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Laparoscopic Indocyanine green sentinel lymph node mapping in early ovarian cancer. A pilot study and review of the literature

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ABSTRACT

Objective: there are still few studies concerning the Sentinel lymph node (SLN) in ovarian cancer. In this pilot study we described the feasibility of SLN mapping with Indocyanine Green (ICG) in five patients affected by early stage ovarian cancer, during laparoscopic surgery.

Material and Methods: the tracer has been injected into the hilum of the ovary through a spinal needle (22-gauge size) connected to 20 cm-long infusion tubing for intravenous fluid delivery advanced inside the lateral trocar (10 mm) through a Johann clamp.

Results: a total of 6 SNs was detected in all patients. The median interval from the tracer injection to the detection of all SNs was 2 minutes (range, 1 - 3 minutes). In three patients, the first detected SN was found in the area of common iliac artery, in two patients at paracaval region. The median number of lymph nodes removed per patient was 2 (range, 0-2). All the detected SLNs were identified ipsilateral to the site of injection. Only in two patients we detected a second SLN, respectively in preaortic region and in under mesenteric region. No allergic reaction has occurred.

Conclusions: ICG laparoscopic SLN mapping in early stage ovarian cancer is a procedure feasible and promising. Additional prospective multicenter studies are needed to evaluate the best technique for staging early stage ovarian cancer patients.

Keywords: sentinel lymph node biopsy, ovarian cancer, Indocyanine Green.

SOMMARIO

Obiettivo: ci sono ancora pochi studi riguardanti il linfonodo sentinella (SLN) nel carcinoma ovarico. In questo studio pilota abbiamo descritto la fattibilità di mappatura SLN con verde di Indocianina (ICG) in cinque pazienti affetti da cancro ovarico fase iniziale, durante la chirurgia laparoscopica.

Materiali e metodi: il tracciante è stato iniettato nell'ilo dell'ovaio attraverso un ago spinale (formato 22-gauge) collegato a 20 cm di lunghezza di tubo di infusione per la somministrazione di liquidi per via endovenosa avanzato all'interno del trocar laterale (10 mm) attraverso una Johann laparoscopica.

Risultati: un totale di 6 SN è stato rilevato in tutti i pazienti. L'intervallo mediano di tempo dalla iniezione tracciante per l'individuazione di tutti i SN era di 2 minuti (range, 1 - 3 minuti). In tre pazienti, il primo rilevato SN è stato trovato nella zona dell'arteria iliaca comune, in due pazienti nella regione paracavale. Il numero mediano di linfonodi rimossi per paziente è stato di 2 (range 0-2). Tutti i linfonodi sentinella rilevati sono stati identificati omolateralmente al sito di iniezione. Solo in due pazienti abbiamo rilevato un secondo SLN, rispettivamente, nella regione preaortica e in regione sotto mesenterica. Nessuna reazione allergica si è verificata.

Conclusioni: il SLN laparoscopico con IDG nel carcinoma ovarico fase precoce è una procedura fattibile e promettente. Ulteriori studi multicentrici prospettici sono necessari per valutare la tecnica migliore da poter eseguire in pazienti affetti da cancro ovarico in stadio iniziale.

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INTRODUCTION

Ovarian cancer is the most unfavorable prognosis gynecological neoplasia. Lymph node involvement is an asymptomatic early phenomenon, able to modify the course of disease. In patients with clinical early-stage disease, an occurrence of occult lymph node metastasis upgrades the disease to FIGO stage IIIC[1]. The incidence of positive lymph node in patients with early stage ovarian cancer is between 5.1-15%[2-3]. Therefore, an accurate surgical staging with systematic pelvic and para-aortic lymphadenectomy, has a significant therapeutic value in early stage ovarian cancer. A lymph node sampling, represent an insufficient procedure because some metastatic lymph nodes could remain undetected[4]. On the other hand, the systematic lymphadenectomy is the gold standard, but the radical procedure implicates too many morbidities such as lymphocists (13%) nerve and vessel injury (4%) increased blood loss and increased surgery times[5].

With the advent of laparoscopic surgery, the concept of sentinel lymph node (SN) biopsy for assessing the regional lymph node status has been studied in all gynecological malignancies. The SN technique is effective in vulvar and breast cancer, whereas in cervical and endometrial cancers are currently ongoing[6]. In ovarian cancer, SN data availability is exiguous, mainly because the tracers dissemination after injection through the cortex is unpredictable and because the risk of tumor spread is very high[7-8]. Another unsolved question concerns the tracer injected during the SN procedure. Usually in other pathologies, node localization is achieved by the combination of injected dye and radioactive tracer. The commonly used dyes are the isomers isosulphan blue and Patent blue V (PBV). Concerning the early-stage ovarian cancer, the radioisotope is considered an inconvenient target to the tracer injections, because require a too long time to be absorbed from the lymphatic system and because a lymphoscintigram is always needed for localizing the SNs[7]. Fluorescent indocyanine green (ICG) dye has been used and tested by several groups in SLN mapping in various solid tumors[9] Near-infrared (NIR) fluorescent dye with ICG has a higher signal-to-background ratio, is cheaper, has fewer adverse effects and less toxicity, and has infrequent allergic reactions[10].

The aim of our paper is to describe the SN procedure in early stage ovarian cancer with tracer injection into the ovarian ligaments during laparoscopic surgery.

MATERIALS AND METHODS

Patients with clinical International Federation of Gynecology and Obstetrics (FIGO) stage I - II ovarian carcinoma, good performance status (PS=0-1), no previous tumors, no previous chemotherapy or radiotherapy, who underwent laparoscopic primary debulking surgery were enrolled in this study. Preoperatively, all patients underwent CT and PET/CT scans to evaluated suspicious nodal involvement that could exclude their case from the nodal mapping.

The SPIES Full HD Image 1S H3-Z FI camera with Karl Storz Near Infrared (NIR/ICG) System (Karl Storz Endoscopy, GmbH, Mittelstrasse, Tuttlingen, Germany) was used and all patients signed an informed consent and the Local Ethic Committees approved the study.

After the induction of pneumoperitoneum, the operative trocars were positioned in the standard manner. The injection procedure was carried out after ascertaining the absence of suspicious gross peritoneal lesions or enlarged lymph nodes.

The ICG (Indocyanine green, PULSION Medical Systems SE, Feldkirchen, Germany) concentration used was 1.25 mg/mL. A 25 mg vial with ICG powder was diluted in 20 cc of aqueous sterile water, and 0.5 to 1 mL of ICG solution was injected near the hilum of the ovary, respectively into the right ovarian pedicle (3 patients), into the right broad ligament (1 patient), into left ovarian parenchyma (1 patient). A percutaneous introduction of the needle was performed with low intra-abdominal pressure (4-5 mmHg) in order to have the ovarian surface close to the abdominal wall during the injection of the tracer. A typical spinal needle (22-gauge size) is made up of an obturator having an end cap, a cannula having a needle bevel at one extremity and a hub at the opposite end. We have disengaged the obturator from the cannula and we have connected the 20 cm-long hub to infusion tubing for intravenous fluid delivery. The infusion tubing is instead connected to indocyanine green infusor. The needle is advanced inside the lateral trocar (10 mm) through a Johann clamp and in this way we can inject the indocyanine in the sites described above (**Figure 1 A-B**). Subsequently, by using the real time fluorescent infrared light of the SPIES camera, the entire retroperitoneal area was explored to find the fluorescent tracer in the lymphatic channels and to identify the anatomical location of the SLNs.

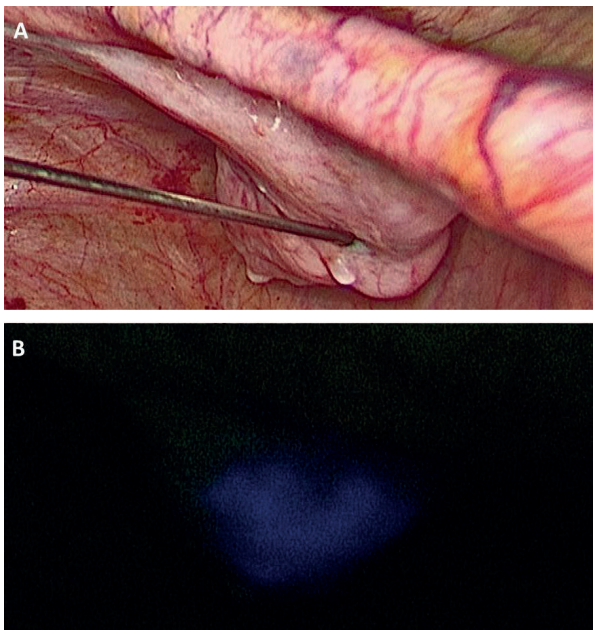

Figure 1.
Inoculation of IDG in the Right ovarian pedicle.
A) Before and B) After inoculation of IDG

RESULTS

Between April 2016 and May 2016, 5 patients (age range 43-67 years; mean 54,7 years) with clinical FIGO stage I - II ovarian carcinoma underwent laparoscopic abdominal hysterectomy (LAH) and bilateral salpingo-oophorectomy (BSO) with radical omentectomy and with pelvic and para aortic lymph node dissection, at the Department of Gynecologic Oncology, Regina Elena National Cancer Institute. (**Table 1**).
The distribution of histology and stage is shown in **Table 2**.

Table 1.
Caracteristics of patients

AGE	43-67 years
DISEASE	Ovarian Cancer
STAGE	IA-IIB
TUMOR DIMENSION	1<X>10 cm
TYPE OF SURGERY	LAH + BSO + Radical Omentectomy + Pelvic and Aortic Lymph Node Dissection

Table 2.
Hystology of patients

	PATIENTS				
HISTOTYPE	SEROUS	ENDOMETRIOID	ENDOMETRIOID	SEROUS	SEROUS
GRADING	3	2	2	3	3
STAGE	IC	IA	IA	IIB	IC

Two epithelial histologies were represented: serous (3 patients) and endometrioid (2 patients). For serous types the grading was 3 in both patients and for endometrioid types grading was 2 in both patiens. A variety of stages were represented, in particular patients with endometrioid ovarian cancer showed stage IA and patients with serous ovarian cancer were affected by stage IIB and IC respectively. LVSI was negative in all of patients. Median time of surgery was 196 minutes (range 180 to 215). Median total nodes removed were 21 (range 18 to 28), in two patient ipsilaterally to the injection site and in two patients bilaterally. A total of 6 SNs was detected in all patients. The median interval from the tracer injection to the detection of all SNs was 2 minutes (range, 1 - 3 minutes). In three patients, the first detected SN was found in the area of common iliac artery, in two patients at paracaval region. The median number of lymph nodes removed per patient was 2 (range, 0-2). All the detected SLNs were identified ipsilateral to the site of injection. Only in two patients we detected a second LSN, respectively in preaortic region and in under mesenteric region. The biopsy of all of 6 SNs was negative: the remaining removed 22 not-sentinel node were negative too. (**Table 3**).

Table 3.
Site of SLN locatio

	TOTAL NODES REMOVED	N° SLN	SLN 1 LOCATION	SLN 2 LOCATION	POSITIVE SLN	LN+ (NOT SLN)
Patient 1	28	2	Common iliac artery	Pre aortic	0	0
Patient 2	20	1	Common iliac artery		0	0
Patient 3	19	1	Para Caval Vein		0	0
Patient 4	18	2	Common iliac artery	Para Aortic (under mesenteric)	0	0
Patient 5	22	1	Para Caval Vein		0	0

No intra o post-operative complications were registered and no allergic reaction occurred. In absence of positive nodes sensitivity could not be calculated. Specificity and negative predictive value (NPV) were 100%.

DISCUSSION

Although the sentinel node biopsy has been applied successfully for breast cancer, vulvar cancer melanoma and other gynecological cancers[6], the role of SLN mapping in ovarian cancer is still unclear. The most common

causes are the different mapping techniques (tracers, injection sites) and the risk of tumor dissemination. Lymph node involvement in early stage ovarian cancer plays a crucial prognostic role. Literature shows that, the prevalence of pelvic and para-aortic node metastases in 689 early stage ovarian cancer is 20%[11]. Systematic pelvic and para-aortic lymphadenectomy (LAE) is an important prognostic factor but, compared with a lymph node sampling, LAE takes prolonged hospitalization, more blood loss and longer surgical time[12].

Kleppe et al. in the 2015, realized a 3D reconstruction of a lymphatic drainage pathways of the ovaries in human fetal pelvis aged 15 weeks, previously sectioned and stained with hematoxylin and eosin or with azan. In addition, series of selected sections were stained with antibodies against Lyve-1 (ReliaTech), S100 (DAKO), and >-smooth muscle actin (SMA; Sigma-Aldrich). Micrographs were made with a microscope and camera and a particular software was used to assemble the 3D volume and to reconstruct the 3D surface meshes. Authors identified 2 major and 1 minor lymphatic drainage pathways of the ovaries: an abdominal pathway, running via the infundibulopelvic ligament to the para-aortic (left side)/paracaval (right side) regions; a pelvic pathway, draining via the lateral parametrium and supraureteral pathway to the internal iliac artery and obturator fossa; and thirdly an inguinal pathway, draining via the round ligament of the uterus to the inguinal regions. Sentinel nodes can be found in any of these regions[13].

Subsequently, the authors determined the feasibility and the safety of the SN procedure performed with tracer injection into the ovarian ligament. Injection of radioactive tracers resulted in the identification of SNs in all patients, suggesting that this procedure could potentially be incorporated into routine clinical practice in patients with early-stage ovarian cancer[4]. Therefore same authors proposed a phase 1 study protocol which provides a blue dye and radioactive colloid injection into the ligamentum ovarii proprium and the ligamentum infundibulo-pelvicum before starting the surgical staging procedure. In the analysis this protocol calculates the percentage of patients in whom is feasible to identify sentinel nodes. Other study parameters are the anatomical localization of the sentinel nodes and the incidence of false negative lymph nodes[14].

Up to the present, a small amount of studies on SNB in ovarian cancer have been published.

Table 4 summarizes the data of these studies in addition to the current one.

Table 4.
Review of literature on SLN in early ovarian cancer

Author (reference)	N	Tracer Used	Injection Site	Overall Detection rate (%)	SLN LOCATION
Negishi 2003 [7]	11	CH40	Cortex ovary	100	a-IMA: 91% ar-IMA: 36% b-IMA: 36% CI: 26% EI: 9%
Nyberg 2011 [8]	16	99mTc blue dye	Left/right ovary	94	a-IMA: 33% b-IMA: 67%
Kleppe 2014 [4]	21	99mTc blue dye	Ovarian and suspensory ligament	96	Aortic/caval: 67% Pelvic: 9% Aortic+Pelvic:24 %
Buda 2016 [20]	10	ICG	Ovarian and suspensory ligament	90	a-IMA: 27% b-IMA: 53% Pelvic: 20%
Present Study 2016	5	ICG	Right ovarian pedicle Right broad ligament Left ovarian parenchyma	100	CI: 25% Paracaval: 25% Preaortic: 25% Mesenteric: 25%

a-IMA: Around the Inferior mesenteric Artery

b-IMA: Below the Inferior mesenteric Artery

CI: Common iliac artery

EI: External Iliac

Three previously published series explored the feasibility of SLN mapping of the ovary performed during traditional open surgery and overall recruited 59 patients. In a pilot study, Negishi et al[7] found SLNs in 11 patients by the injection of a mixture of activated carbon CH40 and polyvinylpyrrolidone into the ovarian cortex with an overall detection rate of 100%. Another two studies injected near the hilum a solution of technetium radiocolloid and blue dye[8-4]. A total of 30 blue SLNs were located in 15 of 16 patients (94%). With great interest, the authors found a statistically significance difference in the level of the right- and left-side SLNs in relation to Inferior Mesenteric Artery (IMA). The right-side injections drained below the IMA, whereas left-side injections located SLNs above the IMA. Kleppe et al[4] in their series of 22 patients injected the tracer close to the ovary on the dorsal and the ventral side of the ovarian ligament and the suspensor ligament. They successfully completed the SLN mapping in 96% of cases. In most patients (67%) the hot spot nodes were detected in the paracaval or in the para-aortic region exclusively, only 9% in the pelvic region, and 24% in both the paracaval/para-aortic and pelvic region. In 19 of 21 patients, SLNs were found ipsilateral to the injection site. The same authors[13] confirmed their preliminary hypotheses with the study of the hystologic lymphatic pathway of the ovary. They

found 3 main lymphatic drainage pathways from the ovaries the abdominal pathway, the pelvic pathway and the inguinal pathway. The first 2 seem to be the major route for lymphatic drainage from the ovary. The inguinal pathway seeems to disappear during embryologic development, but in a limited pecentage of women it may persist and can explain the occurence of isolated inguinal metastases.

The U.S. Food and Drug Administration approved ICG for use in humans since 1959 for cardiac and liver functions, but for tissue ICG, its use is off label. Moreover, ICG is widely used particularly for cholangiography and as a tool to delineate the extrahepatic biliary tree for NIR fluorescence[15]. More recently, the clinical effectiveness of NIR fluorescence using ICG has been evaluated for SLN mapping in gynecologic malignancies, particularly in laparoscopic or robotic approach[16-19]. Buda et al[20] recently published their experience about the feasibility of ovarian SLN mapping during laparoscopy using the fluorescent dye ICG and near-infrared real-time SPIES ICG (Karl Storz). In these patients the same protocol as Kleppe et al[4] has been adopted and the injection has been carried out through a percutaneous approach with a 22-G spinal needle (12 cm). Their results are similar to the previous series published concerning the incidence and the location of nodal involvement in early stage ovarian cancer. 100% of the SLNs were located ipsilaterally to the side of injection. and in 30% the paracaval/para-aortic location was associated with pelvic migration on the common iliac nodes. Most of the right ovarian SLNs were located below the IMA (inferior mesenteric artery), whereas SLNs were located above the IMA when the left ovary was injected.

In our small series, four patients with clinical FIGO stage I and II ovarian carcinoma underwent to laparoscopic SLN mapping using Fluorescent Indocyanine Green injected near the hilum of the ovary, respectively into the right ovarian pedicle (3 patients), into the right broad ligament (1 patient), into left ovarian parenchyma (1 patient). A spinal needle (22-gauge size) was connected to 20 cm-long hub to infusion tubing for intravenous fluid delivery. The needle was advanced inside the lateral trocar (10 mm) through a Johann clamp and the indocyanine was injected into the right ovarian pedicle (3 patients), into the right broad ligament (1 patient), into left ovarian parenchyma (1 patient). Median total nodes removed were 21 (range 18 to 28), in two patient ipsilaterally to the injection site and in two patients bilaterally. A total of 6 SNs was detected in all patients. In three patients, the first detected SN was found in the area of common iliac artery, in one patient at paracaval region.

Only in two patients a second LSN was detected, respectively in preaortic region and in under mesenteric region. The biopsy of all of 6 SNs was negative: the remaining removed 22 not-sentinel node were negative too. Despite the very small series in our experience, we want highlight the innovative laparoscopic injection technique, through a spinal needle connected to infusion tubing. Moreover we used ICG tracer, a new injection agent that relies on near-infrared imaging. Early reports concerning its use in cervical and endometrial cancer suggest very high SLN detection rates[21].

Our paper shows that the laparoscopic Indocyanine Green Sentinel lymph node mapping in ovarian cancer is a feasible, safe and inexpensive technique, suggesting that technique could potentially be incorporated into routine clinical practice. Unfortunately the sample size is very small. Additional prospective multicenter studies are needed to determine the best SN procedure to reduce the morbidity and rate of complications associated with complete pelvic and paraortic lymphadenectomy.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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