
<b>LOCATION LEFT SN</b>			>0,05
Common Iliac Artery Bifurcation	33,3	71,4	
External Iliac Artery	33,3	28,6	
Obturator Fossa	33,3	0	
<b>RIGHT SN STAGE</b>			
Negative	100	100	
Positive	0	0	
<b>LEFT SN STAGE</b>			
Negative	100	100	
Positive	0	0	
<b>ADYUVANT QT</b>	0	20	>0,05
<b>ADYUVANT RT</b>	37,5	88,9	>0,05
<b>TIPE RT</b>			>0,05
RTE	33,3	75	
BT	66,7	25	

HISTOLOGY															>0,05	
Tipo I Endometrioid	100					70										
Tipo II no endometrioid	0					30										
HISTOLOGICAL DEGREE															>0,05	
G1	37,5					30										
G2	62,5					30										
G3	0					40										
LVI	25					30									>0,05	
MELF PATTERN	12,5					20									>0,05	
MOLECULAR PROFILE						**									0,019	
<i>POLE</i> mut	0					0										
MMrd	37,5					60										
NSMP	62,5					0										
P53abn	0					30										
MIOMETRIAL INFILTRATION															>0,05	
<50%	100					60										
>50%	60					40										
PRE-OPERATIVE FIGO STAGE															0,015	
IA	87,5					20										
IB	12,5					80										
POST-OPERATIVE FIGO STAGE															>0,05	
IA	87,5					40										
IB						30										
II	12,5					20										
IIIA						10										
SURGICAL COMPLICATIONS	12,5					40									>0,05	
ADVERSE EVENTS	0					70									0,02	
MORTALITY	0					10									0,05	
QUANTITATIVE VARIABLES																
	N	Min	Max	X	DS	N	Min	Max	X	DS						p
AGE OF Dx (years)	8	45	80	62,62	12,78	10	53	74	65,80	7,26						>0,05
AGE OF MENARCHE (years)	8	11	13	12,38	,74	10	11	15	12,80	1,22						>0,05
MENOPAUSE AGE (years)	6	42	69	53,5	8,68	9	45,0	55	50,44	3,28						>0,05
BMI (kg/m <sup>2</sup> )	8	22,77	31,18	27,99	3,30	10	21,19	33,95	26,63	4,22						>0,05
DURATION (minutes) SURGERY	8	100	150	115,6	17,61	10	205	300	243,5	33,0						>0,05
TIME (minutes) FOR DETECTION SN	5	14	20,0	16,4	2,50	9	14	20	16,22	2,048						>0,05
TUMOR SIZE	8	11	37,0	24,25	8,94	10	20	70	38,90	15,84						>0,05
Nº RIGHT SN	8	0	1	,63	,51	9	,00	1,0	,66	,500						
Nº LEFT SN	8	0	1	,38	,51	10	0	1	,70	,48						
Nº OF n-SLN RIGTH PELVIS	0					10	0	2	,90	,56						
Nº OF TOTAL n-SLN RIGHT PELVIS	6	0	1	,83	,40	10	5	13	7,20	3,01						
Nº OF n-SLN AFFECTED IN RIGHT PELVIS	5	0	0	,00	,00	10	5	14	7,90	2,96						
Nº OF n-SLN LEFT PELVIS	0					10	0	0	,00	,00						
Nº OF TOTAL n-SLN LEFT PELVIS	4	0	1	,75	,500	10	3	10	7,40	2,59						
Nº OF n-SLN AFFECTED LEFT PELVIS	3	0	0	,00	,000	10	4	11	8,30	2,54						
Nº OF n-SLN PARAAORTICS	0					10	0	0	,00	,00						
Nº OF TOTAL n-SLN PARAAORTICS	0					10	0	1	,20	,42						
Nº OF n-SLN PARAAORTICS AFFECTED	0					10	1	20	10,4	6,58						

Qualitative variables are expressed in number and percentage. Quantitative variables are expressed as minimum value, maximum value, mean (X) and standard deviation (N=sample size).

RF: Risk Factors; HTN: Arterial Hypertension; DM: Diabetes Mellitus; PCOS: Polycystic Ovary Syndrome; SD: Syndromes; HRT: Hormone Replacement Treatment; TMX: Tamoxifen; SN: Sentinel Node; n-SLN: non-Sentinel Node; CT: Chemotherapy; RT: Radiotherapy; IVSI: Invasion of the lympho-vascular space; MELF: Microcystic, Elongated and Fragmented; MSI: Unstable Microsatellites; Dx: Diagnosis.

\*\* One patient (10%) did not present differentiation for epithelial, muscular or endometrial stromal tumor, with *FAF1/EP515* Genetic Fusion.



**Figure 4:** FIGO stage of the patients included in the study, pre-operative (left) and post-operative (right).

A total of 26 SLNs were mapped: 24 pelvic and 2 para-aortic; and a total of 273 nodes (sentinel and non-sentinel) were removed: 83 right pelvic, 86 left pelvic and 104 para-aortic. Overall DR was 77.78% (14/18) and bilateral DR, 50% (9/18). If we study it from patient 10 onwards, having probably fulfilled the learning curve and having established a more precise technique to improve depth, we will be able to determine whether the patient’s DR was higher or lower. We observed that in the last 8 patients to whom the technique could be applied, both global DR improved, 100% (8/8) and bilateral DR, 87.5% (7/8), finding statistically

significant differences between the number of mapped CG and the learning curve (p 0.006) (Table 2). The overall DR in relation to the BMI of the patients was as follows: BMI<25 42.9% (6/8), BMI25-30 21.4% (3/4) and BMI>30 35.7%(5/6) (Table 3) and no statistically significant differences were found in this association (p>0.05). Likewise, DR was analysed in relation to age at diagnosis, smoking, risk factors (arterial hypertension, AHT; Diabetes Mellitus, DM and/or polycystic ovary syndrome PCOS), FIGO stage, tumor size and Lymph vascular Invasion (LVI); no statistically significant differences were found (p>0.05).

**Table 2:** Comparison of the cases according to the Learning Curve.

	First 10 patients	Last 8 patients	p
AGE	64,50 ± 11,655	64,25 ± 9,94	>0,05
BMI	27,268±3,32	27,20 ± 4,57	>0,05
Nº OF MAPPED SN	1 (0-3)	2 (1-3)	0,006
Nº OF FAILED SN	1 (0-2)	0 (0-1)	0,003
Nº TOTAL SN REMOVED	17 (1, 42)	21(1-44)	>0,05

Age and BMI are expressed in Mean and Standard Deviation. Nº of mapped and failed SNs are expressed in Median, Minimum Value and Maximum value. BMI: Body Mass Index (kg/m<sup>2</sup>); SN: Sentinel Lymph Nodes.

**Table 3:** Overall detection rate as a function of the BMI of patients.

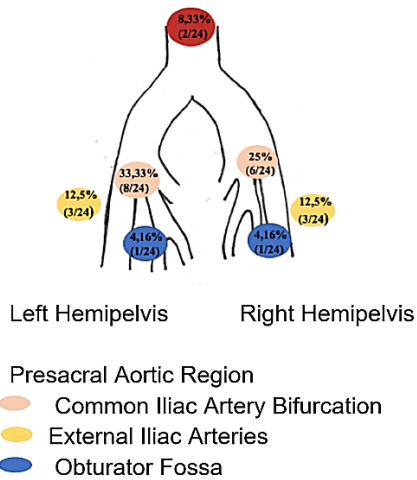
BMI(kg/m <sup>2</sup> )	BMI	Recount	GLOBAL DETECTION		Total
			No	Yes	
BMI<25		Recount	2	6	8
		%	50,0%	42,9%	44,4%
BMI 25-30		Recount	1	3	4
		%	25,0%	21,4%	22,2%
BMI>30		Recount	1	5	6
		%	25,0%	35,7%	33,3%
Total		Recount	4	14	18
		%	100,0%	100,0%	100,0%

BMI: Body Mass Index (kg/m<sup>2</sup>).

The location of the SLN identified can be seen in (Figure 5), being the common Iliac Artery Bifurcation the most frequent location (58.33%). 100% of the non-sentinel lymph nodes did not present metastases in the pathology study, as did 100% of the pelvic and para-aortic lymph nodes, so the false negative rate was 0%. Therefore, the NPV obtained was 100% and the sensitivity of the test was 100%. No adverse effects or

allergic reactions related to the immunofluorescent marker were detected during surgery or post-operatively. Systematic lymphadenectomy is associated with a morbidity and mortality of 40% (4/10) and 10% (1/10), respectively. The surgical complications recorded were, lymphocele 10% (1/10), urethral injury 10% (1/10), reconversion to laparotomy 10% (1/10) and intestinal perforation with exitus 10% (1/10), in the

lymphadenectomy group. The morbidity and mortality associated with performing only SLNB without lymphadenectomy was 0%. The association between morbidity, mortality and surgical procedure was not statistically significant ( $p > 0.05$ ). Likewise, the association between morbidity and mortality and age at diagnosis, BMI, and FIGO stage was not significant in any case ( $p > 0.05$ ), so multivariate analysis was not performed.



**Figure 5:** Location and number of identified sentinel nodes.

Regarding adjuvant treatment, 55.5% (10/18) of the patients had an indication for adjuvant radiotherapy (RT) and 11.1% (2/18) had an indication for chemotherapy. Regarding the impact on quality of life, the incidence of adverse events in patients who underwent systematic lymphadenectomy was 70% (7/10), while the incidence in the group who underwent only SLNB was 0%; this difference being statistically significant ( $p = 0.02$ ). The events recorded were lymphedema 20% (2/10); lymphocele 10% (1/10); nervous alterations with loss of sensitivity 10% (1/10) and urinary alterations 20% (2/10); among which were urinary incontinence with nocturia, without the need for intervention and urgent urinary diversion due to urethral injury during surgery. The association between quality of life and other confounding factors such as age, BMI, HT, DM, PCOS, smoking, histological type, FIGO stage and complications recorded during surgery was analysed, and no statistically significant differences were found in any case.

## Discussion

This study shows that SLNB by immunofluorescence with ICG presents a high DR and a high NPV, if performed with the appropriate technique; with a global and bilateral DR of 77.78% (14/18) and 50% (9/18) respectively, being similar to those recorded in other studies, located at 87% and 65%, respectively; and higher than that of other markers such as Methylene Blue (71% global DR and 44% bilateral DR) [16].

However, we have observed that, at the beginning of the study, bilateral DR was very limited. This could be due to a failure in the tracer injection technique in our first 10 patients, because since we standardized the technique, we obtained a considerable improvement in bilateral detection: 87.5% (before technical standardization 20%). This supports the theory that the technique is the most important factor in detection [16]. On the other hand, it is important to assess the learning curve,

considered an independent factor that can influence the quality of the technique. This has been estimated at a total of 20-30 surgeries, improving with the accumulation of cases per surgeon, although it seems to be independent of experience in minimally invasive surgery [19]. In our study the learning curve occurred in 10 surgeries, since from patient number 11 onwards bilateral DR improved considerably. Although it may seem less than previously published, we must take into account that the same team of surgeons was performing this technique in cervical cancer, so probably the improvement occurred after a larger number of cases, approaching the 20 reported in the literature. We found statistically significant differences between the first 10 surgeries and the last 8 in relation to the number of mapped and failed SLN (Table 2). This means that the learning curve plays an important role in SLN detection. Likewise, there is no link with other factors that may induce detection failure, such as age, FIGO stage, histologic type, SIL and tumor size. We can conclude; therefore, the injection technique is the most important independent factor for adequate SLN determination, and this is due to the number of cases performed by surgeons.

Another factor that should be taken into account when assessing DR is the failure rate in obese patients. Obesity is an independent risk factor for endometrial cancer, with a Relative Risk 2-5; so, a large proportion of these patients will be overweight [1]. That is why it is of particular importance to validate a technique with a good DR in this group of patients. 55.55% (10/18) of our patients were overweight or obese, with a mean BMI of 27 kg/m<sup>2</sup>. The failure of detection in this group of patients could be due to the fact that the adipose tissue surrounding the nodes could hinder the diffusion of the tracers. In our study, we did not find a statistically significant association between DR and BMI, observing that even this is similar in patients with BMI < 25 and BMI > 30 kg/m<sup>2</sup> (42.9% and 35.7% respectively). This coincides with other published articles reporting that ICG has a lower failure rate in obese patients compared to other markers which may be due to a greater diffusion capacity by ICG, or to the greater detection capacity through the use of infrared devices [16, 20]. We can infer, therefore, that obesity is not a factor influencing SLN detection by immunofluorescence.

The injection site of the marker used is another important aspect to consider when designing the technique. Previously, its injection into the uterine fundus was preferred, given its proximity to the tumor and its ability to simulate tumor drainage [16]. However, this injection site ceased to be practical after the introduction of laparoscopy, so cervical injection is currently more widely used, as it is more accessible and has superior sensitivity [7]. On the other hand, peritumoral injection via hysteroscopy seems to be the one that best reflects tumor drainage and has a higher DR for para-aortic nodes [16]. However, given the higher overall DR via cervical (82% vs. 75%) using ICG and the low probability of finding isolated para-aortic metastases in early-stages, in our study we have performed the injection of our immunofluorescent dye at the cervical level [7, 21].

The ability of the SLN technique to detect extra pelvic disease has also been discussed, as most SLN identified are pelvic. However, the presence of isolated para-aortic metastases, without pelvic metastases, occurs in only 5% of endometrial cancer cases [22]. This incidence decreases to 0.9% in Type I Endometrial tumors, while for tumors with Clear Cell, Serous and Carcinosarcoma histology it amounts to 2.5%



[23]. In high-risk tumors systematic lymphadenectomy is not intended to be replaced by SLNB, given the risk of lymphatic involvement intrinsic to this group and that is why we only analysed the study of pelvic sentinel nodes [3]. Even so, in two cases we have detected para-aortic SLN nodes, whose anatomopathological study agrees with that of the pelvic nodes, both being negative for metastasis, in agreement with the literature.

Likewise, it should be taken into account that the anatomopathological study of the lymph nodes includes ultrasonography and immunohistochemistry techniques. This increases the ability to detect Micrometastases or ITC present in 30% of endometrial cancer cases [24]. This implies a better adjustment of adjuvant treatment in patients who would have been FN with classical techniques and, therefore, under staged and undertreated. However, there is still no consensus in the medical literature for the management of Micrometastasis so in our study such treatment has depended on the decision of the gynaecological tumor committee and the HUFJD endometrial protocols: after ultra-staging of the SLN, if the result is Micrometastasis, adjuvant treatment is considered as if it were a Macrometastasis [16]. However, for ITC the same attitude is not considered, not requiring adjuvant treatment on its own, but in the context of the whole surgical specimen. In our study population neither Micrometastases nor ITC have been found.

In relation to associated morbidity and mortality, in our study we observed surgical complications in 40% of the patients who underwent systematic lymphadenectomy, compared to 12.5% in those who only underwent SLNB. This coincides with the data in the literature, so despite not having found statistically significant differences, we consider that this should be evaluated in a subsequent study with a larger sample size. Regarding the impact on quality of life, systematic lymphadenectomy is a technique with well-known adverse effects in gynaecologic oncology for its impact on the quality of life of patients; one of the most frequent events the occurrence of lymphedema in lower limbs, with an incidence of 4.6-47% [5]. We found statistically significant differences ( $p < 0.002$ ) between patients undergoing SLNB versus those undergoing SLNB + Systematic lymphadenectomy, with an incidence of adverse events of 0% and 70%, respectively.

It should be noted that quality of life was assessed one month after surgery, prior to the initiation of adjuvant treatment, so that the appearance of symptoms is independent of subsequent treatment. We did not find statistically significant differences between the appearance of adverse events and other risk factors such as age, BMI, histologic type and FIGO stage; therefore, we conclude that SLNB has a lower impact on quality of life than systematic lymphadenectomy. However, we must emphasize that we started from a selection bias, since from the beginning of the study our patients presented significant differences in relation to FIGO stage ( $p < 0.015$ ), which implies a worse intrinsic prognosis for those who, because they had a higher stage, presented an indication for systematic lymphadenectomy.

Finally, and perhaps the most important aspect to be taken into account when validating our technique, is the NPV, being in our study 100%, coinciding with the values found in other studies [16]. That is to say, we could have applied SLNB, without performing lymphadenectomy, if this was negative; with the certainty of not having left diseased nodes without

biopsying. However, the main limitation of our study is its small sample size, so we cannot conclude this aspect. It is therefore necessary to extend the study to define more precisely the validity of the technique and the influence of the different variables on it.

## Conclusion

The overall DR of SLNB in our study is 77.78% and bilateral, 50%, with a NPV 100%, which allows us to conclude that it is a technique that could be used to predict lymphatic involvement in patients diagnosed with initial stages of Endometrial Cancer, without the need to perform a lymphadenectomy. The morbidity and mortality rate associated with systematic lymphadenectomy was higher than that associated with SLNB although it was not statistically significant. However, the quality of life in our patients was clearly better in those who underwent SLNB alone, with a statistically significant result. These results will be validated later in a prospective study in the same hospital.

## Conflicts of Interest

None.

## Abbreviation

**HT:** Hysterectomy  
**DA:** Double Adnexectomy  
**MRI:** Magnetic Resonance  
**CT:** Computed Tomography  
**SLN:** Sentinel Lymph Node  
**SLNB:** Sentinel Lymph Node Biopsy  
**DR:** Detection Rate  
**NPV:** Negative Predictive Value  
**ICG:** Indocyanine Green  
**ITC:** Isolated Tumor Cells  
**H&E:** Haematoxylin-Eosin  
**BMI:** Body Mass Index  
**AHT:** Arterial Hypertension  
**DM:** Diabetes Mellitus  
**PCOS:** Polycystic Ovary Syndrome  
**LVI:** Lymph Vascular Invasion  
**RT:** Adjuvant Radiotherapy  
**HUFJD:** Fundación Jiménez Díaz University Hospital

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