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*Original Article*

# Added-value of SPECT/CT to lymphatic mapping and sentinel lymphadenectomy in gynaecological cancers

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**Abstract:** Lymphatic mapping and sentinel lymphadenectomy (LM/SL) have been successfully used in pre-treatment nodal staging of gynaecological cancers. We hypothesised the added-value of LM/SL plus SPECT/CT in patients with early stage of cervical cancer and vulvar cancer. A prospective, single-center, diagnostic, open label, active control, non-randomized clinical trial has been conducted in 7 patients with FIGO IA-IB1 cervical cancer and 7 patients with FIGO stage I-II-IIIcN0 vulvar cancer. All patients underwent LM/SL plus SPECT/low-dose CT and complete lymph node dissection (CLND) according to the standard of care. In case of negative hematoxylin-eosin staining, serial sections of the SLNs were analysed by immunohistochemistry and high molecular weight cytokeratin. Primary outcome measures were the detection rate, the sensitivity (SV), the negative predictive value (NPV), the diagnostic accuracy (DA) for anatomic localisation of SLNs, and the impact on management of SPECT/CT guided LM/SL versus CLND. The secondary outcome measure was the patient tolerability and operating time of LM/SL guided SPECT/CT versus CLND. http://clinicaltrials.gov/show/NCT00773071 All 14 patients were enrolled into the 1-day research protocol with dual-tracer LM/SL and SPECT/CT. Additional SLNs were detected on SPECT/CT compared to conventional planar imaging. Hot and cold > 1cm SLNs were detected on SPECT/CT. Detection rate, SV, NPV, DA were 100% in both groups; false negative rate was 0%. Rate of SLN metastases was 28.5% in cervical cancer and 42.9% in vulvar cancer. Impact on treatment was 28.5% and 14.3% in cervical cancer and vulvar cancer patients, respectively. SPECT/CT was well tolerated by all patients and operating time for LM/SL was within 30 min. No adverse events were reported with a time frame of 1-to-3 years. In early stage of gynaecological cancers, SPECT/low-dose CT is technically feasible and of clinical added-value for LM/SL.

**Keywords:** LM/SL, SPECT/CT, vulvar cancer, cervical cancer

## Introduction

Nodal staging is a key-step in pre-treatment assessment of gynaecological cancers [1]. In recent years, lymphatic mapping and sentinel lymphadenectomy (LM/SL) as a minimally invasive pelvic lymph node staging have been successfully evaluated in women with early stage vulvar cancer, cervical cancer, and endometrial cancer [2-4]. In clinical routine, LM/SL may help to avoid the cost and the morbidity of unnecessary lymphadenectomy in the majority of cases with uninvolved sentinel lymph nodes (SLNs). LM/SL has the potential to guide the surgeon to nodal regions that are not routinely dissected (i.e. pre-sacral and para-aortic lymph nodes), and to detect microscopic and sub-microscopic nodal metastases using either immunohistochemistry or molecular biology techniques [5, 6].

So far, within the abdomen and the pelvis, the LM/SL technique alone is often blinded to the accurate localisation of SLNs. The integration of computed tomography (CT) to single photon emission computed tomography (SPECT) devices in a single gantry (SPECT/CT) has allowed a significant gain in terms of diagnostic accuracy and anatomic precision; clinical examples include malignant melanoma, head and neck cancer, breast cancer, and bladder cancer [7-10]. In a seminal series of 26 patients with

**Table 1.** Patient characteristics

| Patients | Age (years) | Primary Tumour Sites | Histology Types | FIGO Stages |
|---|---|---|---|---|
| # 1 | 38 | cervix | SCC | IB |
| # 2 | 40 | cervix | ADC | IA |
| # 3 | 71 | cervix | SCC | IA2 |
| # 4 | 30 | cervix | ADC | IB1 |
| # 5 | 52 | cervix | SCC | IB1 |
| # 6 | 41 | cervix | SCC | IB |
| # 7 | 35 | cervix | ADC | IB1 |
| # 8 | 72 | right labia majora / minora | SCC | I |
| # 9 | 77 | right labia majora | SCC | IA1 |
| # 10 | 68 | right and left labia majora / minora | SCC | II |
| # 11 | 76 | right labia majora / minora | SCC | II |
| # 12 | 42 | left labia majora | SCC | III |
| # 13 | 72 | midline | SCC | I |
| # 14 | 44 | left labia majora | SCC | IIIcN0 |

Abbreviations: FIGO: international federation of gynaecology and obstetrics. SCC: squamous cell carcinoma. ADC: adenocarcinoma.

cervical cancer, SPECT/CT was found superior to conventional planar imaging for detection of SLN and accurate localisation [11]. More recent studies have highlighted the technical feasibility and clinical added-value of a low-dose SPECT/CT to LM/SL in early stage cervical cancer [12-16]. In vulvar cancer, preliminary data highlighted the feasibility of LM/SL in 3D fusion imaging, and a few case reports have showed the usefulness of LM/SL with integrated SPECT/CT [17-19]. In the light of the encouraging data from literature and our own preliminary clinical experience, we hypothesized that the use of SPECT/CT plus LM/SL may be of clinical interest in patients with gynaecological cancers.

## Patients

From 2008 to 2009, 15 patients with histologically proven cervical cancer (n=7) and vulvar cancer (n=8) were enrolled into a prospective, single-center, diagnostic, open label, active control, non randomised clinical trial. This trial was approved by The University of Western Ontario (UWO) Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB – Review Number 12576), and was indexed on ClinicalTrial.gov website from the US National Institutes of Health: http://clinicaltrials.gov/show/NCT00773071 All 15 patients signed the informed consent form; 14 patients completed the research protocol with dual tracer LM/SL plus SPECT/CT. One vulvar cancer patient signed the informed consent but withdrew from the trial. This clinical trial was conducted by a multidisciplinary team including certified nuclear medicine physicians (TB, IR, JLU), gynecologic oncologists (MP, DL, AS, DB), and a pathologist (EH).

All 14 cervical cancer and vulvar cancer patients enrolled into the clinical trial underwent complete lymph node dissection (CLND) according to the standard of care in gynaecological cancers as recommended by the International Federation of Gynaecology and Obstetrics (FIGO) [20, 21]. Eligible patients with FIGO stages IA2 and IB2 cervical cancer (n=7) were scheduled for radical hysterectomy and pelvic lymph node dissection (PLND). Eligible patients with FIGO stages I and II vulvar cancer, and those of patients with FIGO stage III and clinically negative regional lymph nodes (n=7) underwent vulvectomy and inguinal lymph node dissection (ILND). In this clinical trial, 12 patients underwent a laparotomy approach and 2 cervical cancer patients underwent a laparoscopy approach. Patient characteristics are detailed in the **Table 1**.

## Methods

### *Lymphatic mapping and sentinel lymphadenectomy*

A one-day dual-tracer protocol was used in this clinical trial. Pre-operatively, 2 to 4 peri-tumoral injections (3h, 6h, 9h, 12h) of $^{99m}$Tc-cysteine rhenium colloid (10-12 nm average size, 1 cc, 1 mCi/37MBq) were performed in the department of nuclear medicine by the gynaecologic oncologists (MP, AS, DL, MB). $^{99m}$Tc-cysteine rhenium colloid is a modified formulation of $^{99m}$Tc- sulfur colloid that had been licensed by Health Canada and developed by the radio-

pharmacist (PZ) to meet all USP pharmaceutical requirements and quality control specifications. It has a smaller particle size (over 90% less than 0.1 *u*m) ideal for LM/SL with excellent sentinel nodal trapping [22]. Intra-mucosal peri-cervical injections were performed in cervical cancer patients, and intra-dermal injections were performed in vulvar cancer patients. Dynamic lymphoscintigraphy (30 images, 20 sec per frame, 128 x 128 matrix), and static anterior, posterior, and lateral views (10 min per view, 350 Kcnts minimum per view) were performed. Immediately after the planar imaging, a SPECT/low-dose CT acquisition (Infinia-Hawkeye™, GE healthcare) was performed on the Infinia-Hawkeye™ -1 (n=13) and the Infinia-Hawkeye™ -4 (n=1) from mid-thighs to the liver centered over the abdomino-pelvic region, the arms above the head. Dual-head SPECT acquisition parameters included step-and-shoot mode, automatic body contours, 128x128 matrix, 360° angle, 3° per step, 25 sec per step, zoom = 1, H rotation = 0, low energy high resolution (LEHR) collimator. Low-dose CT acquisition parameters included 512 x 512 matrix, 2.5 mA, 140 kV, effective dose < 2 mSv, 10 mm (Hawkeye -1) or 5 mm (Hawkeye -4) slice thickness, E-T = 180°, step-and-shout rotation (Hawkeye -1) or helical half-rotation (Hawkeye -4), 5 min (Hawkeye -4) or 10 min (Hawkeye -1) scan time duration. No contrast-agent was injected for the low-dose CT acquisition. SPECT images were reconstructed using the ordered subset expectation maximisation (OSEM) iterative method. SPECT images were corrected for soft-tissue attenuation artifact using the low-dose CT. Non-corrected and corrected SPECT images, CT images, and fused SPECT/CT images were displayed on the Xeleris™ workstation 1.1v or 2.05v. Intra-operatively, five minutes before incision, 2 to 4 peri-tumoral injections (3h, 6h, 9h, 12h) of 2 cc of patente blue-dye (THERAPLEX™, Montreal, Canada) were performed. Lymph node dissection was carried out first. Resection of the primary tumour was carried out after completion of lymph node dissection by the gynaecologic oncologists (MP, DL, AS, MB). Intra-operative gamma probe guidance (Navigator GPS™, Tyco Healthcare) was performed for detection of the SLNs (GPG). A standard lymphatic mapping probe (14 mm tip diameter, 35° tip angle) was used in twelve patients, and a laparoscopic lymphatic mapping probe (10 mm tip diameter, 0° tip angle) was used in two cervical cancer patients. Removal of SLNs was performed separately for sophisticated pathological analysis.

*SLN detection*

SLNs were identified as hot and/or blue lymph nodes. Pre-operatively, the hot SLNs were defined as the first lymph nodes detected within 30 min post-tracer injection during the lymphoscintigraphy and the SPECT/CT. Intra-operatively, all blue-stained and/or hot lymph nodes with a radioactivity greater than 10% of the hottest lymph node were considered as SLNs. Cold SLNs or non radioactive and non blue-stained SLNs massively invaded by tumour were also detected on low-dose CT from SPECT/CT as supra-centimetric (> 1 cm) lymphadenopathies. Dynamic lymphoscintigraphy, static views, and SPECT/CT images were analysed and interpreted by nuclear medicine physicians (TB, IR, JLU). GPG was performed by the nuclear medicine physician and the gynaecologic oncologist. Pre-operative and intra-operative SLN features were precised: 1) patterns (hot and/or blue and cold), 2) number, 3) anatomical localisation, 4) in-vivo and ex-vivo counting rates.

*Pathologic analysis*

SLN and non SLNs were analysed by the pathologist referee (HE) with controls. Serial sections of the SLNs were analysed by hematoxylin and eosin staining (H-E) at three levels. In cases of negative H-E, SLNs were further analysed by immunohistochemistry (CKAE1/CKAE3) and high molecular weight cytokeratin (CK 34BE12). Non SLNs were analysed as usual in routine by H-E. SLN features were precised including the SLN size, the SLN involvement (macro-metastases > 2 mm; micro-metastases ≤ 2 mm; isolated tumor cells), the percentage of SLNs involved (small < 25%; moderate = 25-75%, massive > 75%). SLN and non SLNs will be stored in the department of pathology. The specimens and data will be kept indefinitely.

*Primary outcome measures*

LM/SL plus SPECT/CT was compared to LM/SL alone to assess: 1) SLN detection rate (DR) pre-operatively and intra-operatively, 2) diagnostic accuracy (DA) for anatomic localisation of SLNs detected pre-operatively versus surgery, 3)

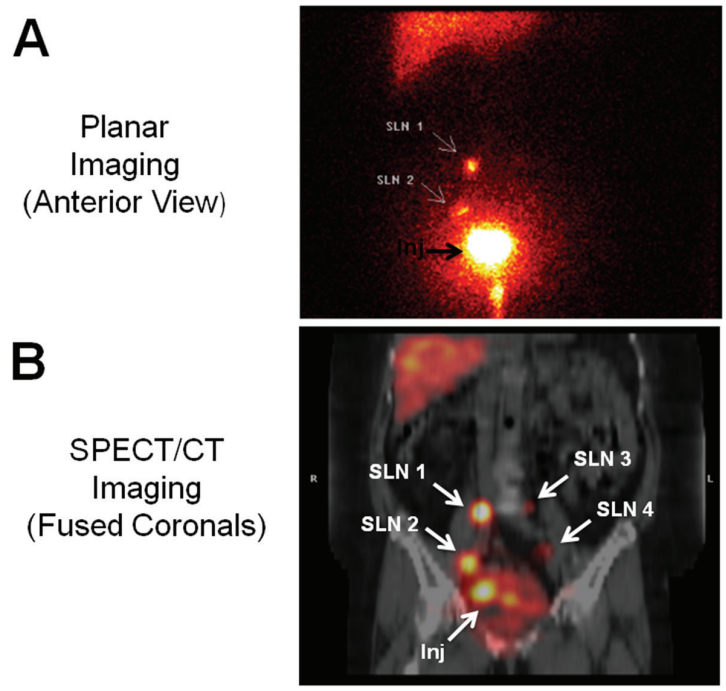


**Figure 1.** A FIGO stage IB1 cervical cancer with unilateral pelvic SLNs on planar imaging and bilateral pelvic SLNs on SPECT/CT. A. Planar imaging showed 2 SLNs (SLN1 and SLN2, white arrows) on the right side of the pelvis. B. SPECT/low-dose CT evidenced 4 SLNs (SLN1, SLN2, SLN3, SLN4, white arrows) on both sides (2 right and left external iliac SLNs and 2 right and left common iliac SLNs), which were precisely localised at surgery as blue-stained and hot SLNs. At the final pathology, 5 SLNs and 15 non-SLNs were analysed, which were all free of tumour. SLN: sentinel lymph node. Inj: injection site.

sensitivity (SV) for detection of SLN metastases detected by H-E or immunohistochemistry, 4) negative predictive value (NPV) for assessing the regional lymph node status (negative non-SLNs when the SLN is negative), 5) false negative rate (non-SLN metastases with a negative SLN), 6) impact on patient's management.

*Secondary outcome measures*

Safety of LM/SL + SPECT/CT was assessed: 1) patient's tolerability during the SPECT/CT study, 2) duration of operating time for SPECT/CT guided LM/SL, 3) complications rate.

*Time frame*

All 14 patients enrolled into the clinical trial were followed by their referring gynaecologists who were co-investigators in this research study for a time frame varying from 1 year to 3 years after the LM/SL plus SPECT/CT study. Patient initials, birth date, and OHIP number (Ontario Health Insurance Plan) were used only for follow-up with the referring gynaecologist.

*Statistical analysis*

Patients' age was expressed in mean age ± SD. A positive SLN involved by tumour with a negative or positive non-SLN was considered as a true-positive (TP). A negative SLN free of tumour with a negative non-SLN was considered as a true-negative (TN). A negative SLN free of tumour with a positive non-SLN was considered as a false-negative (FN).

SLN detection rates (DR) were reported at the patient's level. Rates of metastatic SLNs per patient were reported in percentage. SV (TP/TP+FN) and NPV (TN/TN+FN) were calculated per patient according to the classical definitions. Diagnostic performance per patient was reported with 95% confidence intervals (CI). Overall survival (OS) and progression-free survival (PFS) rates were described at 1-year, 2-year, and 3-year follow-up in cervical cancer patients (n=7) and vulvar cancer patients (n=7) who completed the clinical trial. OS was defined as the absence of death or survival in the presence of recurrence, and PFS was defined as survival in the absence of recurrence and death.

## Results

*Patient characteristics*

In this clinical trial, 7 patients with FIGO stages IA2-IB1 cervical cancer and 7 patients with FIGO stages I, II, and IIIcN0 vulvar cancer were enrolled into the SPECT/CT guided LM/SL research protocol versus CLND. In patients with cervical cancer, the mean age ± SD was 43.9 ± 13.7 years (30-71); the histology types were squamous cell carcinoma (SCC) in 4 patients and adenocarcinoma (ADC) in 3 patients. In patients with vulvar cancer, the mean age ± SD was 64.4 ±14.9 years (42-77); the histology type was squamous cell carcinoma (SCC), and the primary tumour sites were the right labia majora (n=1), the left labia majora (n=2), the

**Table 2.** LM/SL plus SPECT/CT results in cervical cancer patients – SLN sites

| SLN sites at surgery | Number of SLNs (%) |
|---|---|
| Obturator | 5 (19%) |
| External iliac | 12 (46%) |
| Internal iliac | 1 (4%) |
| Common iliac | 5 (19%) |
| Pre-sacral | 1 (4%) |
| Para-aortic | 2 (8%)* |
| Total | 26 |

*The 2 para-aortic SLNs were not removed.

right and left labia majora and minora (n=1), the right labia majora and minora (n=2), and the midline (n=1). Patient characteristics are detailed in the **Table 1**.

*Primary outcome*

In cervical cancer patients, 56 SLNs were resected (8 SLNs per patient) and 92 non-SLNs were resected (13 non-SLNs per patient). The SLN detection rate (DR) was 85% on planar imaging (6/7 patients) and 100% on SPECT/CT imaging (7/7 patients). On dynamic lymphoscintigraphy and static views, 15 hot SLNs were detected; on SPECT/CT, 23 hot SLNs were detected. Bilateral SLNs were found in 5 patients, and unilateral SLNs were found in 2 patients (**Figure 1**). At surgery, 26 SLNs (1 blue, 12 hot, and 13 hot and blue SLNs) were removed, and 56 SLNs were analysed at the final pathology. SLNs sites were found in predictable pathways including the 5 obturator (19%), 12 external iliac (46%), and 1 internal iliac (4%) SLNs, and unpredictable pathways including the 5 common iliac (19%), 2 para-aortic (8%), and 1 pre-sacral (4%) SLNs; the 2 para-aortic SLNs were not removed (see **Table 2**). SLN metastases were detected in 2 out of 7 patients with negative non-SLNs (28.5%); one patient (# 4) had 1 SLN positive at H-E with a micro-metastasis (< 2 mm) and < 25% tumour involvement, and the other patient (# 7) had 7 SLNs positive at H-E and 1 SLN positive at IHC with isolated tumour cells (1 SLN), < 2 mm micro-metastases (5 SLNs) and > 2 mm macro-metastases (2 SLNs), < 25% (7 SLNs) and 25-75% tumour involvement (1 SLN). In the 5 out of 7 patients with 30 negative SLNs, 76 non-SLNs were also negative. Results of pathologic analysis in cervical cancer are detailed in the **Table 3**. SV was 100%, NPV was 100%, DA was 100%, and FN rate was 0%. In cervical cancer patients, SPECT/CT guided LM/SL had an impact on treatment in two patients (# 4, # 7) with a positive SLN and negative non-SLNs (28.5%); concurrent chemo-radiation therapy (CCRT) and CCRT plus brachytherapy, respectively.

In vulvar cancer patients, 22 SLNs (3 SLNs per patient) were resected and 64 non-SLNs were resected (9 non-SLNs per patient). The SLN DR was 85% (6/7 patients) on planar imaging, and 100% on SPECT/CT imaging (7/7 patients). Bilateral SLNs were detected in 3 patients, and unilateral SLNs were detected in 4 patients. At surgery, 19 SLNs (3 blue, 9 hot, 5 hot and blue, and 2 cold > 1 cm SLNs) were removed (**Figure 2**), and 22 SLNs were analysed at the final pathology; LM/SL plus SPECT/CT SLN patterns included 15 hot and hot and blue SLNs < 1 cm (68%), 2 hot and blue SLNs > 1 cm (9%), 2 cold > 1 cm SLNs (9%), and 3 blue SLNs (14%). In one vulvar cancer patient (FIGO stage IIIcN0) with a cold right inguinal SLN invaded by tumour measuring 1.6 cm in the short axis on low-dose CT, a hot focus was also detected in the right groin on planar imaging and SPECT/CT but not found at surgery; this hot focus likely corresponded to a lymph vessel stasis with a re-routing of the lymph flow caused by the cold SLN > 1 cm. In one patient with a FIGO stage II vulvar cancer, dynamic lymphoscintigraphy and static views did not show any hot SLN. SPECT/CT evidenced two hot left inguinal and inguinal femoral SLNs, and GPG allowed to detecting an additional hot right inguinal SLN; all three hot SLNs were invaded by tumour at the final pathology (**Figure 2**). SLNs sites were found in predictable pathways including the 13 inguinal SLNs (68.5%) and 4 inguino-femoral SLNs (21%), and unpredictable pathways including the 1 obturator SLN (5.25%) and 1 external iliac SLN (5.25%). SLN sites in vulvar cancer are detailed in the **Table 4**. SLN metastases were detected in 3 out of 7 patients (42.9%); 2 patients (# 10, # 14) with positive non-SLNs had 4 SLNs positive at H-E with macro-metastases (> 2 mm), < 25% (2 SLNs) and > 75% (2 SLNs) tumour involvement. Another patient (# 11) had 4 positive SLNs at H-E with negative non-SLNs; SLN metastases were < 2 mm micro-metastases (1 SLN) and > 2 mm macro-metastases (3 SLNs), < 25% (1 SLN) and > 75% (3 SLNs) tumour involvement. In the 4 out of 7 patients with 13 negative SLNs, 25 non-SLNs were also nega-

**Table 3.** LM/SL plus SPECT/CT results in cervical cancer patients – Pathologic analysis

| Patients | Non-SLNs | Non-SLNs + (H-E) | SLNs (blue and/or hot) | SLNs + (H-E) | SLNs + (IHC) | SLNs + Size | SLNs + % Involvement |
|---|---|---|---|---|---|---|---|
| # 1 | 21 | 0 | 5 | 0 | 0 | | |
| # 2 | 10 | 0 | 3 | 0 | 0 | | |
| # 3 | 17 | 0 | 2 | 0 | 0 | | |
| # 4 | 2 | 0 | 15 | 1 | 0 | < 2mm | < 25% |
| # 5 | 15 | 0 | 5 | 0 | 0 | | |
| # 6 | 13 | 0 | 15 | 0 | 0 | | |
| # 7 | 14 | 0 | 11 | 7 | 1 | ITC (1)<br>< 2mm (5)<br>> 2mm (2) | < 25% (7)<br>25-75% (1) |
| Total | 92 | 0 | 56 | 8 | 1 | | |

Abbreviations: H-E: hematoxylin and eosin staining. IHC: immunohistochemistry. ITC: isolated tumour cells.

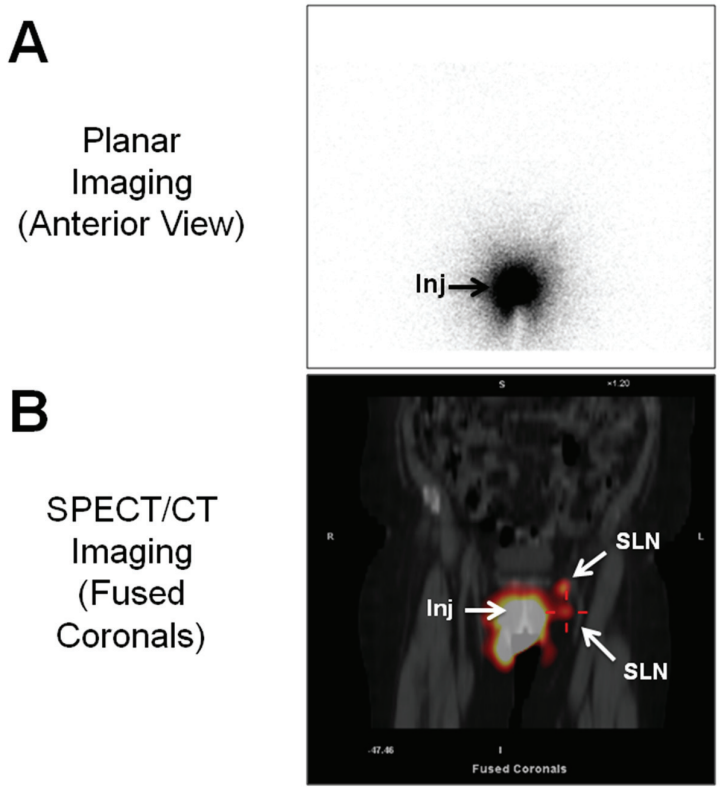


**Figure 2.** A FIGO stage II vulvar cancer with hot SLNs on SPECT/CT not seen on planar imaging. A. Planar imaging showed no SLN. B. SPECT/low-dose CT revealed 2 hot left inguinal femoral SLNs (white arrows), which were invaded by tumour with macro-metastases > 2 mm at the final pathology. SLN: sentinel lymph node. Inj: injection site.

tive. Results of pathologic analysis in vulvar cancer are detailed in the **Table 5**. SV was 100%, NPV was 100%, DA was 100%, and FN rate was 0%. In vulvar cancer patients, SPECT/CT guided LM/SL had an impact on treatment in one patient (# 11) with a positive SLN and negative non-SLN (14.3%); intensity modulated radiation therapy (IMRT). The two other patients (# 10, # 14) with positive non-SLNs and positive SLNs received radiation therapy and CCRT, respectively. Results per patient of SPECT/CT guided LM/SL in gynaecological cancers are detailed in the **Table 6**.

*Secondary outcome*

LM/SL plus SPECT/CT study was well tolerated by all 14 patients enrolled into the clinical trial. SPECT/CT study was performed within 30 min. Operating time for SPECT/CT guided LM/SL was within 30 min. No pre-operative, intra-operative, and post-operative complications related to LM/SL plus SPECT/CT was reported with a follow-up time frame from 1 to 3 years.

*Overall survival and progression-free survival*

All cervical cancer patients were alive with no evidence of disease after 1-year (n=7), 2-year (n=6), and 3-year (n=4) follow-up; no nodal or cervical recurrence was detected during this time frame. OS and PFS were 100% at 1-year, 2-year, and 3-year follow-up.

In vulvar cancer patients, 3 patients with a positive SLN (2 FIGO stage II and 1 FIGO stage

**Table 4.** LM/SL plus SPECT/CT results in vulvar cancer patients – SLN sites

| SLN sites at surgery | Number of SLNs (%) |
|---|---|
| Obturator | 1 (5.25%) |
| Inguinal | 13 (68.5%) |
| Inguino-femoral | 4 (21%) |
| External iliac | 1 (5.25%) |
| Total | 19 |

IIIcN0) died from nodal recurrence and 4 patients with a negative SLN (3 FIGO stage I and 1 FIGO stage IIIcN0) were alive after 3-year follow-up with no nodal recurrence; 1 patient had a vulvar recurrence resected at 1-year after LM/SL with no evidence of disease at 3-year follow-up, and 1 patient had a brain metastasis from unknown origin and is still alive at 3-year follow-up. OS was 57% at 1-year, 2-year, and 3-year follow-up; PFS was 43% at 1-year, 53% at 2-year and 3-year follow-up.

## Discussion

In patients suffering from an early stage of gynaecological cancer with no clinical evidence of nodal disease, there is theoretically no reason to perform systematically a complete lymph node dissection (PLND or ILND) as demonstrated for other types of lymphophilic cancers such as malignant melanoma, breast cancer, and head and neck cancer [23, 24]. CLND morbidity is associated to leg lymphoedema, nerve or great vessel or ureteral injuries, infection, and blood loss. Delayed operating time, recovery time, and hospitalisation stay are costly [25, 26]. In the selected cervical cancer and vulvar cancer indications, there is a strong clinical rationale for the use of LM/SL in the staging of regional lymph nodes [1, 5]. Systematic reviews of the literature and meta-analyses evidenced the highest sensitivity and specificity of LM/SL for prediction of lymph node status in comparison to fine needle aspiration, ultrasound, computed tomography, magnetic resonance imaging, and $^{18}$F-fluorodeoxyglucose positron emission tomography [27-30]. In an interim analysis of 60 out of 120 patients with cervical cancer FIGO stage IA-IIA, $^{18}$FDG PET had 10% sensitivity only for detection of pelvic SLN metastases. As a conclusion, $^{18}$FDG PET was not recommended in the early stage protocol [31].

In recent multicenter clinical trials, LM/SL as a minimally invasive surgery has been validated as a robust alternative to CLND in cervical cancer and vulvar cancer [32-36]. In the AGO Study Group including 590 patients with cervical cancer, the German multicenter trial evidenced the validity of the SLN concept in tumours ≤ 2.0 cm size with a DR of 94%, a SV of 90.9%, and a NPV of 99.1% [32]. In the SENTICOL study, the French multicenter trial, LM/SL had a 97.8 % DR, a 92% SV and a 98.2% NPV in 139 patients with early stage IA1-IB1 cervical cancer [33]. In the prospective multicenter observational study including 403 patients with early stage vulvar cancer > 1 mm invasion (T1-2 < 4.0 cm, cN0), the Netherlands multicenter trial, LM/SL was found a safe alternative to elective inguinal femoral dissection (EIFD) with low morbidity rate, low recurrence rate, and excellent survival; a quality controlled multidisciplinary group was required [34]. In the German multicenter study including 127 patients with early stage vulvar cancer (T1-3, cN0), DR was 98.3%, SV was 92.3%, and FN rate was 7.7%; LM/SL was recommended in cN0 stage 1 < 4.0 cm vulvar cancer [35]. In the Gynecologic Oncology Group study (GOG 173 protocol), a US multicenter study including 452 patients, LM/SL was found a reasonable alternative to EIFD in early stage vulvar cancer > 1 mm invasion (T ≥ 2 cm and ≤ 6 cm, cN0) with a DR of 92.5%, a SV of 94.1%, and a NPV of 97.1%; FN rate was 2.0% in tumours < 4.0 cm [36].

The originality of this prospective single center clinical trial relies upon the use of hybrid imaging in LM/SL for gynaecological cancers. SPECT/low-dose CT allowed to obtaining both functional and anatomic information in a single study [37]. The radiation dose from the low-dose CT (Effective Dose < 2 mSv) is in the order of the yearly natural background (~ 3 mSv) [38]. Of note, all selected patients presented with a histologically proven cancer. Therefore, the radiation exposure expected from diagnostic modalities may be considered as not critical in this clinical setting. All fourteen patients who completed the clinical trial well tolerated the dual-tracer LM/SL with SPECT/low-dose CT. No adverse events have been reported with a time frame follow-up of one year to three years. In our 1-day LM/SL plus SPECT/CT protocol, additional hot and/or blue SLNs were detected compared to conventional LM/SL protocol. In

**Table 5.** LM/SL plus SPECT/CT results in vulvar cancer – Pathologic analysis

| Patients | Non-SLNs | Non-SLNs + (H-E) | SLNs (hot and/or blue and cold) | SLNs + (H-E) | SLNs + (IHC) | SLNs + Size | SLNs + % Involvement |
|---|---|---|---|---|---|---|---|
| # 8 | 9 | 0 | 2 | 0 | 0 | | |
| # 9 | 5 | 0 | 6 | 0 | 0 | | |
| # 10 | 21 | 3 | 3 | 3 | NA | > 2mm (all 3) | > 75% (2)<br>< 25% (1) |
| # 11 | 2 | 0 | 4 | 4 | NA | > 2mm (3)<br>< 2mm (1) | > 75% (3)<br>< 25% (1) |
| # 12 | 4 | 0 | 1 | 0 | 0 | | |
| # 13 | 7 | 0 | 4 | 0 | 0 | | |
| # 14 | 16 | 5 | 2 | 1 | NA | > 2mm | < 25% |
| Total | 64 | 8 | 22 | 8 | | | |

Abbreviations: H-E: hematoxylin and eosin staining. IHC: immunohistochemistry. NA: not applicable.

**Table 6.** Results per patient of SPECT/CT guided LM/SL in gynaecological cancers

| Diagnostic Performance [95%CI] | Cervical Cancer (n=7) | Vulvar Cancer (n=7) |
|---|---|---|
| DR | 100% [59.0% – 100%] | 100% [59.0% – 100%] |
| % SLN + | 28.5% [2.7% – 71.0%] | 42.9% [9.9% – 81.6%] |
| SV | 100% [59.0% – 100%] | 100% [59.0% – 100%] |
| NPV | 100% [59.0% – 100%] | 100% [59.0% – 100%] |
| DA | 100% [59.0% – 100%] | 100% [59.0% – 100%] |
| Treatment Impact | 28.5% [2.7% – 71.0%] | 14.3% [0.4% – 57.9%] |

Abbreviations: [95% CI]: 95% confidence intervals. DR: SLN detection rate. %SLN +: rate of positive SLNs. SV: sensitivity. NPV: negative predictive value. DA: diagnostic accuracy for SPECT/CT localisation of SLNs.

two vulvar cancer patients, cold SLNs massively invaded by tumour were detected on low-dose CT as supra-centimetric lymphadenopathies; this reduced the FN rate to 0% (**Figure 3**). In one of these cold SLNs, SPECT/CT evidenced a re-routing of the lymph flow to a hot focus, which was likely a lymph stasis. In breast cancer with suspicious non radioactive and non blue metastatic lymphadenopathies, the FN rate is significantly reduced by using the definition of truly positive SLN [39]. In penile cancer with enlarged SLNs and blockage of lymph vessels by tumour, the re-routing of lymph flow to neo-SLNs has been reported on SPECT/CT with hot foci related to lymph fluid stasis [40].

In cervical cancer patients, LM/SL plus SPECT/CT allowed to detecting 31% of SLNs in unpredictable lymphatic pathways (i.e. common iliac, para-aortic, and pre-sacral SLNs). In two cervical cancer and one vulvar cancer patients, seven micrometastases (≤ 2 mm) and one submicrometastases or ITC (≤ 0.2 mm) out of seventeen positive SLNs (47%) were detected on serial H-E staining and IHC; percentage of tumour involvement was < 25% in eleven out of seventeen involved SLNs (64.7%). Additional SLN metastases were detected on SPECT/CT guided LM/SL with an actual impact on treatment in 28.5% of cervical cancer patients and 14.5% of vulvar cancer patients. In this study, NPV was 100%. However, CLND was performed in all patients according to the standard of care as recommended by the FIGO [20, 21]. In Ontario, gynaecological cancers are not yet reimbursed clinical indications for LM/SL such as malignant melanoma and breast cancer. Potential impact is the cost-effectiveness of LM/SL plus SPECT/CT in vulvar cancer and cervical cancer, thereby, avoiding the cost and the morbidity of unnecessary CLND in the majority of patients with negative SLNs. In our protocol, three-dimensional (3D) SPECT/CT imaging allowed precise anatomic localisation of cold and hot SLNs with a 100% diagnostic accuracy compared to two-dimensional (2D) planar imaging. This is of clinical interest for a laparoscopic or robotic approach with a minimal morbidity [41, 42]. A recent multicenter prospective SENTICOL study concluded to the feasibility of LM/SL in 139 cervical cancer patients undergoing planar lymphoscintigraphy with laparoscopic surgery; DR was 97.8%, SV was 92%,

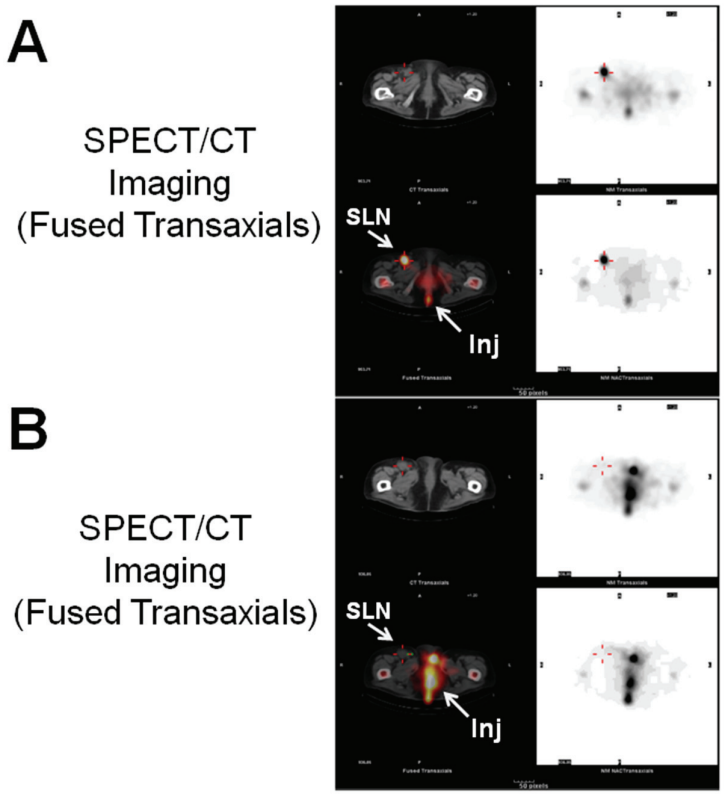


**Figure 3.** A FIGO stage II vulvar cancer with hot and 'cold' SLNs > 1 cm on SPECT/CT. A. a right inguinal femoral hot SLN > 1 cm (white arrow) and (B) a right inguinal femoral 'cold' SLN > 1 cm (white arrow), which were massively invaded by tumour (> 75% tumour involvement) at the final pathology. SLN: sentinel lymph node. Inj: injection site.

and NPV was 98.2% [43]. Also, CT-based attenuation correction allowed improved detection of SLNs with a better signal-to-background ratio on SPECT attenuation corrected images compared to planar imaging and SPECT non-attenuation corrected images. In early stage cervical cancer (IA-IIA), five prospective and one retrospective clinical trials including 130 patients have also concluded to the added-value of SPECT/CT in LM/SL with a mean DR of 98% (95% to 100%), a mean SV of 97% (87.5% to 100%), a mean NPV of 99% (95.2% to 100%), a mean DA of 98% (96.3% to 100%), and a mean FN rate of 3% (0% to 14.3%) [11-16]. In a series of 10 patients with vulvar carcinoma (cT1 and cT2), 3D fusion imaging (SPECT lymphoscintigraphy with CT/MRI image fusion) detected more SLNs, and was found more precise for anatomical localization than planar imaging [17]. In vulvar carcinoma and vulvovaginal melanoma, three case reports have more recently showed the added-value of integrated SPECT/CT to LM/SL for the detection of unpredictable lymphatic pathways and accurate anatomic localisation of SLNs [18, 19]. A recent review of the literature also highlighted the potential applications of SPECT/CT to LM/SL in cervical cancer and vulvar cancer [44].

In this study, some limitations have been noted. This is a single center non-randomized clinical trial. The learning curve was not defined. Also, the small sample size (n=14) limits the statistical analysis. In this diagnostic clinical trial, OS and PFS of SPECT/CT guided LM/SL were assessed with a limited follow-up. However, the purpose of this trial was to assess the technical feasibility and clinical added-value of LM/SL plus SPECT/low-dose CT in early stage of gynaecological cancers. An experienced multidisciplinary team has assessed the LM/SL plus SPECT/CT research protocol in vulvar cancer and cervical cancer. SPECT/low-dose CT is routinely used in London hospitals for LM/SL in malignant melanoma and breast cancer. Also, the number of non-SLNs and SLNs analysed at the final pathology (234 lymph nodes) was statistically high. Importantly, the number of patients required for the learning curve is not clearly defined in the literature for LM/SL in vulvar cancer and cervical cancer [1, 24, 36]. The encouraging preliminary results of this single center study may help design a prospective multicenter randomised clinical trial.

## Conclusion

Preliminary results indicate the technical feasibility and clinical added-value of SPECT/CT guided LM/SL in patients with early stage of cervical cancer and vulvar cancer. Hybrid imaging improved the detection and 3D anatomic localisation of hot and cold SLNs in predictable and unpredictable pathways compared to 2D planar imaging. LM/SL plus SPECT/CT allowed 100% DR, 100% SV, and 100% NPV for accurate staging of regional lymph nodes with an actual and potential impact on management.

## Conflict of interest

The authors declare that they have no conflict of interest.

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