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Sentinel Lymph Node Biopsy in Cervical Cancer

Results and Implications of a Systematically Developed Algorithm

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Sentinel Lymph Node Biopsy in Cervical Cancer

Results and Implications of a Systematically Developed Algorithm

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on October 10th, 2025, at 09.00 at the Department of Obstetrics and Gynaecology, Klinikgatan 12, Lund

Faculty opponent
Professor Fabrice Lécuru, Institut Curie, Paris, France

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Title and subtitle: Sentinel Lymph Node Biopsy in Cervical Cancer – Results and Implications of a Systematically Developed Algorithm

Abstract: The general aim was to develop and examine a strict anatomically based sentinel lymph node (SLN) algorithm to assess pelvic nodal metastatic spread in women with early-stage cervical cancer (CxCa).

Study I: To investigate if combining two tracers, Indocyanine Green (ICG) and radiocolloid Technetium (Tc^{99m}), increased the SLN detection rate in CxCa. The bilateral detection rate for ICG was 98.5% and for Tc^{99m} was 60.0%. ICG correctly identified all node-positive women. Combining the tracers did not improve bilateral detection rates, as compared to using ICG alone.

Study II: To investigate the occurrence of lymph nodes and lymph node metastases (LNMs) in the parauterine lymphovascular tissue (PULT) in women with early-stage cervical cancer. Of 145 women, 52.4% had lymph nodes present in the PULT. Pelvic LNMs were found in 19 (13.1%) women, of which 6 had LNMs in the PULT. Three (2.1%) women had LNMs isolated to the PULT. The PULT should be dissected and treated as SLN-tissue in women with CxCa.

Study III: To compare the anatomical distribution of SLNs and metastatic SLNs in CxCa and endometrial cancer (EC). Bilateral mapping rates were 97.9% in CxCa and 95.0% in EC. There were no significant differences between the groups regarding SLNs at typical positions (interiliac and obturator fossa) (CxCa 78.1% vs. EC 82.1%, p=.09), nor regarding metastatic SLNs at the obturator fossa, 54.1% and 48.6% of all positive SLNs. All node-positive women were correctly identified by at least one positive SLN. There are no significant differences between the anatomical postions of SLNs and positive SLNs regarding CxCa and EC, and sensitivity results for an SLN algorithm can be pooled.

Study IV: To assess the sensitivity and negative predictive value (NPV) of a systematically developed anatomically based SLN algorithm using ICG as a tracer, allowing for reinjection, in CxCa. Included were 181 women. An interim analysis was predetermined at 29 node-positive women. As the algorithm correctly identified all, H_0 was rejected, and inclusion stopped. One additional node-positive woman was awaiting histology at study closure and was included. Results show a sensitivity of 100% and NPV 100%. Of node-positive women, 40.0% had isolated metastases to the obturator fossa and 10% to the PULT.

Conclusion: This thesis describes the development and evaluation of a strict anatomically-based SLN algorithm using ICG as a tracer, allowing for reinjection, that has demonstrated a sensitivity of 100% and NPV of 100% in women with early-stage CxCa. The algorithm suggests that the PULT should be treated as SLN tissue and, in particular, stresses the importance of carefully evaluating the obturator fossa.

Keywords: Cervical cancer, sentinel lymph node biopsy, lymphadenectomy, lymphatic metastases, lymphatic system, lymphatic complications, indocyanine green, robotic surgery.

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Results and Implications of a Systematically Developed Algorithm

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MADE IN SWEDEN

To my wife, Lotta, and my children, Valdemar and Otto.

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Thesis at a Glance

Study	Aim	Results	Conclusion
Study I: Combining Indocyanine Green and Tc99-nanocolloid does not Increase the Detection Rate of Sentinel Lymph Nodes in Early Stage Cervical Cancer Compared to Indocyanine Green Alone	To investigate if combining Indocyanine Green (ICG) and Tc^{99m} increases the sentinel lymph node (SLN) detection rate in women with early-stage cervical cancer.	In the 65 analyzed women, the bilateral mapping rate was 98.5% for ICG and 60% for Tc^{99m} ($p<0.01$). In 3 women (5%), Tc^{99m} identified ICG-negative non-metastatic SLNs without impact on the bilateral detection rate. Eight women (12%) had lymph node metastases (LNMs). All had at least one metastatic SLN. Seven (35%) of the 20 metastatic SLNs were detected by ICG only and 12 (60%) were ICG and Tc^{99m} positive.	SLN detection rate was significantly higher using ICG compared with Tc99. ICG identified all patients with LNMs. Combining ICG and Tc99 did not improve the bilateral detection rate of SLNs.
Study II: Resection of the Upper Paracervical Lympho-vascular Tissue Should be an Integral Part of a Pelvic Sentinel Lymph Node Algorithm in Early Stage Cervical Cancer	To investigate the prevalence of lymph nodes and lymph node metastases (LNMs) in the upper para-cervical lymphovascular tissue (UPLT) in early-stage cervical cancer.	A total of 145 women were analysed. Pelvic LNMs occured in 19 (13.1%) women, all identified by at least one metastatic SLN. In 76 women (52.4%), at least one UPLT lymph node was identified. Metastatic UPLT lymph nodes were identified in 6 women, of whom 3 women (2.1% of all and 15.8% of node-positive women) were without lateral pelvic LNMs. In 13 women, lateral pelvic SLN LNMs with either no (n=5) or benign (n=8) UPLT lymph nodes were identified. No intraoperative complications occurred due to the removal of the UPLT.	Removal of the UPLT and treating it as SLN-tissue should be an integral part of the SLN concept in early-stage cervical cancer.
Study III: Similar Distribution of Pelvic Sentinel Lymph Nodes and Nodal Metastases in Cervical and Endometrial Cancer. A Prospective Study Based on Lymphatic Anatomy	Comparing the anatomical distribution of metastatic and non-metastatic pelvic sentinel lymph nodes (SLN) in cervical and endometrial cancer (CxCa and EC).	For CxCa and EC respectively, bilateral mapping rate was 97.9% and 95.0% (p=.16). Typically positioned (interiliac and proximal obturator fossa) SLNs along the UPP was proportional between groups (78.1% vs 82.1%, p=.09), and the rate of metastatic SLNs in the obturator fossa was 54.1% and 48.6% respectively (p=.45). All node positive women (CxCa n = 19, EC n = 37) had at least one metastatic SLN. Anatomically typical positions could not be defined along the LPP.	The anatomical location of SLNs and SLN metastases are similar in CxCa and EC, suggesting that sensitivity results for an SLN concept in endometrial cancer and cervical cancer can be accumulated.
Study IV: A Prospective Study Evaluating an Optimized Sentinel Node Algorithm in Early Stage Cervical Cancer: The PROSACC-study	A single-center prospective non-randomized study to assess a systematically developed anatomically-based sentinel lymph node (SLN) algorithm in cervical cancer.	181 women were included for analysis. Median tumor size was 14.0mm (range 2–80mm). Bilateral mapping rate was 98.3%. An interim analysis rejected H_0 and inclusion stopped at 29 node positive women, all identified by at least one metastatic ICG-defined SLN. One woman awaiting histology at study closure was node positive and included in the analysis. Sensitivity was 100% (95% CI, 88.4%–100%) and NPV 100% (95% CI, 97.6%–100%). In node positive women, the proximal obturator position harbored 46.1% of all SLN metastases representing the only position in 40% and 10% had isolated metastases in the PULT.	The proposed anatomically based SLN-algorithm accurately identified pelvic nodal metastases in early-stage cervical cancer, using ICG as a tracer. Assessing the obturator compartment in particular and treating the PULT as SLN-tissue is essential.

Original Studies

This thesis is based on the following original studies, which will be referred to in the text by their Roman numerals. The original studies are appended at the end of the thesis in reprints made with permission from the publisher.

I. Lührs O, Ekdahl L, Lönnerfors C, Geppert B, Persson J. Combining Indocyanine Green and Tc99-nanocolloid does not increase the detection rate of sentinel lymph nodes in early stage cervical cancer compared to Indocyanine Green alone. Gynecologic Oncology 156 (2020) 335-340

II. Lührs O, Ekdahl L, Geppert B, Lönnerfors C, Persson J. Resection of the upper paracervical lymphovascular tissue should be an integral part of a pelvic sentinel lymph node algorithm in early-stage cervical cancer. Gynecologic Oncology 163 (2021) 289-293

III. Lührs O, Bollino M, Ekdahl L, Lönnerfors C, Geppert B, Persson J. Similar distribution of pelvic sentinel lymph nodes and nodal metastases in cervical and endometrial cancer. A prospective study based on lymphatic anatomy. Gynecologic Oncology 165 (2022) 466-471

IV. Persson J, Lührs O, Geppert B, Ekdahl L, Lönnerfors C. A prospective study evaluating an optimized sentinel node algorithm in early-stage cervical cancer: The PROSACC-study. Gynecologic Oncology 187 (2024) 178-183

Populärvetenskaplig sammanfattning

Livmoderhalscancer drabbar årligen cirka 550 kvinnor, oftast yngre, i Sverige. Det är en relativt låg befolkningsandel jämfört med många utvecklingsländer. En starkt bidragnade orsak är att Sverige 1967 införde screening med cellprover för att hitta förstadier till cancer. Livmoderhalscancer uppstår nästan uteslutande efter en infektion med ett virus, humant papillomvirus (HPV), som överförs vid sexuell kontakt. Därför är screeningen idag i första hand inriktad på att hitta kvinnor som bär på så kallat högrisk-HPV. Vid positivt HPV-prov utreds dessa kvinnor vidare för eventuella cellförändringar. Vid uttalade cellförändringar genomgår kvinnan en konisering i lokalbedövning, där cellförändringarna skärs bort. Detta är tänkt som ett botande ingrepp avseende cellförändringarna men eliminerar inte virusinfektionen varför fortsatt uppföljning är nödvändig.

Vaccination mot HPV erbjuds alla flickor i skolåldern sedan 2012 och pojkar sedan 2020. På sikt bedöms det att antalet som kommer att drabbas av livmoderhalscancer kommer att minska kraftigt. Även andra HPV-beroende cancerformer förväntas minska, till exempel penis- och analcancer.

Om cellförändringarna har övergått i cancer avgörs behandlingen av tumörens storlek och om det finns tecken till spridd cancer. Vid mindre tumörer kan det räcka med att ta bort en del av livmoderhalsen och fertiliteten kan då bevaras. Vid större tumörer behöver hela livmoderhalsen och livmodern med omkringliggande vävnad tas bort. Om tumören inte bedöms vara möjlig att operera bort med tillräckliga marginaler till omkringliggande vävnader (radikalt) eller om tumören är spridd utanför livmodern strålbehandlas kvinnan utan föregående operation.

Den vanligaste spridningsvägen för cancern är till lymfkörtlar i bäckenet (lymfkörtelmetastaser) vilket hittills inte kunnat uteslutas utan en lymfkörtelutrymning. En sådan omfattande körtelutrymning orsakar kronisk lymfsvullnad i benen hos minst en av tre kvinnor. Tillståndet påverkar livskvaliteten mycket negativt hos de drabbade. Oupptäckt spridning till lymfkörtlar, vilket kan ske även vid mindre tumörer, leder till återfall i tumörsjukdomen vilket inte alltid kan botas.

Att kunna diagnostisera lymfkörtelmetastaser genom att operera bort ett fåtal körtlar dit spridning sker först, och därmed minska risken för lymfsvullnad, vore därför önskvärt. Dessa körtlar kallas portvaktskörtlar eller Sentinel Lymph Nodes (SLN). Forskargruppen i Lund, som ligger bakom studierna i den här avhandlingen, har sedan tidigare genomfört inledande studier hos kvinnor med livmodercancer avseende livmoderns lymfavflöde, en reproducerbar kirurgisk teknik samt en tydlig definition av vilka körtlar som är äkta portvaktskörtlar. Studierna har lett till införandet av porvaktkörteldiagnostik, som har ersatt full körtelutrymning i bäckenet vid livmodercancer. Införandet genomfördes 2020 och innebar ett paradigmskifte för denna cancerform.

Den viktigaste egenskapen för ett kirurgiskt koncept för portvaktskörteldiagnostik är att metoden har en mycket hög känslighet (sensitivitet) för att korrekt identifiera de kvinnor som har körtelmetastaser och även har en hög teknisk lyckandegrad. Den tekniska lyckandegraden beror på vilket spårämne som används för att identifiera portvaktskörtarna.

Tidigare spårämnen såsom radioaktivt Technetium (radiotracer) eller olika för ögat direkt synliga färgämnen hade inte tillräcklig hög och/eller långvarig ”infärgning” av lymfkörtelsystemet för att vara kliniskt optimala. En ny fluoroscerande substans, Indocyaningrönt, ICG, blev tillgänglig i början av 2010-talet. ICG har egenskapen att fluoroscera osynligt rött ljus vid belysning av en definierad kortare våglängd och har fördelen att både ge ett säkrare upptag i lymfsystemet och en längre passagetid i lymfkärl och lymfkörtlar. Med rätt utrustning, som finns integrerad vid robotassisterad kirurgi, framställs lymfsystemet tydligt mot en kontrasterande mörkare bakgrund.

Mot bakgrund av erfarenheterna av ICG och grundläggande studier vid livmodercancer planerades en strukturerad vidareutveckling av portvakskörtelkonceptet även vid livmoderhalscancer, då man inte kunde förutsätta att resultaten vid livmodercancer var direkt överförbara.

I **studie 1** jämfördes teknisk lyckandegrad och i viss mån känslighet mellan ICG och radioaktivt Technetium som användes parallellt på samma kvinnor. Studien visade att en ICG ensamt var tekniskt lyckat (visade portvaktskörtlar på båda bäckenväggarna) hos 98.5% av kvinnorna och att Technetium inte bidrog till att öka den tekniska lyckandegraden eller känsligheten att hitta körtelmetastaser. Denna prospektiva studie var den första att visa detta och en grund till att fortsatt rekommendera ICG ensamt som tracer.

I **studie 2** undersöktes om den vävnad som för lymfan från livmoderhalsen till körtlar på bäckenväggarna innehöll lymfkörtlar och körtelmetastaser. Denna parauterina lymfatiska vävnad (PULT) ingår i den större vävnadsbrygga som omger livmoderhals och livmoder men tas inte bort vid mindre livmoderhalscancrar. Genom att vi i studien anatomiskt definierade och histologiskt undersökte PULT som en del av portvaktskörtelkonceptet visade vi att 2% kvinnor med små livmoderhalstumörer hade isolerade körtelmetastaser i detta annars inte undersökta område. Detta är en mycket viktig insikt som har fått stor internationell uppmärksamhet.

I **studie 3** kunde vi visa att de anatomiska lokalisationerna av portvaktskörtlar med och utan metastaser inte skiljde sig mellan livmoder- och livmoderhalscancer. Beräkning av en portvaktsmetods känslighet kräver statistiskt ett stort antal kvinnor med metastaser i lymfkörtlarna. Livmoderhalscancer är ovanligare och har mer sällan körtelmetastaser, varför studien ger en möjlighet att kombinera känslighetsdata från de båda tumörformerna för en högre statistisk säkerhet.

I den slutliga **studie 4** bedömdes såväl känslighet, teknisk lyckandegrad och anatomisk lokalisation av metastaser baserat på en strikt kirurgisk algoritm och en strikt definition av äkta portvaktskörtlar. Genom att en full körtelutrymning, för närvarande rekommenderat av vårdprogram, utfördes efter borttagande av PULT och portvaktskörtlar i bäckenet utgjorde kvinnorna sina egna kontroller. Alla kvinnor med metastaser i bäckenet hade metastaser i portvaktskörtlarna, inte sällan enbart i dessa, vilket ytterligare styrker konceptet. Metoden lyckades identifiera samtliga kvinnor med lymfkörtelmetastaser. Studien gav också avgörande information kring kriterier för optimalt utförd portvaktskörteldiagnostik, av synnerlig vikt vid ett senare nationellt införande som ersättning till full körtelutrymning.

Sammanfattningsvis har vi genom en systematisk utveckling av portvaktskörtelkonceptet skapat en grund för att systematiskt, strukturerat och säkert kunna införa detektion av portvaktskörtlar som ersättning till full körtelutrymning vid livmoderhalscancer. Det faktum att ett litet antal portvaktskörtlar, jämfört med det stora antalet vid full körtelutrymning, tillåter en noggrannare histologisk undersökning med fynd av annars oupptäckta mindre metastaser gör att införandet av portvaktskörteldiagnostik har potential att inte bara minska lymfkomplikationer men även förbättra överlevnaden vid denna cancersjukdom.

Abstract

Background

The yearly incidence of cervical cancer in Sweden is approximately 11 in 100.000 women, corresponding to more than 500 women diagnosed. A prior Human Papillomavirus infection causes a vast majority of all cervical cancer cases.

In early-stage cervical cancer, surgical treatment is recommended. A radical hysterectomy, or radical trachelectomy to preserve fertility, and pelvic lymphadenectomy is recommended.

A pelvic lymphadenectomy constitutes a risk of postoperative morbidity, mainly through the development of lymphedema. The first lymph node along each lymph vessel that drains the cancer-affected organ, i.e., the uterus, is known as the sentinel lymph node (SLN). Since lymph node metastases occur sequentially, an SLN will be affected before any secondary echelon nodes are.

The aim of this thesis is to develop and evaluate a reproducible SLN algorithm for the determination of pelvic lymph node metastases in early-stage cervical cancer based on the lymphatic anatomy of the uterus using Indocyanine Green (ICG) as a tracer, allowing for reinjection, and to estimate the sensitivity, negative predictive value, anatomic positions of SLNs and metastatic SLNs, and mapping rates in said algorithm.

Aims of the Studies

Study I: To investigate if combining two independent tracers, ICG and Tc^{99m}-nanocolloid, increased the detection rate of SLNs in early-stage cervical cancer.

Study II: To investigate the presence of lymph nodes and lymph node metastases in the parauterine lymphovascular tissue (PULT) in relation to lateral pelvic SLN status in women with early-stage cervical cancer.

Study III: To compare mapping rates and anatomical distribution of non-metastatic and metastatic SLNs between women with early-stage cervical cancer and high-risk endometrial cancer, using ICG as a cervically injected tracer.

Study IV: To evaluate the sensitivity and negative predictive value (NPV) of a structurally developed optimized SLN algorithm based on studies of uterine lymphatic anatomy, on optimal tracer, strict definition of SLNs, and including the PULT as SLN tissue.

Material and Methods

In these prospective studies, women diagnosed with early-stage cervical cancer underwent robotic radical hysterectomy (RRH) or robotic radical trachelectomy (RRT) at one academic center, using a structurally developed SLN algorithm, using

cervically injected ICG (and Tc^{99m} in study I), allowing for reinjection of ICG in case of non-mapping, and including separate removal of the PULT, which was treated as SLN tissue.

Study I: Eighty consecutive women diagnosed with cervical cancer FIGO 2009 IA2-IB1, and selected cases IA1 or IB2-IIA, were assessed for inclusion between November 2014 and March 2017. Included were 65 women who underwent an RRH or RRT and an SLN procedure followed by a complete pelvic lymph node dissection.

Study II: Histopathological results for the PULT regarding the presence of lymph nodes and lymph node metastases of 145 consecutive women between 2014 and 2020 with early-stage cervical cancer were analyzed. Included were women with FIGO 2009 stages IA1-IB1, and selected stages IB2-IIA.

Study III: Consecutive women with early-stage cervical cancer between 2014 and 2020 (n=145) and women with high-risk endometrial cancer (n=201) were included for data analysis regarding mapping rates and anatomic locations of metastatic and non-metastatic SLNs. Included were women with cervical cancer stages FIGO 2009 IA1-IB1, and selected cases IB2-IIA, and in the endometrial cancer cohort FIGO grade 3, deep myometrial invasion, and non-endometroid histology.

Study IV: Consecutive women diagnosed with early-stage cervical cancer, stage FIGO 2009 IA2-IIA1, between September 2014 and January 2023, were approached for inclusion. Included for final analysis were 181 women of 236 assessed for eligibility. Further enrollment was stopped at the planned interim analysis, since the algorithm correctly identified all 29 node-positive women. One additional node-positive woman was awaiting final histology at the time of the interim analysis and was included in the final analysis.

Results

Study I: The bilateral mapping rates were 98.5% for ICG and 60% for Tc^{99m} (p<0.01). Eight (12%) of the 65 included women had lymph node metastases, all identified by at least one metastatic SLN. Twenty metastatic SLNs were retrieved in total, of which 12 (60%) were ICG and Tc^{99m} positive and seven (35%) were positive for ICG only. Combining the two tracers did not improve the bilateral detection rate.

Study II: Of 145 women analysed, 76 (52.4%) had at least one lymph node in the PULT. Nineteen (13.1%) women had pelvic lymph node metastases, all correctly identified by at least one positive SLN or a positive node in the PULT. Six women had metastatic lymph nodes in the PULT, of which three had metastatic lymph nodes in the PULT without lateral pelvic lymph node metastases. The three isolated PULT metastases correlate to 2.1% of all included women, or 15.8% of all node-positive

women. Thirteen node-positive patients had either no (*n*=5*)* or benign (*n*=8) PULT lymph nodes.

Study III: There were no significant differences in bilateral mapping rates between women with cervical cancer (97.9%) and endometrial cancer (95.0%) (*p*=.16). Typically positioned SLNs, at the interiliac and proximal obturator fossa positions, were proportionally similar between the two cohorts, 78.1% in cervical cancer patients and 82.1% in endometrial cancer patients (*p*=.09). All node positive women (cervical cancer *n*=19, endometrial cancer *n*=37) were correctly identified by at least one positive SLN. The rate of metastatic SLNs in the obturator fossa were similar, 54.1% (cervical cancer) and 48.6% (endometrial cancer) (*p*=.45).

Study IV: In 181 women included for analysis, the median tumor size at histology was 14.0 mm (range 2-80 mm), and the bilateral mapping rate was 98.3%. An interim analysis conducted at the predetermined 29 node-positive patients mark showed that all node-positive patients were correctly identified by at least one metastatic SLN defined by ICG, and H_0 was rejected. One additional node-positive woman with pending histology results at the time of the interim analysis was included, resulting in a total of 30 node-positive patients. The SLN algorithm had a sensitivity of 100% (95% CI, 88.4%-100%) and NPV 100% (95% CI, 97.6%-100%). The proximal obturator fossa held 46.1% of all SLN metastases, and was the sole position for positive SLNs in 40% of node-positive women. The PULT had isolated metastases in 10% of women with metastatic disease.

Conclusions

This thesis describes the structural development of a reproducible SLN algorithm, based on studies of uterine lymphatic anatomy and the optimal tracer for identifying SLNs; furthermore, a strict definition of SLNs, i.e., the juxtauterine mapped lymph nodes along identified lymphatic pathways, was fundamental to the described SLN concept. When using ICG as a tracer and allowing for reinjection in case of failed mapping, the algorithm demonstrates a sensitivity and NPV of 100% in the prospective studies included in this thesis. By strictly adhering to the described algorithm, similar results should be achievable by an experienced surgeon. Additionally, the anatomical distribution of metastatic SLNs must be stressed. Both carefully assessing the obturator compartment and removal of the PULT, treating it as SLN tissue, are imperative.

Abbreviations

BT	Brachytherapy
CT	Computed tomography
CCRT	Concomitant chemoradiotherapy
CRT	Chemoradiotherapy
dCRT	Definitive chemoradiotherapy
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
EBRT	External beam radiation therapy
ESGO	European Society of Gynaecological Oncology
FIGO	International Federation of Gynecology and Obstetrics
FZ	Frozen section
Gy	Gray
HIV	Human immunodeficiency virus
HPV	Human Papillomavirus
HR	Hazard ratio
HSIL	High-grade intraepithelial lesions
IMA	Inferior mesenteric artery
ICG	Indocyanine green
IPP	Infundibulopelvic pathway
ITC	Isolated tumor cells
LACC	Laparoscopic Approach to Cervical Cancer
LN	Lymph node
LNM	Lymph node metastases
LND	Lymph node dissection
LPP	Lower paracervical pathway
LVSI	Lymphovascular space invasion
MIM	Micro metastases
MIS	Minimally invasive surgery

MRI	Magnetic resonance imaging
NACT	Neoadjuvant chemotherapy
NCCN	National Comprehensive Cancer Network
ORH	Open radical hysterectomy
OS	Overall survival
PET-CT	Positron emission tomography and computed tomography
PLND	Pelvic lymph node dissection
PULT	Parauterine lymphatic tissue
RH	Radical hysterectomy
RRH	Robotic radical hysterectomy
RRT	Robotic radical trachelectomy
SLN	Sentinel lymph node
SQRGC	Swedish Quality Registry for Gynecologic Cancer
TNM	Classification of Malignant Tumors
UPP	Upper paracervical pathway

Introduction

In 2022, the median age for a cervical cancer diagnosis in Sweden was just over 50 years, with one in three women diagnosed being under 40 years old [1]. Women under 40 are primarily diagnosed with early-stage cancer. In contrast, women over 70, hence no longer part of the Swedish screening population for cervical dysplasia, are diagnosed at more advanced stages [2, 3]. The vast majority of cervical cancer occurs as a consequence of a prior HPV infection, where certain strains of HPV are more likely to cause cancer. In Sweden, the annual incidence of cervical cancer is approximately 11 per 100,000 women [2, 3]. The incidence is kept down by the public screening program for HPV infection and dysplasia, offered to all women ages 23 through 69 [3]. Furthermore, an HPV vaccination program for girls was implemented in 2012 and for boys in 2020. All children are offered a 9-valent vaccine at the age of eleven. The 9-valent vaccine was introduced in 2019 and is now the only one on offer. In children born in 2009, and hence the first cohort where both sexes were offered the vaccine, the vaccination coverage is 87.8% for girls and 82.1% for boys [3]. Treatment is dependent on the stage of the disease, as classified according to the FIGO staging system, which was defined in its latest iteration in 2018 [4]. In the early stages, the affected women are selected for surgery [2]. An essential part of the surgical procedure in cervical cancer patients has been a full pelvic lymphadenectomy (PLND), as nodal involvement is one of the most important prognostic factors in early-stage disease [5, 6]. In many other forms of cancer, including breast, melanoma, penile, vulvar, and endometrial cancer, the planned lymphadenectomy is less extensive and limited to the lymph nodes most adjacent to the cancer-affected organ, the sentinel lymph nodes (SLN) [7-10]. Any spread of tumor cells through the lymphatics will pass the SLNs before reaching subsequent lymph nodes. If the SLNs are free of metastatic cells, given a defined high-sensitivity SLN algorithm, undetected nodal spread is minimized. The concept is attractive, as a well-developed SLN algorithm provides the surgeon with information that supports perioperative decision-making. Furthermore, ultrastaging and immunohistochemistry of SLNs, which are not performed on the larger amounts of lymph nodes following a full PLND due to resource reasons, will increase the overall detection rate of pelvic nodal metastases by identifying small-volume metastases, potentially improving prognosis. The patients also gain from a less extensive procedure, decreasing the risk of peri- and postoperative complications, first and foremost chronic lower limb and/or trunchal lymphedema with severe negative impact on women´s quality of life [11-13].

According to the Swedish, European Society of Gynaecological Oncology (ESGO), and National Comprehensive Cancer Network (NCCN) guidelines, SLN-only procedures are practiced in the least advanced stages eligible for surgery, i.e., selected cases of Stage IA1 and Stage IA2. In more advanced stages, a PLND with or without an SLN biopsy is performed [2, 14, 15]. Over the last 15 years, prospective studies have been presented, proposing SLN algorithms for women with cervical cancer [16-20]. There are significant variations in the described surgical approaches and techniques, preoperative evaluation, choice of tracers to identify SLNs, injection technique, and pathology assessment of SLNs in the current studies [21]. Furthermore, there are inconsistencies in the actual definition of the term SLN and in the anatomical definitions regarding the lymphatic system. The lack of consensus results in difficulties in implementing a standardized SLN algorithm.

This thesis describes the systematic prospective development of a reproducible SLN algorithm based on lymphatic anatomy and strict definitions of SLNs. The algorithm, when performed by experienced surgeons, has demonstrated bilateral mapping rates of 98.3% using indocyanine green as a tracer. The SLN algorithm successfully identified all women with lymph node metastases, achieving a sensitivity and negative predictive value of 100%. The importance of incorporating the parauterine lymphovascular (PULT) tissue in the SLN algorithm was demonstrated for the first time.

Background

Cervical Cancer

Epidemiology and Etiology

Worldwide, uterine cervical cancer is the fourth most common cancer form and the fourth most common cause of cancer-related deaths in women, with approximately 660,000 new cases diagnosed and 350,000 reported deaths in 2022 [22, 23]. About 85% of cases occur in low-income countries. Sub-Saharan Africa, parts of South America, and southeastern Asia are particularly affected, with cervical cancer being the primary form of cancer in both incidence and mortality [2, 22]. The incidence is considerably lower, up to tenfold, in countries with long-term access to cervical cancer screening programs [22]. The reported incidence in 2022 varied from 40.4/100,000 in the most affected regions, i.e., Western Africa, down to 4.1/100,000 in areas that have the least reported cases, i.e., Western Asia [22]. The low incidence in Western Asia and the ”10 countries—all in the Eastern Mediterranean” is not further explained in the 2022 GLOBOCAN report. A possible explanation is a very conservative standpoint on premarital relationships and a predominantly monogamous way of life, minimizing the risk for HPV infections. In Sweden, 533 women were diagnosed with cervical cancer in 2021, corresponding to an annual incidence of 10.6/100,000 [1, 24]. The median age at diagnosis was 51 years, with one in three being under 40. Following a steady decrease in incidence from approximately 20/100,000 in 1970 to a plateau of just under 10/100,000 between 2000 and 2013, there has been an increase in incidence to over 11/100,000 in 2014 and the following years. Since 2021, the trend is that the number of women diagnosed with cervical cancer has again decreased.

In the mid-1970s, the idea that certain subtypes of human papillomaviruses (HPV) had a role in cancer first surfaced [25-29]. In 2008, Harald zur Hausen was awarded the Nobel Prize based on an 1983 article proving the role of HPV 16 in cervical cancer [30]. Two decades later, high-risk HPV was considered the leading risk factor for developing several forms of cancer [31-33]. The term “high-risk” refers to the most oncogenic strains of HPV, especially types 16, 18, and 45, which are present in 80% of all cervical carcinomas and in 94% of cervical adenocarcinomas [2, 34, 35]. Human papillomaviruses are double-stranded DNA viruses that are transmitted sexually and infect mucosal cells in the genital tract [33, 36]. High-risk HPV

carries oncoproteins E6 and E7, which have a high affinity for p53 and pRB, respectively [34, 36]. This inhibits the host cells' ability to undergo apoptosis, promoting unregulated proliferation and genomic instability. Over time, damaged DNA accumulates without the possibility of repair or induction of apoptosis, leading to a buildup of mutations and the eventual transformation into cancerous cells [34, 36]. This process typically occurs over a time frame of 10 to 20 years, reflected in the time that dysplasia progresses to cancer [34, 37].

Although most sexually active women acquire HPV infections at some point in life, 90% resolve within two years of transmission [38-41]. Among women with high-grade intraepithelial lesions (HSIL), 12% to 31% will develop cervical cancer if left untreated [42-44]. Significant risk factors that increase the likelihood of persistent HPV infection include acquired immunodeficiency (e.g., due to HIV infection or medication) or congenital immunodeficiency. Additionally, smoking and other sexually transmitted diseases heighten the risk of persistent infection. Sexual debut at a young age, having many sexual partners or partners who have had many sexual partners, and multiparity are all associated with an increased risk of cervical cancer, however likely due to an increased risk for HPV infections [45-49].

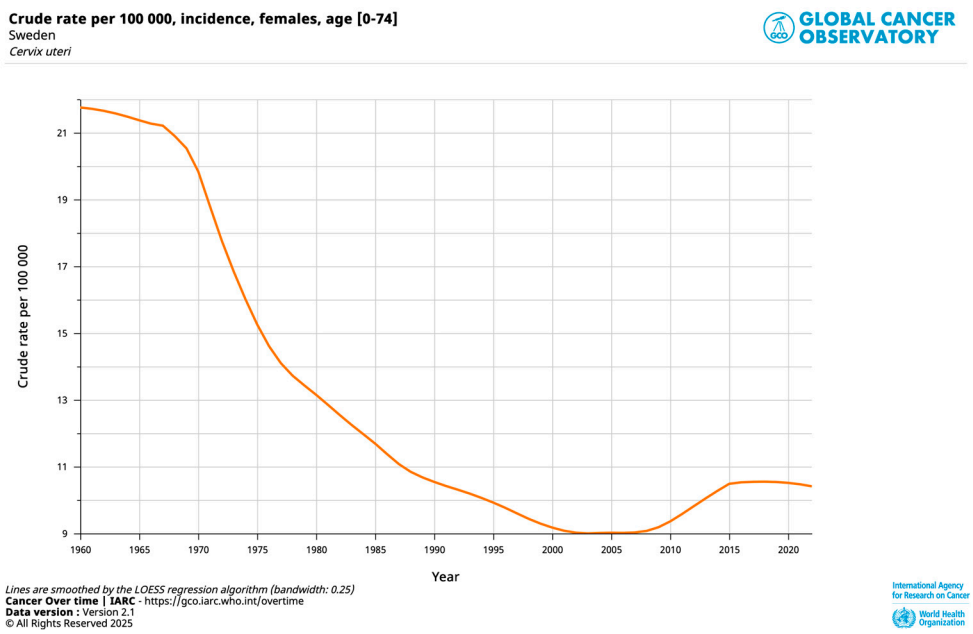


Figure 1
Incidence of cervical cancer/100,000 women in Sweden 1960 – 2022. Data source: Cancer Over Time, IARC https://www-dep.iarc.fr/overtime/en

Prevention

Introduced in Sweden in 1967, cervical cancer screening was gradually implemented in the then county councils (now regions) over a decade and was offered nationwide in 1977 [2, 50]. Screening decreased the incidence from 25/100,000 in 1965 to about 10/100,000 (Figure 1).

The reasons for the aforementioned increase in incidence that occurred in 2014 and onwards are not fully understood [51]. Analysis suggests that the fluid-based cytological testing procedures, introduced a few years before the increase, underperformed, and new routines have been implemented at the Swedish laboratories since [51, 52]. Following this, there has been a trend of falling numbers in cervical cancer incidence. The time span is too short to draw any certain conclusions regarding a permanent decline [52].

The Swedish guidelines, recommended by Socialstyrelsen (the National Board of Health and Welfare) since 2022, state that all women aged 23 to 49 should be offered HPV testing every five years, while women aged 50 to 64 should receive HPV testing every seven years. In cases of HPV positivity, a cytological analysis is performed. Women with HPV positivity are scheduled for screening every three years until age 49 and every five years between ages 50 to 64 until they test HPV-negative. Women over 64 who test HPV-positive remain in the screening program until they are HPV-free. HPV testing is free of charge and is typically conducted at a maternal care unit. Self-testing is also available as an alternative to testing at a maternal care unit, and the method offered is at the discretion of each health region [53].

The national children's vaccination program introduced primary prevention through vaccination for girls in January 2012 and for boys in the autumn of 2020. It is offered to children ages 10 to 12 [3]. Since 2019 the only vaccine on offer in Sweden is a 9-valent variant. Vaccination has proven to significantly lower the risk of high-grade cervical lesions and cervical cancer [54, 55]. The vaccination coverage is 87.8% for girls and 82.1% for boys born in 2009, the first cohort for whom both sexes were offered the HPV vaccine [3]. Data retrieved on June 1st, 2025, show that 60.6% of all women targeted in a catch-up vaccination campaign among women born 1994 – 1999 had received the 9-valent vaccine [56]. The goal of the campaign is to achieve 70% coverage within the group.

In 2021, the Swedish Parliament adopted a strategy to eliminate cervical and other HPV-related cancers through vaccination programs. Elimination is defined as an incidence of fewer than 4 cases of cervical cancer per 100,000 women, which is expected to be achieved with a vaccination coverage of 70% and the attainment of herd immunity. The primary goal is that no woman born in 2009 or later should be diagnosed with cervical cancer [57].

Symptoms and Diagnosis

Early symptoms of cervical cancer frequently involve irregular bleeding. This can include post-coital bleeding or bleeding during physical activity. Prolonged watery discharge may also occur. Sometimes, the discharge carries a foul odor due to necrosis and secondary anaerobic infection. Locally advanced tumors and lymph node metastases can induce pain, edema, or hydronephrosis by compressing nerves, veins, or ureters. Symptoms from the urinary bladder or the rectum are caused by pressure from a large tumor mass or tumor overgrowth [2]. Notably, smaller early-stage tumors and intracervical tumors may have few initial symptoms.

Cytology alone is insufficient for diagnosing cervical cancer. A guided biopsy or cervical cone biopsy, along with a histological analysis, are required to verify a cancer. Swedish guidelines require a preoperative pelvic MRI and thoracic and abdominal CT scans for all cases classified as FIGO stage >IA2 or IA1 with lymphovascular space invasion (LVSI). If needed for additional information, an optional vaginal ultrasound by a highly specialized ultrasonographer may be conducted. Patients who are not candidates for surgery and are planned for primary chemoradiotherapy (CRT) undergo further evaluation with positron emission tomography and computed tomography (PET-CT). This protocol also applies to women with highly malignant histologies such as small-cell neuroendocrine or clear cell histologies. To finally decide primary treatment modality, a gynecologic oncologist and a gynecologic tumor surgeon examine the patient under general anesthesia [2].

Histopathology and Staging

The most common histological type of cervical carcinoma is squamous cell carcinoma, which accounts for 75% of all diagnosed tumors. The remaining 25% are primarily adenocarcinomas or adenosquamous carcinomas [2, 15]. Clear cell, mucinous, and neuroendocrine tumors occur much less frequently but are considered to have a worse prognosis [2].

The International Federation of Gynecology and Obstetrics (FIGO) system for classifying cervical carcinomas is routinely used in all staging, except for occult cervical cancer, for which the Classification of Malignant Tumors (TNM) method is utilized exclusively. The latest iteration of the FIGO classification system was released in 2018 and implemented in Sweden in January 2020. Compared to the 2009 version, FIGO 2018 is more comprehensive, considering additional variables based on clinical examination, cystoscopy, rectoscopy, and a chest X-ray. These variables include lymph node status, as determined by both imaging and histopathology. The TNM protocol can also be used in combination with the FIGO staging system (Table 1) [4, 58].

Table 1.
Staging of cervical cancer according to FIGO 2009, 2018, and TNM classification. Updates in FIGO 2018 are highlighted. Adapted from Pecorelli et al. 2009 and Bhatla et al 2018.

FIGO 2009	FIGO 2018	TNM	Description
I		T1	Carcinoma strictly confined to the cervix (extension to the uterine corpus should be disregarded).
IA	-	T1a	Invasive cancer identified only by microscopy (all gross lesions even with superficial invasion are Stage IB cancers). Stromal invasion is limited to maximal depth of 5 mm and wifth of 7 mm.
IA1	-	T1a1	Stromal invasion ≤ 3 mm in depth and ≤ 7mm in width.
-	IA1		Stromal invasion < 3 mm in depth.
IA2	-	T1a2	Stromal invasion > 3 mm and ≤ 5 mm in depth and ≤ 7 mm in width.
-	IA2		Stromal invasion ≥ 3 mm and < 5 mm in depth.
IB	-	T1b	Microscopic lesions > IA or macroscopic lesions limited to the cervix.
-	IB		Invasive carcinoma limited to the uterine cervix, with measured deepest invasion ≥ 5 mm (greater than stage IA).
IB1	-	T1b1	Clinical lesions no greater than 4 cm in size.
-	IB1		Invasive carcinoma with ≥ 5mm stromal invasion and < 2 cm in its greatest dimension.
IB2	-	T1b2	Clinical lesions > 4 cm in size.
-	IB2		Invasive carcinoma ≥ 2 cm and < 4 cm in its greatest dimension.
-	IB3	T1b3	Invasive carcinoma ≥ 4 cm in its greatest dimension.
II		T2	Carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall.
IIA		T2a	Limited to the upper two-thirds of the vagina without parametrial involvement.
IIA1		T2a1	Tumor measures < 4 cm in greatest dimension.
IIA2		T2a2	Tumor measures ≥ 4 cm in greatest dimension.
IIB		T2b	With parametrial involvement but not up to the pelvic wall.
III		T3	Carcinoma involves the lower thirds of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney and/or involves pelvic and/or paraaortic lymph nodes.
IIIA		T3a	Involves the lower third of the vagina, with no extension to the pelvic wall.
IIIB		T3b	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney from tumor.
-	IIIC	TXN1	Involvement of pelvic and/or paraaortic lymph nodes, irrespective of tumor size and extent.
-	IIIC1	-	Pelvic lymph node metastasis only.
-	IIIC2	-	Paraaortic lymph node metastasis.
IV		T4	Carcinoma has extended beyond the true pelvis or has involved (biopsy-proven) the mucosa of the bladder or rectum.
IVA		T4a	Spread to adjacent pelvic organs.
IVB		T4b	Spread to distant organs.

Treatment

Treatment Protocols

The main primary treatment options for cervical cancer are surgery or chemoradiation, with the choice depending on the stage, histopathology, and comorbidities. According to Swedish guidelines, women diagnosed with early-stage cervical cancer receive surgical treatment, while those with more advanced stages are chosen for CRT. Primary surgery is the preferred curative approach for stages IA1 through IB2 and selected cases stage IIA1. In stages IB3 and IIA2 through IVB, CRT is the treatment of choice [2].

Neoadjuvant chemotherapy (NACT) followed by radical surgery lacks sufficient scientific support for cervical cancer and is generally not practiced [2]. Two phase III studies compare NACT followed by radical surgery to concomitant chemoradiotherapy (CCRT) in women with stages IB2 to IIB [59, 60]. Neither the study by Kenter et al. nor the one by Gupka et al. showed any difference in overall survival (OS); the former demonstrated a marginally better disease-free survival (DFS) in the CCRT cohort, while the latter showed significantly higher DFS in the CCRT group [59, 60]. Adjuvant treatment after primary surgical treatment is suggested in certain cases, partially depending on findings related to the Sedlis criteria, i.e., stromal invasion, LVSI, and tumor size [2, 61]. Further indications are lymph node metastases, extra-cervical growth, small cell neuroendocrine histopathology, and surgically small vaginal margins less than 5 mm [2]. In case of positive SLNs, the lymphadenectomy is extended to one station further cranially from the positive nodes, i.e., paraaorta below the IMA. The surgery is then aborted and the patient is planned for definitive CRT. There is no evidence that completing the surgical procedure before CRT has a positive effect on OS or DFS, but there is an increase in morbidity [2, 6].

For women eligible for fertility-sparing surgery with tumors larger than 2 cm in diameter, NACT is an alternative. After the neoadjuvant treatment, Swedish guidelines recommend an initial SLN biopsy to reduce the risk of lymph node metastases. However, due to the limited experience with long-term oncological outcomes of trachelectomy following NACT, great caution should be exercised before selecting this option [2].

Surgical Treatment

According to Swedish guidelines, the recommended surgical approach varies based on the FIGO stage. For stage IA1 without LVSI, a cone biopsy or simple hysterectomy can be performed at a secondary hospital without lymph node dissection. Stage IA1 with LVSI and stages IA2 through IB2 and selected IIB1 that are eligible for primary surgery should be treated at a tertiary hospital by gynaecologic oncology surgeons [2]. For women diagnosed with stages IA1 with LVSI or IA2, regardless of LVSI, a simple hysterectomy along with sentinel lymph node (SLN) biopsy or

pelvic lymphadenectomy (PLND) is recommended. Reconization can be an option for preserving fertility. Tumors classified as stage IB1, with an invasion depth of less than 10 mm or less than 50% stromal invasion on MRI scans, are treated with either simple hysterectomy or fertility-sparing simple trachelectomy, alongside PLND, with or without SLN biopsy. If the invasion depth exceeds 10 mm or stromal invasion is more than 50% on MRI in stage IB1, a radical hysterectomy (RH) or a radical trachelectomy (RT), for women planning future pregnancies, is performed using an otherwise identical procedure as in other stage IB1 patients. Stages IB2 and selected cases of IIA1 undergo RH and PLND, with or without SLN biopsy. Salpingectomy is recommended in all hysterectomies, while oophorectomy is routinely performed only on post-menopausal women [2]. The exceptions to this are high-risk histopathologies and adenocarcinoma, where individual assessments are made due to a higher risk of ovarian metastases [62]. Alternatives for surgical approach in Sweden include open abdominal surgery or minimally invasive surgery (MIS) via robotic surgery, the only MIS approach applied and usually reserved for smaller tumors [2]. In the perspective of the LACC (Laparoscopic Approach to Cervical Cancer) trial, current recommendations are that MIS/robotic radical hysterectomy (RRH) in stages IB1 and higher are primarily to be conducted in the context of clinical trials [2, 63].

The international LACC trial by Ramirez et al. is a phase III multicenter randomized study comparing MIS to laparotomy in women with cervical cancer FIGO 2009 stages IA1 – IB1. Distributed across 33 centers worldwide over nine years, a total of 631 women were enrolled and randomized into two groups. A laparotomy group with 312 patients was randomly assigned to it, and a MIS group containing 319 women. Of the women assigned to the MIS group, 84.4% underwent laparoscopic surgery, and 15.6% had robotic surgery performed. The study showed that MIS was associated with a lower rate of DFS (HR 3.74, 95% CI, 1.63 – 8.58) and OS (HR 6.00, 95% CI, 1.77 – 20.30) [63]. The study is underpowered to draw any conclusions regarding “low-risk” tumors; <2cm; no LVSI; <10mm invasion depth; and no lymph node involvement [63]. In a final analysis of the LACC trial cohorts, published in 2024, all women had completed a follow-up of 4.5 years with similar results as in the original publication, DFS (HR 3.91, 95% CI, 2.02 – 7.58) and OS (HR 2.71, 95% CI, 1.32 – 5.59) [64]. The conclusion drawn by the authors is that RH in women with cervical cancer should undergo open surgery and that MIS is only suitable in clinical trials.

Sert et al. found no significant difference in DFS or OS when comparing women with cervical cancer and surgical treatment, either by RRH (n=259) or open radical hysterectomy (ORH) (n=232), performed at three different centers between 2005 and 2011 [65]. Neither were there any significant differences in DFS or OS in 864 women having had primary surgical treatment due to early-stage cervical cancer with either open surgery or robot assisted MIS between 2011 – 2017, in data retrieved from the Swedish Quality Registry for Gynecologic Cancer (SQRGC) [66].

Similar to this, a Danish study collecting data on 1125 women who had surgery due to early-stage cervical cancer between 2005 and 2017, 530 through laparotomy and 595 through robotic MIS. Again, there were no differences in OS and DFS between the groups. Even so, a national Swedish study adding early cohorts of RRH performed before the SQRGC identified a learning effect with a lower recurrence rate with increased experience, a plausible explanation to the discrepancy between studies [67]. Ongoing studies are comparing RRH and ORH in women with cervical cancer, e.g., the multicenter RACC (ClinicalTrials NCT03719547) measuring outcomes in DFS and OS, and the non-inferiority multicenter trial ROCC (ClinicalTrials NCT04831580), recruiting 800 and 840 women, respectively.

Table 2.
Classification of radical hysterectomy according to Querleu-Morrow. Adapted from Ekdahl 2021 (ISBN: 978-91-8021-146-8) (Querleu et al. 2008, Querleu et al. 2017).

Type	Resection	Mobilization of the Ureter	Lateral Dissection	Vaginal Resection	Involvement of the Sacrouterine Ligament	Involvement of the Vesicouterine Ligament
A	Extrafascial	None	Close to the cervix.	Minimal resection	Dissection close to the cervix.	Dissection close to the cervix.
B	Modified radical	Partial	Medial to the ureter.	10 mm	Partial resection.	Partial resection.
C1	Classic radical	Complete	Lateral to the ureter. At the iliac vessels caudal part preserved.	15–20 mm	Transection at the rectum. Nerve sparing.	Transection at the bladder. Nerve sparing.
C2	Classic radical	Complete	Lateral to the ureter. At the iliac vessels including caudal part.	15–20 mm	Transection at the rectum. Hypogastric nerve is sacrified.	Transection a the bladder. Bladder nerves sacrified.
D	Lateral extended	Complete	At the exit of the a. iliac interna, with exposure of the root to n. ischiadicus.	15–20 mm	Transection at the rectum.	Transection at the bladder.

A four-scale classification for radical hysterectomy, based on distinct anatomical landmarks and a nerve-sparing technique, was described by Querleu and Morrow (QM) in 2008 and further developed in 2017 [68, 69]. This classification serves as the principal basis for the surgical approach in cervical cancer (Table 2). Nerve-sparing procedures type A – C1 is suggested in surgery for stages IB1, IB2, and IIA1. Although uncommon, if there are signs of disseminated disease in the abdomen or pelvis during surgery, a biopsy is sent for analysis with frozen section (FZ). In the rare cases of spread disease, the operation is aborted, and the patient is planned

for suitable oncologic treatment. The same applies for instances where lymph node metastasis (LNM) is discovered during surgery via an SLN procedure.

Following an intraoperative diagnosis of an SLN metastasis, further lymph node dissection is then limited to any suspicious LNs, and removal of cranial common iliac and inframesenteric paraaortic LNs. As a rule, the operation is aborted without a hysterectomy, and the patient is planned for CRT [2]. If possible, in pre-menopausal women <45 years of age, the ovaries are transposed to prevent premature menopause and related health issues [70, 71]. In informed, primarily younger sexually active women, the hysterectomy is completed in smaller cervical cancers to preserve vaginal function better, i.e sparing the vagina from negative brachytherapy effects. Furthermore, in younger women, if the uterus is left in place some endometrium may still be active after external radiotherapy causing a diagnostic problem as a recurrence must be ruled out in case of bleeding.

Clinical trials examining simple hysterectomy as compared to RH in women with cervical cancer running a relatively low risk of disseminated disease have been conducted. Most notable are the SHAPE and ConCerv trials.

The multicenter, randomized, non-inferiority SHAPE trial includes 700 equally randomized patients into two groups. Included were women with FIGO 2009 stages IA2 and IB1; squamous-cell carcinoma, adenocarcinoma, and adenosquamous carcinoma; tumors smaller than 2 cm; less than 10 mm invasion depth; less than 50% stromal invasion on MRI scan; no evidence of lymph node metastases; LVSI was accepted. Open surgery and MIS were both accepted, and the choice of operating method was at the surgeon's discretion. There was no significant difference in patient characteristics between the two groups. The recurrence rates were 2.52% and 2.17% for simple hysterectomy and RH, respectively. Median follow-up times were 4.5 and 4.6 years. There was no significant difference in recurrence rates, and simple hysterectomy was deemed non-inferior to RH [72]. In a subsequent publication from 2025, Plante et al. compared the oncological outcome of MIS and open surgery in the group who had a simple hysterectomy [73]. The results indicates that MIS is non-inferior to open surgery in patients with early-stage cervical cancer when performing a simple hysterectomy, but they stress that the study design was not aimed at evaluating surgical approach and the study was underpowered. They recommend further studies in the area to reach a conclusion.

The ConCerv trial is a prospective multicenter study including 100 women with early-stage cervical cancer FIGO 2009 stages IA2 – IB1 with squamous cell or adenocarcinoma histologies (grade 1 or 2 only), tumors less than 2cm, less than 10 mm invasion depth, no LVSI, and negative imaging for metastatic findings. Of included women 44% had a conization, 40% had a simple hysterectomy performed, and 16% had an "inadvertent" simple hysterectomy. A PLND and/or SLN+PLND procedure was performed in 96% of the women and SLN only in 4%. In 96 patients MIS was performed, where of 83 were laparoscopic interventions, and 13 were

robotic surgery. In 5% of the women, LNMs were retrieved. The 2-year recurrence rate was 3.5%. The authors conclude that less extensive surgery is safe and feasible in women with early-stage, low-risk cervical cancer [74].

The SHAPE and ConCerv trials in conjunction with the utilization of the Sedlis criteria, have set an ongoing trend that more patients are selected for primary CRT, and patients deemed fit for surgery more often have smaller tumors and less frequently LNMs than earlier, and less radical surgery is called for [61]. This trend has also led to an increased demand for a safe and feasible SLN algorithm.

Chemoradiotherapy

Definitive chemoradiotherapy (dCRT) is indicated in stages IB3, and IIA2 – IVA (FIGO 2018) according to Swedish guidelines [2]. The treatment consists of brachytherapy (BT), external beam radiation therapy (EBRT), and chemotherapy. Optimally, the total treatment dose of BT and EBRT combined is $D_{90\%} > 85$ Gy, where $D_{90\%}$ means that at least 90% of the target organ has received the prescribed dose in full. In combination with radiation, the patient should receive weekly Cisplatin 40mg/m^2 for optimally six cycles [2]. In cases where lymph node metastases are suspected, a higher radiation dose is given as a boost, between 55 – 64 Gy, depending on the size of the lymph node.

Adjuvant treatment is indicated in some cases, e.g., surgical margins < 5 mm, LNM, tumor size > 40 mm, growth outside the cervix, and small-cell neuroendocrine histology. External beam radiation therapy > 45 Gy and weekly Cisplatin 40mg/m^2 for five cycles is strived for.

Sentinel Lymph Node Biopsy

Development

Ernst A. Gould coined the expression “sentinel node” in 1960, referring to the first lymph node with tumor spread in parotid cancer. Still, already in 1923, the British surgeon Braithwaite identified “glands sentinel” while studying the lymphatic drainage of the omentum [75-77].

The word “sentinel” refers to a soldier or guard who keeps watch, which is a fitting description of the first draining lymph nodes that function as dense filters, trapping tumor cells released into the lymphatic vessels from the main tumor, obligatory for tumor cells to drain to subsequent nodes, as lymph nodes are connected in series via the lymphatic vessels. This principle leads to the proposition that if the sentinel lymph node is cancer-free, consecutive lymph nodes further along the same lymphatic pathway are also cancer-free.

The first description of an SLN concept was published in 1977 by Cabanas regarding penile carcinoma [7]. In the years that followed, between 1987 and 1994, publications on SLN biopsy for testicular cancer, melanoma, and breast cancer were released [8, 9, 78]. In 1996, Burke et al. presented a pilot study on lymphatic mapping in endometrial cancer, and in 2012, Barlin et al. published a surgical algorithm for SLN biopsy in endometrial cancer [79, 80]. This algorithm specified the need to remove any mapped SLNs and suspicious lymph nodes (LNs), and perform side-specific pelvic lymph node dissection (LND) in cases without mapping. The Barlin algorithm however lacks a clear definition of an SLN a such.

The Rationale for Sentinel Lymph Node Biopsy

To consider implementing an SLN algorithm, high bilateral mapping and high sensitivity are required to detect nodal spread. An optimally performing algorithm facilitates the surgeon's ability to make decisions based on perioperative ultrastaging and immunohistochemistry. The risk of underdiagnosis of spread disease can be significantly reduced, enabling more accurate intraoperative decisions regarding the transposition of the ovaries or the potential abortion of surgery in preparation for CRT if nodal metastases are discovered. Furthermore, a well-designed surgical algorithm based on lymphatic anatomy and an understanding of the typical positions of SLNs, at the hands of experienced surgeons, is likely to improve both the technical success rate and the detection rate of LNMs. Even though implementing an SLN algorithm requires the attending pathologist to allocate more time to examine the removed specimens, the ultrasectioning and immunohistochemistry of fewer SLNs, as compared to the amount of LNs following a full PLND subject for standard sectioning and hematoxylin and eosin staining only, increases the possibility to identify small volume metastases, i.e., micro metastases (MIM) and isolated tumor cells (ITC).

Under the circumstances described above, an SLN algorithm leads to an increased detection rate of pelvic nodal metastases, a decreased risk of perioperative complications, and most importantly, a reduced risk of lymphatic complications [12].

A Systematic and Structured Development of a Sentinel Lymph Node Algorithm

Developing an SLN algorithm for cervical and endometrial cancer requires a systematic and logical process. The initial step should be *in vivo* studies of the uterine lymphatic anatomy and the pathways along which the SLNs are located. Then, a reproducible surgical algorithm is formulated with a clear definition of an SLN, i.e., the mapping lymph node closest to the uterus in any relevant lymphatic pathway. The algorithm should also specify that macroscopically suspect and juxtauterine

non-mapping nodes along a dyed lymphatic pathway must be removed and treated as SLNs. The reproducible surgical algorithm is ideally based on studies of surgical methodology, such as the optimal tracer, injection site, injection technique, and surgical technique. However, any controversies regarding the injection site of tracers concerning endometrial cancer are non-pertinent in women with cervical cancer, as cervical injection is peritumoral in all cases. In 2017, studies of the lymphatic anatomy by Geppert et al. and a description of a standardized surgical algorithm by Persson et al. were published. [81, 82]. Both authors are from our institution and research group.

Based on these initial foundations, adequately powered studies on the sensitivity and NPV of the SLN algorithm should be conducted to investigate its performance in detecting and ruling out pelvic nodal metastases. To achieve high internal validity, only a few highly trained surgeons should perform the procedure to demonstrate the algorithm's potential. The results can later serve as a benchmark for other centers to aim for during implementation. During these studies a complementary adequate PLND must be performed as a means to evaluate the sensitivity and NPV of the algorithm accurately.

Given that the SLN algorithm proves to have sufficient sensitivity and NPV, implementation on a national and international scale should proceed. Continuous educational efforts will be essential, alongside a thorough evaluation of the results. This evaluation involves mapping rates and the number of removed SLNs to indirectly assess adherence to the protocol, as well as the ability to explore all relevant pathways. Furthermore, comparing the proportion of SLN metastases in well-defined groups can be considered the best way to evaluate the accuracy of the SLN algorithm, both during implementation and over a longer perspective. Any unexpected deviations from the anticipated results should be scrutinized, and their causes addressed. Typically, inadequate understanding of lymphatic anatomy, insufficient knowledge of the SLN algorithm, and lack of surgical training are likely causes of any deviations.

The SLN algorithm presented here has been developed in parallel to an SLN algorithm in endometrial cancer, which has been described in the thesis by Geppert from 2018 and further developed and described by Bollino in 2024 (Figure 2) [83, 84].

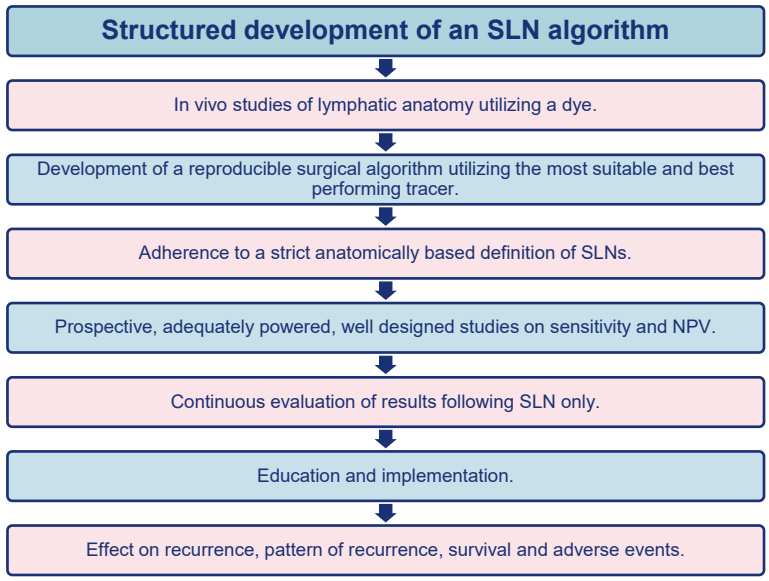


Figure 2.
Ideal development of an SLN algorithm. Adapted from Bollino 2024.

Lymphatic Anatomy of the Uterus

Lymph node status is the most important prognostic factor in cervical cancer [5, 6]. Clinical studies describing LND and SLN procedures in uterine cancers have been abundant over the last decades [19, 85-93]. In order to develop an SLN algorithm with high sensitivity that is reproducible, a clear definition of the lymphatic anatomy and the anatomic position of the "true" SLNs, i.e., the position of the juxtauterine lymph nodes along the lymphatic vessels that drain the uterus, should be fundamental. Yet, few clinical studies have been conducted in recent years to understand the lymphatic anatomy further. During the late 19th and first half of the 20th centuries, many clinical studies examined the lymphatic anatomy of the female genitalia through autopsies on women's corpses, using quicksilver and Prussian blue as tracers to identify the lymphatic vessels [94-100]. The following lymphatic pathways were described:

1. The external iliac pedicle, pre-ureteral pedicle, or primary pedicle follows the uterine artery and drains the external iliac and obturator lymph nodes. The pathway drains the paraaortic lymph nodes further on.
2. Posterior pedicle, hypogastric pedicle, or retro-ureteral pedicle follows the uterine vein to the hypogastric or presacral lymph nodes.
3. The infundibulopelvic pathway (IPP), along the infundibulopelvic ligament, drains directly to the paraaortic lymph nodes.

Although the idea of lymphatic cancer spread was initially presented by Virchow in 1863, the concept of "sentinel nodes" was first introduced by Gould et al. in 1960, as mentioned previously [75, 101, 102]. The early studies describing the lymphatic anatomy were not executed in the context of today's advances in surgical technology nor current surgical treatment protocols, and without the conception of SLNs. In their 2017 publication, Geppert et al., examined and described the uterine lymphatic anatomy, identifying the three aforementioned pathways by injecting Indocyanine green (ICG) and visually observing the lymphatic spread over 15 minutes using the da Vinci® Surgical System (Intuitive Surgical Inc., Sunnyvale, Ca, USA) and its Firefly mode [81]. The ICG was injected either in the fundus (n=30) or cervically (n=60) with no difference in defining the pelvic pathways. The mapping was recorded on an anatomical chart, as was the position of the juxtauterine lymph node in each pathway (Figure 3). The pathways were defined as follows:

1. The upper paracervical pathway (UPP), corresponding to the external iliac pedicle, runs along the uterine artery via and within the parauterine lymphovascular tissue into the medial external and/or obturator lymph nodes. It then crosses the external iliac artery and continues laterally to the common iliac artery, extending to the lateral precaval and paraaortic areas.
2. The lower paracervical pathway (LPP), corresponding to the posterior pedicle, runs along the upper edge of the sacrouterine ligament, extending to the presacral area medial to the internal iliac artery. This is followed by internal iliac and/or presacral draining nodes. The LPP continues medial to the common iliac artery, reaching the medial paraaortic and precaval areas. Small left-to-right crossover vessels at a level just cranial to the promontory were also observed.
3. The IPP with a course along the fallopian tube and upper broad ligament via the Infundibulopelvic ligament to paraaortic lymph nodes. The IPP was primarily visible after the fundal injections.

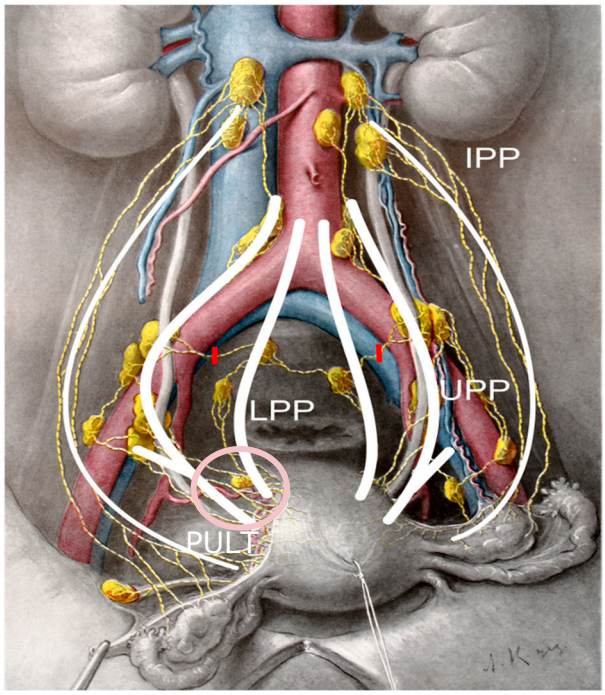


Figure 3
The principal lymphatic pathways draining the uterus. Modified by Geppert, Persson, and Lührs after Döderlein and Krönig, 1912. With kind permission of Theme Medical Publishers, Germany.

Cervical injection of ICG resulted in a significantly higher bilateral detection rate compared to fundal injection, as demonstrated in studies on endometrial cancer [81]. The "true" SLNs that drain the uterus are situated along the UPP and LPP, typically positioned along the UPP at the obturator and/or external iliac positions. Typical positions along the LPP could not be defined due to a longitudinal variation and left-to-right cross-over of lymphatic vessels, always necessitating a deep presacral dissection. Paraaortic LNs are almost exclusively secondary echelon nodes and should be considered as SLNs only if no pelvic SLNs are identified or if the IPP is clearly visualized after injection with ICG [81, 82]. Even though the relevance was unclear at the time, it was suggested that the upper paracervical lymphovascular tissue may hold a true SLN and that the tissue should be separately removed and treated as SLN tissue [82]. A change in nomenclature regarding the upper paracervical lymphovascular tissue was subsequently proposed by Querleu, suggesting that the term parauterine lymphatic tissue (PULT) is more adequate to describe the anatomical structure [103]. The phenomenon of disseminated disease to the parametrium and the existence of metastatic parametrial lymph nodes have been described earlier, although not in the context of sentinel node biopsy, as pointed out by Querleu [104-108]. The lymphatic anatomy described by Geppert et al. and the clinical advantages of cervical injection were later verified in a study by Zuo et al. [109].

Tracers for Sentinel Lymph Node Mapping

A tracer is injected peritumorally submucosally in the cervix uteri to enhance perioperative lymph node identification and SLN mapping. Dye-like tracers aid in visualizing lymphatic vessels and/or lymph nodes. Radiotracers are not visible to the eye but are detectable through probes. The radiotracer ideally accumulates in lymph nodes, and the probe reacts to the accumulation. The most common dyes are isosulfan blue, patent blue, or methylene blue, and in later years, the fluorescent dye Indocyanine Green (ICG). Blue dye has the advantage of being visible without any additional technical equipment, and it is relatively cheap. Still, the passage through the lymphatics is relatively quick and swiftly stains second-echelon nodes [110, 111]. Indocyanine Green is an option both in MIS and in open surgery but requires auxiliary near-infrared imaging equipment, e.g., PINPOINT® (Stryker, Kalamazoo, MI, USA), SPY Elite® (Novadaq Technologies, Bonita Springs, FL) or Fire-Fly® technology built into the robotic da Vinci Surgical System® (Intuitive Surgical Inc., Sunnyvale, Ca, USA), to enable visualization. The fluorescent signal in lymph nodes is very strong, facilitating mapping (Figure 4). Rapid extravasation of ICG, when the surgeon starts dissecting, is a potential problem since the image becomes cluttered, and any SLNs can be harder to identify, requiring a structured surgical opening of avascular planes, keeping lymphatics intact [111, 112]. In earlier years, the most commonly used radiotracer was technetium-99 nanocolloid (Tc^{99m}). Due to differences in routines between medical centres, the timing of the cervical injection in relation to the start of surgery varies from up to 18 hours before surgery to immediately before surgery. Like the other tracers, it is distributed through the lymph vessels and theoretically may have a less extensive spread beyond the juxtauterine lymph nodes, i.e., SLNs, which is a potential advantage with Tc^{99m}. The main drawback is that lympathic channels, cruzial for definition of paralell lympathics and defintition of SLNs are not visualized. Furthermore injecting and handling a radioactive substance means exposure for both patient and personnel, it requires additional logistics, as does the injection 18 hours before planned surgery, and additional technical equipment. Additionally, the probe cannot be directed against the cervix as the primary injection activity will override the signal; hence, only parietal lateral nodes may be detected. Finally, the probe will occupy one of the trocars, leaving one less trocar for other instruments [110, 111].

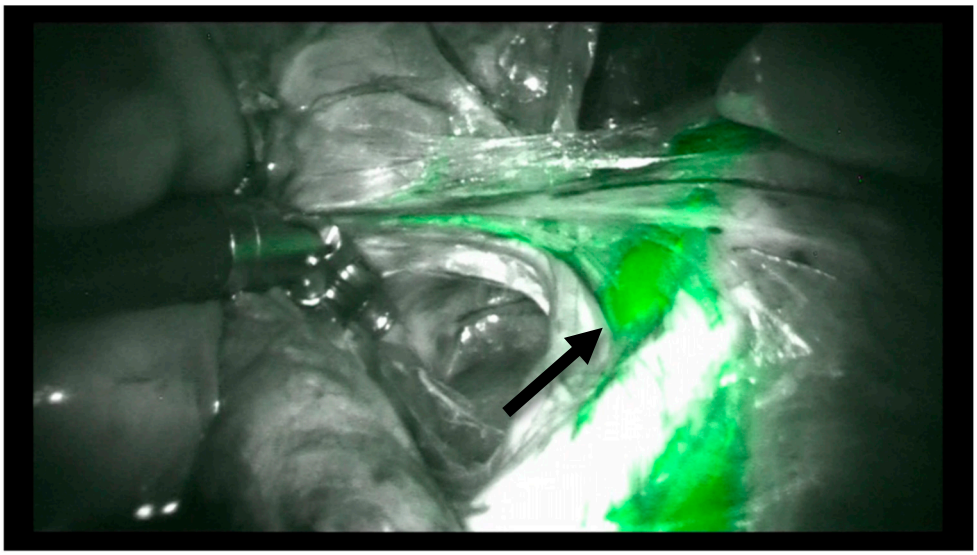


Figure 4.
Indocyanine green displays the UPP and an SLN type 1 defined by an afferent lymphatic vessel along the UPP in the medial external iliac ("interiliac") position. The SLN is marked with an arrow. (Picture J. Persson 2022).

Obtaining bilateral mapping is key to developing an SLN algorithm with high sensitivity and negative predictive value (NPV). Combining tracers has been one way to increase mapping, and multiple studies have compared different tracers and combinations of these. In the 2018 FILM study, Frumovitz et al. established that ICG is non-inferior to blue dye and to a combination of blue dye and ICG [113]. They found that ICG alone had an overall detection rate of 92%, while ICG + blue dye had 92% and blue dye alone 76%. The bilateral detection rate was not disclosed for the ICG + blue dye combination, but for ICG and blue dye alone it was 81% and 32% respectively. Noteworthy is that blue dye had a false negative count of eight SLNs while ICG had none. Jewell et al. 2014 retrospective publication concludes that blue dye and ICG combined does not add to bilateral mapping compared to ICG alone, 77% and 79% respectively [114]. Cabrera et al. found a higher bilateral mapping rate in combining Tc^{99m} + ICG as compared to Tc^{99m} + blue dye, 68.6% and 40.8% respectively [111]. In retrospective studies by Buda et al. and one by Di Martino et al. ICG proved to have a higher bilateral mapping rate, ranging from 85% to 98.5%, as compared to Tc^{99m} + blue dye, ranging from 58% to 76.3% [115-118]. In one of the studies blue dye alone was accounted for and proved a bilateral mapping rate of 50% [115]. In 2017, Tanaka et al. do not describe bilateral mapping results but conclude that for ICG, the sensitivity was 80% and the overall mapping was 61.6%, the corresponding numbers for Tc^{99m} were 70.3% and 85.5%, and for blue dye 87.5% and 20.2% [119]. False negative rates were higher for Tc^{99m}, with n=8

(5.7%), than for the other tracers. Blue dye and ICG each had one false negative SLN, corresponding to 2.7% and 2.0%, respectively. Nonetheless, the Tanaka et al. conclude that Tc^{99m} is the most feasible alternative in an SLN algorithm. This is contradictory to the other previously mentioned studies, which all conclude that ICG or ICG+ Tc^{99m} are the most promising tracer alternatives in an SLN procedure [111, 113-118]. The studies are summarized in Table 3. However, an unresolved issue is the anatomic rationale/background on how to define an SLN in case of use of more than one tracer in case of mapping by one and non-mapping of the other.

Bizzarri et al. found no significant difference regarding bilateral mapping nor sensitivity when comparing ICG as tracer in MIS and open surgery SLN procedures in patients with cervical cancer [120]. In a review article from 2021, Baeten et al. observed that ICG had higher bilateral mapping than Tc^{99m} + blue dye and that the promising results could induce a shift towards ICG only in SLN procedures in cervical cancer, although with a reservation that more and larger prospective studies are needed regarding bilateral mapping and false negative rates, to have sufficient support for a definitive change [121]. In their 2016 review article on SLN mapping for endometrial and cervical cancer, Rocha et al. also call for larger randomized studies on SLN mapping using ICG but conclude that its use is promising and that the ICG and near-infrared fluorescence technique is safe, time-efficient, and seemingly reliable [122].

Table 3. **A summary of studies on tracers used in cervical and endometrial cancer.**

Study	Study type	Type of Cancer	Number of Centres	Number of Patients	Type of Tracer 1	Unilateral Mapping Rate	Bilateral Mapping Rate	Sensitivity / False Negative	Type of Tracer 2	Unilateral Mapping Rate	Bilateral Mapping Rate	Sensitivity / False Negative	Type of Tracer 3	Unilateral Mapping Rate	Bilateral Mapping Rate	Sensitivity / False Negative	Comments
Frumovitz et al. 2018	Prospective, Non-inferiority	Endometrial + Cervical	8	180	BD+ICG	92%	n/a	n/a	ICG	98%	81%	n/a / 0	BD	76%	32%	n/a / 8	
Cabrera et al. 2020	Prospective, Non-randomized	Endometrial	1	84	Tc99m+BD	93.9%	40.8%	n/a / 1	Tc99m+ICG	91.4%	68.6%	n/a / n/a	-	-	-	-	
Jewell et al. 2014	Retrospective	Endometrial + Cervical	1	275	BD+ICG	93%	77%	n/a / n/a	ICG	95%	79%	n/a /n/a	-	-	-	-	
Buda et al. 2016	Retrospective	Cervical	5	144	Tc99m+BD	96%	76.3%	96% / 1	ICG	100%	98.5%	96% / 1	-	-	-	-	Sens and false neg not specified to group
Di Martino et al. 2017	Retrospective	Cervical	4	95	Tc99m+/-BD	91.5%	66.0%	n/a / 0	ICG	100%	91.7%	n/a / 3	-	-	-	-	
Tanaka et al. 2016	Retrospective	Cervical	1	119	Tc99m	85.5%	n/a	70.3% / 8	BD	20.2%	n/a	87.5 / 1	ICG	61.6%	n/a	80.0 / 1	Tracer combinations inadequately described.
Buda et al. 2016	Retrospective	Endometrial + Cervical	1	163	Tc99m+BD	97%	58%	n/a / n/a	BD	89%	50%	n/a / n/a	ICG	100%	85%	n/a / n/a	
Buda et al. 2017	Retrospective	Cervical	2	65	Tc99m+/-BD	95.7%	69.6%	100% / 0	ICG	100%	95.2%	100% / 0	-	-	-	-	

Lymphatic Complications Following Pelvic Lymphadenectomy vs. Sentinel Lymph Node Biopsy

One incentive to implement an SLN algorithm is the presumed decreased risk of developing long-term complications related to a PLND, specifically lymphedema. It can manifest in the lower abdomen, lumbar region, and lower extremities, causing discomfort, dysfunction, and disabilities for those affected and has an adverse long-term effect on quality of life [13, 123]. In their 2017 review article, Lindqvist et al. reported a prevalence of 0% to 50% of lower limb lymphedema (LLL) in women having had hysterectomy and PLND related to endometrial cancer (EC). They saw great inconsistencies in the reporting of LLL and therefore hesitate to draw any conclusions, due to the risk of reporting bias. However, they conclude that there appears to be a causal association between LLL and lymphadenectomy, the number of LNs removed, and radiation therapy [13]. Another review article, by Helgers et al. (2021), compares lymphedema and post-operative complications in women with SLN only vs. PLND in endometrial cancer. They found that the risk of developing LLL was significantly reduced in women who underwent SLN only. They also identified the number of LNs removed and radiation as risk factors for developing LLL, as well as obesity. Furthermore, they found that SLN only significantly reduced surgery time, blood loss, and hospital stay. They do stress that the oncological outcome has not been considered in their review, and that there are additional clinical factors to consider when deciding on the extent of the lymphadenectomy [12].

Aims

General Aims

The general objective of this thesis is to develop and evaluate a reproducible minimally invasive surgical SLN algorithm for the determination of pelvic lymph node metastases in early-stage cervical cancer based on the lymphatic anatomy of the uterus using ICG as a tracer, allowing for reinjection, and to estimate the sensitivity, negative predictive value, anatomic positions of SLNs and metastatic SLNs, and mapping rates in said algorithm.

Specific Aims

Study I

- A prospective study to investigate whether combining two independent tracers, ICG and Tc^{99m}, increases the SLN detection rate in women with early-stage cervical cancer.
- To examine any intraoperative or up to 30 days postoperative adverse events.

Study II

- A prospective study to examine the presence of lymph nodes in the PULT in women with early-stage cervical cancer.
- To examine the presence of lymph node metastases in the PULT in women with early-stage cervical cancer.
- To assess the presence of lymph nodes and lymph node metastases in the PULT in relation to lateral pelvic SLN status in women with early-stage cervical cancer in a strictly defined SLN surgical procedure.
- To evaluate possible adverse events related to removing the PULT as SLN tissue.

Study III

- A prospective study to compare the anatomical distribution of non-metastatic and metastatic SLNs in cervical and endometrial cancer using cervically injected ICG as a tracer.

Study IV

- A prospective study describing an optimized SLN algorithm in early-stage cervical cancer using ICG as a tracer and a strict definition of SLN based on previous anatomy studies of the lymphatic pathways, including the PULT as SLN tissue.
- To assess the sensitivity and NPV of said algorithm.
- To provide a detailed description of the anatomic distribution of SLN metastases.

Material and Methods

Common Traits Study I – IV

The SLN algorithm utilized in all studies was developed at our institution based on strict anatomical definitions of the uterine lymphatic pathways and of SLNs (Figure 5) [81]. Three highly experienced surgeons performed all surgical procedures. At the end of these studies, a total experience of 1300 or more pelvic SLN procedures in endometrial and cervical cancer combined was achieved.

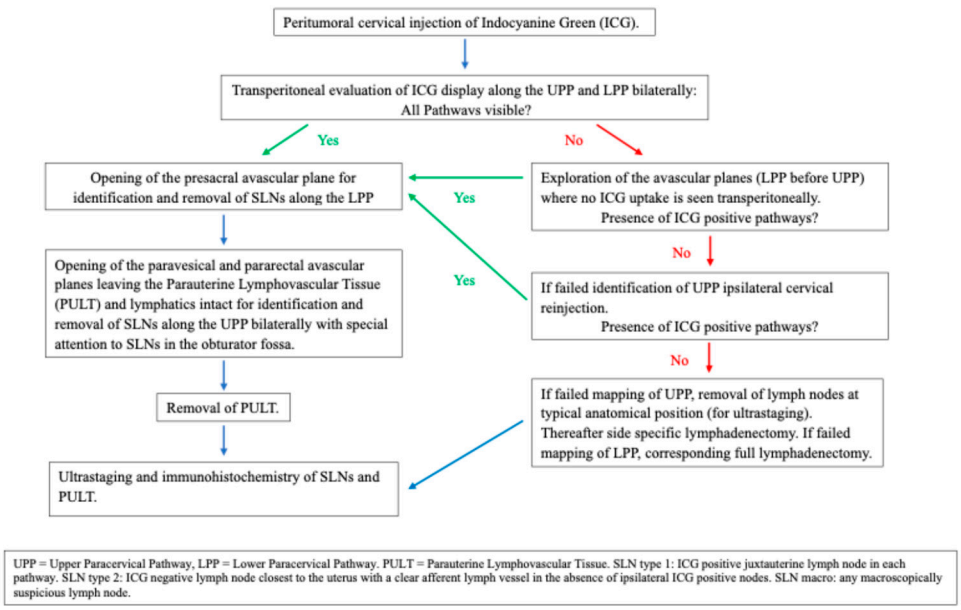


Figure 5.
Surgical algorithm for identification of sentinel lymph nodes (SLN) in early-stage cervical cancer.

Inclusion criteria were early-stage cervical cancer (FIGO 2009) stages IA1 with LVSI, IA1-IB1, selected cases IB2-IIA, multifocal disease, mixed squamos cell cancer and adenocarcinoma or unclear margins at a cone biopsy and a uterine size allowing for vaginal extraction. Exclusion criteria were inability to understand written

patient information, comorbidity, age >80 years, bleeding disorder, allergy to iodine, ongoing pregnancy, and any contradiction to robot-assisted laparoscopy.

Based on anatomy studies by Geppert et al., the algorithm defines two main lymphatic pathways, the upper (UPP) and lower (LPP) paracervical pathways. The juxtauterine lymph nodes along these pathways were defined as SLNs, subdivided into three types:

- SLN type 1 is the juxtauterine ICG-positive node with an afferent lymphatic channel.
- SLN type 2 is a juxtauterine ICG-negative node along an ICG-positive lymphatic channel and in the absence of an ICG-positive node along the same channel.
- SLN-macro is any macroscopically suspect node regardless of ICG uptake.

Indocyanine green (ICG, Pulsion medical system, PICG0025SE, Feldkirchen, Germany) was injected cervically and used as tracer, where a total of 1 ml 2,5 mg/ml ICG solution was injected in the cervix at 2, 4, 8, and 10 o'clock. The full volume was injected peritumorally submucosally. A minimum of 10 minutes of observation was allowed to assess the ICG uptake. In case of non-mapping of any of the pathways, an ipsilateral reinjection of 0,25 ml ICG solution was performed submucosally at 3 or 9 o'clock. While keeping the lymphatic vessels (via the PULT) connecting the uterus with parietal lateral pelvic nodes intact, the retroperitoneal avascular presacral, pararectal, and paravesical planes were developed. Parallel lymphatic vessels, frequently to the obturator and external iliac positions, were actively looked for. Any lymph nodes defined as SLN type 1, SLN type 2, or SLN-macro were retrieved. Furthermore, the PULT was removed *en bloc*, dissected separately, and treated as SLN tissue. The entire PULT tends to be dyed green after the ICG injection, and the lymph nodes are often relatively small and hard to identify separately. The PULT was not sent for frozen section. All SLNs and their respective type were marked on an anatomical chart. A chart with predefined nodal positions and type and prelabelled jars accompanied the pathology report (Figures 6 and 7).

In women stages ≥IA2, a PLND followed the SLN procedure. If metastases were evident on FZ, the PLND was extended to include the inframesenteric paraaortic area. In appropriate cases, the ovaries were transposed. If no mapping of SLNs occurred, a side-specific PLND was performed. Unless metastases were detected during FZ, an RRH or RRT was performed.

The attending designated gyneoncologic pathologist examined the retrieved SLNs. Sentinel lymph node tissue exceeding 3 mm in thickness was embedded and bisected. If the SLN tissue had a maximum diameter of >1 mm, Hematoxylin and Eosin staining (H&E) was used for ultrastaging in five sections at three different levels, spaced 200 µm apart. Immunohistochemistry staining (IHC) using antibodies

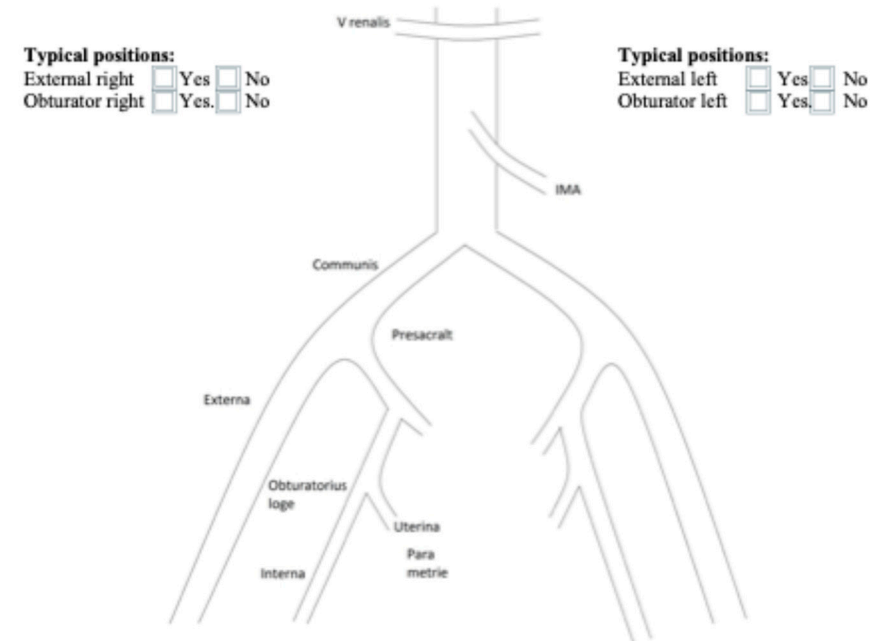
Anatomical plan for localization of sentinel lymph nodes

Display after first injection:

Display after reinjection: ☐ yes ☐ no

	UPP	LPP	IPP
Right			
Left			

	UPP	LPP	IPP
Right			
Left			



Mark position and type of SLN on anatomical chart with number of corresponding to postion and number on list and on seperate jars for each SLN

○ = ICG positive Sentinel node **(SLN1)**

☐ = ICG negative juxtauterine lymph node with afferent lymphatic vessel **(SLN 2)**

X = Tumor suspect lymph noder egardless of mapping **(SLN-macro)** with information on ICG positivity or not.

Figure 6
The anatomical chart used perioperatively to depict SLN positions in studies II-IV, SLN type, injection, and reinjection. The first study had a similar chart, though it also included alternatives for Tc^{99m}. UPP=upper paracervical pathway, LPP=lower paracervical pathway, IPP=infundibolopelvic pathway, ICG=Indocyanine green. Translated from Swedish.

List of preparations endometrial and cervical cancer KK Lund. Patient ID, date

If preparation is not retrieved, cross over corresponding line below. Numbers for jars are kept for retrieved preperations.

KK Jar no.	Lymph node position	Patol jar no	Dosa no	No of pieces	Mikro no of nodes	Of which metast
1	Uterus, right ovary and tube, left ovary and tube					
2	Lgl Iliaca externa dx					
4	Lgl Obturatorius dx					
6	Lgl Iliaca communis dx					
8	Lgl Presacral dx					
10	Lgl Iliaca externa sin					
12	Lgl Obturatorius sin					
14	Lgl Iliaca communis sin					
16	Lgl Presacral sin					
18	Lgl paraaorta below IMA					
20	Lgl paraaorta above IMA					
21	Omentum					
22	SLN PULT dx					
23	SLN PULT sin					
24	SLN type 1 Prescral dx					
25	SLN type 1 Presacral sin					
26	SLN type 1 Iliaca externa dx					
27	SLN type 1 Obturatorius dx					
28	SLN type 1 Iliaca externa sin					
29	SLN type 1 Obturatorius sin					
30	SLN type 1					
31	SLN type 1					
32	SLN type 2					
33	SLN type 2					
34	SLN Macro ICG pos ICG neg					
35	SLN Macro ICG pos ICG neg					
36	SLN anatom Iliaca externa dx					
37	SLN anatom Iliaca externa sin					
38	SLN anatom Obturatorius dx					
39	SLN anatom Obturatorius sin					
40	SLN anatom communis dx					
41	SLN anatom comunis sin					
42	SLN anatom					
43	SLN anatom					
44						

Figure 7
List of preparations of nodal specimens used perioperatively in studies II-IV. The first study had a similar preparations list, though it also included alternatives for Tc^{99m}. Translated from Swedish.

cytokeratin MNF116 and pancytokeratin was performed. The embedding and microscopic examination of the node-like tissue was conducted through gentle palpation, if no lymph glands were macroscopically located in the SLN tissue the whole tissue was embedded. The PULT was embedded as a whole and treated as SLN tissue. Non-SLNs were stained for H&E only. To classify metastatic disease, the American Joint Committee on Cancer staging definitions for axillary nodes in breast cancer were modified (macro-metastases = tumor >2.0 mm in diameter, micro-metastases = tumor cell aggregates between 0.2 and 2.0 mm in diameter, isolated tumor cells (ITC) = individual tumor cells or aggregates that are <0.2 mm in diameter and <200 cells) [124].

All women diagnosed with early-stage cervical cancer went through a staging process that included a computed tomography (CT) scan of the thorax and abdomen, a pelvic magnetic resonance imaging (MRI), and a gynecologic examination under general anesthesia.

All surgical procedures were performed using the da Vinci Si or Xi robots (Intuitive Surgical inc., Sunnyvale, Ca, USA).

All women gave their written informed consent to participate. The studies were registered at Clinicaltrials.gov (NCT03680833) and approved by the Regional Institutional Review Board (Dnr 2013/163).

Study I

Consecutive women diagnosed with early-stage cervical cancer between November 2014 and March 2017 were assessed for eligibility. Included women were planned for a robotic radical hysterectomy (RRH) *ad modum* Querleu-Morrow B2-C2 [68] or a robotic radical trachelectomy (RRT). Women with FIGO stage <IA2 had an SLN procedure performed and stages IA2 through IIA had an SLN procedure followed by a PLND.

The retrieved SLNs were defined as SLN-ICG, SLN-ICG+Tc^{99m}, and SLN-Tc^{99m}. Any SLN-macro were removed regardless of uptake of tracers, but information on the mapping of any tracer was documented.

Perioperative exploration of the UPP and LPP for SLN detection was performed using the Fire-Fly® technology and the Neo2000© laparoscopic probe (Neoprobe Corporation Dublin OHIO). At the onset of surgery, 0,25 mL of a 2,5 mg/mL ICG solution and 20MBc Tc^{99m} was injected submucosally peritoumorally at 2, 4, 8 and 10 o'clock respectively with a 23G × 1½″ needle, achieving a total dose of 2,5 mg ICG and 80MBq Tc^{99m}. The nanocolloid was prepared at the radionuclide unit 1–2 h before surgery. After injection, a compression-free fornix presenter without an intracervical device was placed. Following a minimum of ten minutes of observation time, the respective tracers' uptake along the UPP and LPP was assessed.

As described above, the retroperitoneal avascular presacral, pararectal, and paravesical planes were developed. Any type of lymph node described in the ICG protocol and/or radio-positive lymph node exhibiting a minimum of five times the background radioactivity count was retrieved. The positions of the retrieved SLNs were marked on an anatomical chart, along with their characterization as described above. If no radio-positive SLNs were detected, a second pathway-wise scanning using the gamma probe was performed after the initial SLN removal. The PULT was removed per protocol but was not included in the analysis.

Any adverse events related to injection of tracers and the SLN procedure as such were registered. Complications occurring within 30 days after surgery was also registered and categorized according to the Clavien-Dindo classification [125].

Statistical Plan and Analysis

A bilateral success rate for Tc^{99m} of 59%, based on previous data, served as the null hypothesis, and for ICG of 85%, based on pilot studies from our institution [90]. To reject the null hypothesis with a power of 80% and a significance level of 5%, 65 patients needed to be included. Included women served as their own controls when comparing the independent tracers. Fifteen additional women were planned for inclusion to compensate for dropouts and protocol violations. The number of eligible patients was continuously monitored. For statistical analyses we used Fisher's exact test. A value of $p < 0.05$ was considered statistically significant. Calculations were performed with PS Power and Sample Size Calculations version 3.1.2.

Study II

Given the definition of an SLN as a juxtauterine lymph node draining the uterus, a lymph node in the PULT qualifies under that description as the UPP passes through the PULT, often in parallel pathways, to the external iliac and obturator areas. Even though it has been recognized earlier that the parauterine and paracervical tissue can contain lymph nodes and that metastases occur, the PULT and any lymph nodes therein have never been defined as SLNs/SLN tissue [16, 104, 107, 108, 126, 127]. The PULT is the tissue between the broad ligament and the obliterated umbilical artery, caudal to the supravesical artery and ventral to the ureter. The PULT does not contain any autonomic nerves, and dissection while recognizing the anatomical landmarks described above is unrelated to an increased morbidity risk as opposed to more extensive approaches in dissecting the “parametria”.

Defined surgical steps to perform the separation and removal of the PULT:

1. While keeping the broad ligament intact, develop the pararectal and paravesical spaces.

2. The ureter is lateralized.
3. The uterine artery and the avascular plane to the superior vesical artery are visualized/ developed.
4. Dissect and remove the PULT with the superior vesical artery as the ventral limit, the broad ligament as the medial limit, the ureter as the dorsal limit, and the obliterated umbilical artery as the lateral limit.

In case of a trachelectomy, the uterine arteries are spared.

Any intraoperative and postoperative complications were registered, and any possible associations with the PULT removal were investigated further.

Study III

Prior, and parallel to the studies presented in this dissertation, the involved group has conducted studies and presented the SHREC study, an SLN algorithm for high-risk endometrial cancer with a sensitivity of 100% and bilateral mapping of 95% using ICG as a tracer. The study by Persson et al. describes an algorithm strictly based on the uterine lymphovascular anatomy and a clear definition of the SLNs draining the uterus [23]. Considering that cervical injection of ICG, rather than peritumoral injection, in endometrial cancer results in adequate visualization of lymphatic pathways and SLNs, the conclusion can be drawn that fundamental studies on anatomy applied in the development of a SLN concept in endometrial cancer are also relevant to cervical cancer [81, 82]. The anatomy of the lymphatic system is not altered due to a uterine tumor. The anatomically defined positions of SLNs are depicted in Figure 8.

Given the comparatively low incidence of cervical cancer relative to endometrial cancer, conducting adequately powered prospective studies on sensitivity and false negative rates in cervical cancer patients is time-consuming, especially if one wants to concentrate the trial in one or a few centers to ensure a strict methodology to avoid performance bias [128]. The ability to pool sensitivity data from women with different uterine cancers regarding studies of SLN concepts is appealing, as adequately powered studies can be conducted in a much shorter time frame if we can establish that the function of the lymphatic system and patterns for metastasizing are unaltered regardless of the type of cancer affecting the uterus.

Women with presumed high-risk endometrial cancer, FIGO grade 3 OR deep myometrial invasion OR non-endometroid histology, who underwent robotic MIS, were included in the endometrial cancer arm. Consecutive women with early-stage cervical cancer, as described above, between 2014 and 2020, were approached for

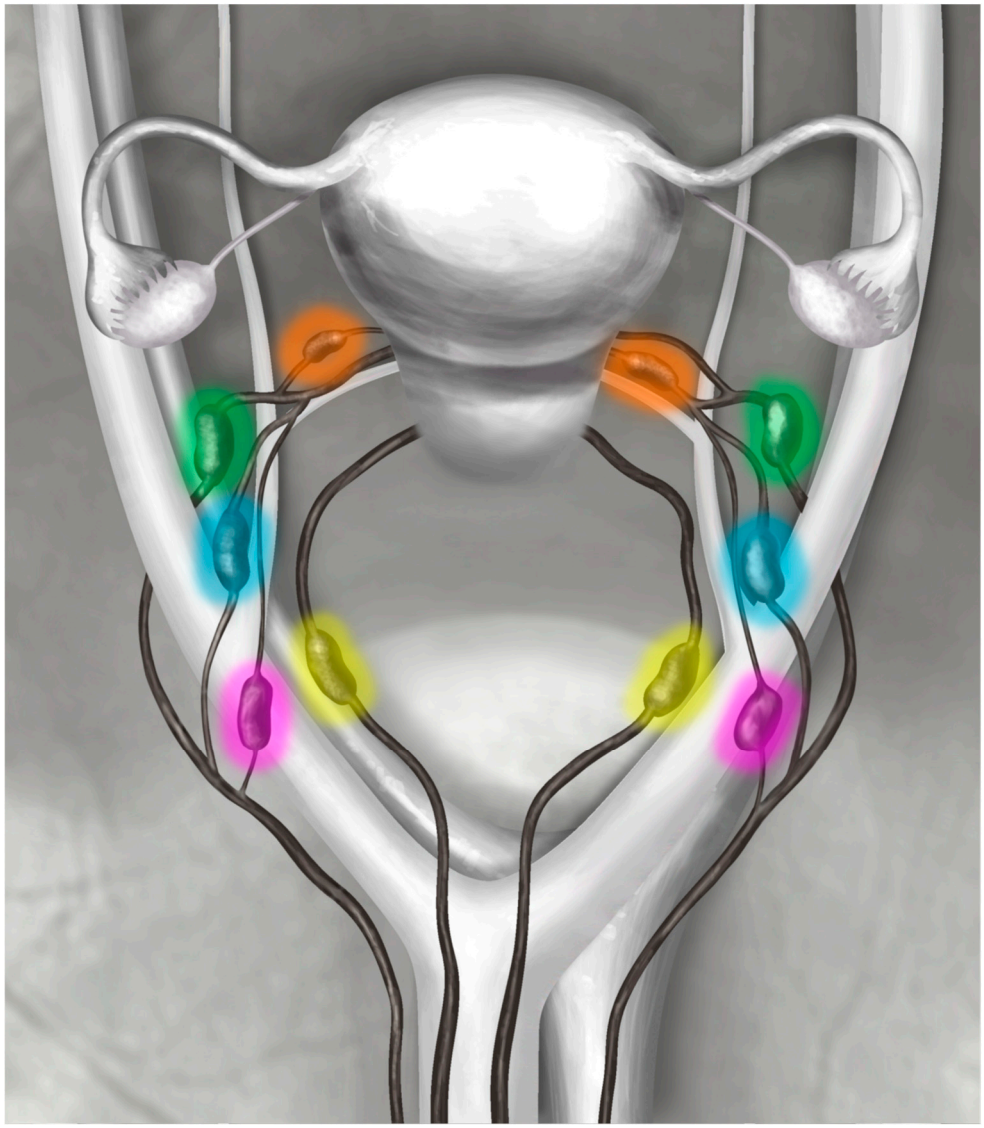


Figure 8
Sentinel lymph nodes are marked by color. **Orange:** The PULT. **Green:** The proximal obturator fossa position. **Blue:** The external iliac position. **Purple:** The common iliac postion. **Yellow::** Presacral.

inclusion in the cervical cancer arm. In both groups, ICG was injected cervically; however, in the endometrial cancer group, half the volume was injected submucosally and half into the cervical stroma. Otherwise, the SNL algorithm was identical between the groups. The positions and types of SLNs were depicted on an anatomical chart perioperatively. At the proximal obturator fossa and/or the inter iliac

positions, defined as typical SLN positions in previous work from the research group [129].

Following the SLN removal, a full PLND was performed in women with cervical cancer stages ≥ IA2, while stage IA1 was limited to an SLN-only procedure. Women with endometrial cancer had a full PLND, including the common iliac area to the level of the aortic bifurcation.

The studies were registered at clinical trials (NCT03680833 and NCT02690259) and approved by the Institutional Review Board (DNR 2013/163). All included women gave their written informed consent.

Statistical Plan and Analysis

A comparison of the distribution and anatomical position of all SLNs and all metastatic SLNs between cancer types was performed, along with mapping rates per pathway and per hemipelvis. Chi-square analysis or Fisher's exact test was used for statistical analysis of mapping rates and anatomical distribution. A value of $p < .05$ was considered statistically significant. The proportion of typically positioned SLNs along the UPP in women with endometrial cancer has previously been reported to be 72%. [129]. Based on this information, a sample size analysis was performed. Rejecting a 10% proportional inferiority of typically positioned SLNs in cervical cancer (null hypothesis), with a power of 80%, required 145 cervical cancer patients. Calculations were performed with PS Power and Sample Size Calculations version 3.1.2.

Study IV

Consecutive women diagnosed with early-stage cervical cancer stages IA2 through IIA1 (FIGO 2009) and planned for surgery between September 2014 and January 2023 were approached for inclusion [58]. Planned surgery was a SLN procedure followed by a completion PLND and RRH or RRT. Open surgery was performed in two women, and for the initial SLN procedure, the robot was docked via the laparotomy, without portholes.

Retrieved SLNs were marked on an anatomical chart regarding the type of SLN and position.

All perioperative adverse events (AE) or serious adverse events (SAE) and possible relations to using ICG, the SLN procedure, the PLND procedure, or hysterectomy/trachelectomy were recorded. Postoperative complications occurring within 30 days of surgery were noted.

Since each studied SLN procedure was followed by a completion PLND, each woman served as her own control regarding nodal status.

The SLN-ICG algorithm was assessed separately from the overall SLN algorithm, which also includes SLN-macro.

Statistical Plan and Analysis

To determine sample size, interim analysis, and decision to stop recruitment based on sensitivity, the Fleming two-stage design was used. The null-hypothesis with a sensitivity of 82% was tested against a one-sided alternative with a desired sensitivity of 95%. When 29 women with ICG-mapped LNMs were identified, an interim analysis was planned. At the time of interim analysis, all enrolled women, including those with pending results from final histology, were included in the final analysis. The interim analysis gave the following conditions:

- If 26 or fewer women with pelvic LNMs were correctly identified by at least one SLN-ICG or isolated node metastasis to the PULT, the study would be stopped for futility.
- If 27 – 28 women with pelvic LNMs were correctly identified by at least one SLN-ICG or isolated node metastasis to the PULT, the study would continue until 38 women with pelvic LNMs were included.
- If all 29 women with pelvic LNMs were correctly identified by at least one SLN-ICG or isolated node metastasis to the PULT, the hypothesis of inefficacy would be rejected, and enrolment should stop.

If the study were to be continued until 38 node-positive women were included, a total of 35 women with LNMs correctly identified by at least one SLN-ICG or isolated to the PULT, would be required to reject the hypothesis of inefficacy. This design yielded a type 1 error of 0.05 and a power of 80% when the true sensitivity is 95%. The IBM SPSS software v.29 was used for analyses.

Ethical considerations

All women gave their written informed consent to participate in the trials and to the robot-assisted surgical approach. All studies were approved by the Regional Institutional Review Board at Lund University (Dnr 2013/163).

Results

Study I

Of the 80 consecutive women eligible to participate, 74 were included, and 65 remained for final analysis. All women approached to participate accepted.

Of the included women, 58 had a PLND performed, and the remaining seven had a SLN-only procedure.

The median tumor size was 17 mm (1-70). As defined by the attending pathologist the median number of SLN-ICG was 6 (2-22) and SLN-Tc^{99m} was 3 (1-10).

Defining bilateral mapping rate as at least one ICG-defined SLN per hemipelvis, either in the UPP or the LPP, the mapping rate for ICG was 98.5%, for Tc^{99m} it was 60%, where in one case, a malfunctioning gamma probe led to no mapping of Tc^{99m} ($p < .01$). Bilateral mapping of both the UPP and the LPP was found in 64.6% for ICG and 1.5% for Tc^{99m} ($p < .001$). Though it did not affect the bilateral mapping rate for ICG in the specific cases, three women (4.6%) had non-metastatic SLN-Tc^{99m} only. Combining ICG and Tc^{99m} did not contribute to bilateral detection.

All eight (12.3%) women with LNMs had at least one metastatic SLN-ICG. The total number of metastatic SLNs was 20. Twelve (60.0%) were identified as SLN-ICG, seven (35.0%) were SLN-ICG-Tc^{99m}, and one (5%) was a bulky node, i.e., SLN-macro. None were SLN-Tc^{99m} only ($p < .05$, *OR* 12.7, 95% CI: 1.4, 114.4). No isolated LNMs were found along the LPP, in the presacral area, but three (37.5%) women had metastases here.

In one woman, an LNM was discovered where no mapping occurred unilaterally, but metastatic SLN-ICG was retrieved from the contralateral hemipelvis. If calculated per hemipelvis this would count as a false negative.

Of the 65 women, six had SLN type 2, of whom two had SLN type 2 along more than one pathway. One of the six women had metastasis in an SLN type 2.

Two women had metastatic findings in SLN-macro. One of the women had metastases in SLN-macro from two different pathways, one negative for both tracers and one positive for both tracers. The other woman had one SLN-macro, which was positive for both tracers. Both women had other metastatic lymph nodes, either SLN-ICG or SLN-ICG-Tc^{99m}.

No adverse events related to ICG injections or the SLN procedure were recorded. No intraoperative complications or conversions to laparotomy occurred. Postoperative complications within 30 days of surgery were documented in seven (10.7%) of the women. Of these, six (9.2%) were classified as grade I-II according to the Clavien-Dindo classification, comprising two vault hematomas, two lower urinary tract infections, and two blood transfusions. One woman had a grade III complication (port site hernia). No one had a grade IV complication.

Three women (4.6%) were readmitted, and one (1.5%) underwent a reoperation within 30 days of surgery.

Study II

Included for analysis were 145 women, of whom 120 underwent robotic radical hysterectomy and 25 had a robotic trachelectomy performed. In all women, the PULT was dissected and treated as SLN tissue. Twenty women had an SLN-only procedure, and the remaining 125 had an SLN procedure followed by a supplementary PLND.

The median tumor size, as described by the attending pathologist, was 12.0 mm (range 0.3–70 mm). Histologically, 57.9% were diagnosed as squamous cell carcinomas, and 33.8% had adenocarcinomas.

Indocyanine green was used as a tracer, and the algorithm achieved a bilateral mapping rate of 97.9%. The median number of SLNs (SLN type 1–2), as perceived by the surgeon, was 5 (range, 1–9).

Seventy-six women (52.4%) presented with an LN in the PULT, of whom 25 (17.2%) had bilateral LNs.

Lymph node metastases were identified in 19 women (12.3%), all of whom were confirmed by at least one positive SLN as defined by the algorithm. Of these women, 16 had at least one lateral pelvic SLN. Six women (4%) had metastatic nodes in the PULT, of whom three (2%) had metastases isolated to the PULT, i.e without any lateral pelvic LNMs. This translates to 16% of all node-positive patients and 2 % of all women. Details on all women with PULT metastases are available in Table 4. Women with PULT metastases had a median tumor size of 21.5 mm (range 12–30 mm) as compared to 24.7 mm (range 4–45 mm) in all node-positive patients ($p < .05$). All women with PULT metastases were LVSI positive.

The removal of the PULT was not related to any intraoperative complications.

Study III

Included for analysis were 145 women diagnosed with cervical cancer and 201 diagnosed with endometrial cancer, for a total of 346 consecutive women.

The bilateral mapping rates per hemipelvis were similar, 97.9% and 95.0% for cervical and endometrial cancer, respectively ($p = .16$). When subdivided into the UPP and LPP, the responding results were 97.2% and 92.0% ($p = .06$) for the UPP. The mapping along the LPP was higher in cervical cancer (69.7%) than in endometrial cancer (45.8%) ($p = .05$).

As stated earlier, typical positions along the LPP cannot be defined due to longitudinal variations and cross-over lymph vessels, but along the UPP, the typical anatomical positions are the interiliac and the proximal obturator fossa. With 78.1% and 82.1% SLNs at the typical positions among the included women, for cervical and endometrial cancer, respectively, there was no proportional difference ($p = .09$).

All women with nodal metastases ($n = 56$) were identified through the algorithm, having at least one metastatic pelvic SLN or a positive node in the PULT, yielding a sensitivity of 100% (95% CI, 93.6%-100%) and NPV of 100% (95% CI, 98.7%-100%). The anatomical distribution of metastatic SLNs was similar between the groups. The most common position was the proximal obturator fossa, 54.1% of women with cervical cancer and 48.6% of those with endometrial cancer had a positive SLN in said position ($p = .45$). Furthermore, 13 women (3.8% of all, and 23.3% of node-positive patients) had metastases isolated to the obturator position. The second most common position was the interiliac position (31% in cervical and 35.8% in endometrial cancer, $p = .56$). Of the metastatic SLNs, 85.2% (cervical cancer) and 83.4% (endometrial cancer) were retrieved at either typical position ($p = .75$).

In 7.6% and 7.0%, cervical and endometrial cancer, respectively, SLNs at atypical positions (lateral external/distal common iliac/ distal obturator fossa) were retrieved in the absence of SLNs at typical positions. Sentinel lymph-node metastases were found in these atypical positions in 4.8% of cervical and 5.7% of endometrial cancer patients ($p = .48$). Among women with endometrial cancer, two had metastatic SLNs isolated to atypical locations, both at the distal common iliac site.

In women with cervical cancer, 91.3% of all metastatic SLNs occurred along the UPP; the corresponding number for endometrial cancer was 91.4%. Metastatic SLNs isolated to the UPP were observed in 84.2% and 83.8%, respectively ($p = .96$)

Only one woman with endometrial cancer had a metastatic SLN isolated along the LPP; all others with positive SLNs along the LPP also had metastases along the UPP. Metastases in SLNs retrieved along the LPP occurred in 8.7% and 8.6% for cervical and endometrial cancer, respectively.

Study IV

Of 236 consecutive women assessed for eligibility, 194 were enrolled, and 181 were eligible for final analysis. Inclusion stopped for an interim analysis performed at the predetermined mark of 29 node-positive women. As the SLN algorithm correctly identified all node-positive women, further enrollment stopped. One additional node-positive woman was included as the final histology was pending at the study closure. Included women were stages IA2 (*n*=28, 15.4%), IB1(*n*=131, 72.4%), IB2 (*n*=17, 9.4%), and IIA1 (*n*=5, 2.8%) (FIGO 2009).

Of the included women, 149 (82.3%) had a Querleu Morrow B2-C1 hysterectomy, and 32 (17.7%) had a radical trachelectomy performed.

The median tumor size, as described by the attending pathologist, was 14 mm (range 2.0–80.0). The median number of SLNs was 6 (range 2–9), as perceived by the surgeon, and 8 (range 2–22) according to the attending pathologist at final histology. The median pelvic LN count, including SLNs, at final histology was 24 (range 7–71). In 50.8% of all women, at least one unilateral lymph node in the PULT was discovered.

Lymphovascular space invasion (LVSI) was positive in 51 (28.2%) women, negative in 105 (58.0%), and the status was not described in 25 (13.8%) women.

The overall bilateral mapping rate, before and after re-injection, and defined as at least one ICG-SLN type 1-2 per hemipelvis, was 94.5% and 98.3%, respectively. The remaining 1.7% mapped unilaterally, and no failed mapping occurred.

All 30 women with LNMs were correctly identified as having at least one metastatic SLN-ICG, as defined by the SLN-ICG algorithm, resulting in a sensitivity of 100% (95% CI, 88.4%-100%) and an NPV of 100% (95% CI, 97.6%-100%). Hence, the SLN-ICG algorithm and the overall SLN algorithm had identical results. Details on all node positive women are presented in Table 4.

A total of 951 SLNs were retrieved, of which 65 were found to be metastatic, of which 89.5% were SLN-type 1, 1.9% were SLN-type 2, and 4.3% were defined as SLN-macro.

The predominant position for positive SLNs was the proximal obturator fossa, where 46.1% of all metastatic nodes were located. It was the sole position for metastases in 40.0% of all node-positive women. The PULT carried isolated metastatic LNs in 1.7% of all women, or 10.0% of node-positive women. No isolated metastatic SLNs were found in atypical positions, i.e., the common iliac area, nor along the LPP. Of the retrieved metastatic SLNs, 6.0% were found in the LPP and 6.0% were located in the common iliac area; again, none of them were isolated.

Table 4. Data on all women with pelvic nodal metastases included in the prospective study IV on SLN detection in early-stage cervical cancer. Distribution, type, and size of lymph metastases in relation to pelvic lymphatic pathways and defined anatomical positions. Indocyanine green was injected cervically, allowing for reinjection.

CRF Number of metastatic SLN/LN	Clinical data	Right UPP				Left UPP				Right LPP	Left LPP	Paraaortic		
		External iliac artery	Obturator fossa	Common iliac artery	PULT	External iliac artery	Obturator fossa	Common iliac artery	PULT	Presacral	Presacral	Infra-mesenteric	Supra-mesenteric	Frozen Section +/-
CRF 2 SLN+: 4 LNM: 0	Stg=IB1, Op=RH, Sz=45, Inv=5 LVSIu, Hist=Sq	SLN1+ nLN	0SLN nLN	SLN1+ nLN	SLN-	0SLN nLN	SLNm+ nLN	SLN1+ nLN	0LT	SLN1- nLN	SLN1- nLN	nLN	0LND	+
CRF4 SLN+: 1 LNM: 0	Stg=IB1, Op=RH, Sz=7, Inv=3 LVSIy, Hist=Sq	0SLN nLN	SLN1- nLN	SLN1- nLN	0LT	0SLN nLN	SLN1+ nLN	0SLN nLN	SLN-	SLN1- 0LND	SLN1- 0LND	0LND	0LND	-
CRF 7 SLN+: 2 LNM: 3	Stg=IB1, Op=RH, Sz=20, Inv=5 LVSIy, Hist=Mix	0SLN nLN	0SLN LNMm+	0SLN nLN	0LT	SLN1+ nLN	0SLN nLN	0SLN LNM	SLN-	0SLN nLN	SLN1+ 0LND	0LND	0LND	+
CRF 21 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=22, Inv=20 LVSIy, Hist=Sq	SLN1- nLN	SLN1+ nLN	0SLN nLN	SLN-	SLN1- nLN	SLN1+ nLN	0SLN nLN	0LT	0SLN nLN	SLN1- nLN	0LND	0LND	-
CRF 22 SLN+: 5 LNM: 1	Stg=IB1, Op=RH, Sz=40, Inv=10 LVSIy, Hist=Sq	0SLN LNM	SLN1+ 0LND	0SLN nLN	0LT	SLNm+ 0LND	SLNm+ 0LND	0SLN nLN	0LT	SLN1+ nLN	SLN1- 0LND	LNM	0LND	+
CRF 31 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=34, Inv=13 LVSIn, Hist=Sq	SLN1- nLN	SLN2+ nLN	0SLN nLN	0LT	0SLN nLN	SLN1+ nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	nLN	0LND	+
CRF 47 SLN+: 3 LNM: 0	Stg=IB1, Op=RH, Sz=40, Inv=8 LVSIy, Hist=Muc	0SLN nLN	SLN1+ nLN	0SLN nLN	SLN-	SLN1+ nLN	0SLN nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	nLN	0LND	-

CRF Number of metastatic SLN/LN	Clinical data	Right UPP				Left UPP				Right LPP	Left LPP	Paraaortic		
		External iliac artery	Obturator fossa	Common iliac artery	PULT	External iliac artery	Obturator fossa	Common iliac artery	PULT	Presacral	Presacral	Infra-mesenteric	Supra-mesenteric	Frozen Section +/-
CRF 55 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=40, Inv=5 LVSIu, Hist=Sq	SLN1- nLN	SLN1+ nLN	0SLN nLN	0LT	SLN1- nLN	SLNm+ nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	0LND	0LND	+
CRF 61 SLN+: 7 LNM: 8	Stg=IB1, Op=Trach, Sz=14, Inv=4 LVSIy, Hist=Ad	SLN1+ LNM	SLN1+ LNM	0SLN LNM	SLN+	SLN1+ nLN	SLN1+ nLN	0SLN nLN	0LT	SLN1+ nLN	SLN1- nLN	LNM	0LND	+
CRF 67 SLN+: 1 LNM: 0	Stg=IB1, Op=RH, Sz=38, Inv=5 LVSIu, Hist=Ad	0SLN nLN	SLN1- nLN	0SLN 0LND	0LT	SLN1- nLN	SLN1+ nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	nLN	0LND	+
CRF 81 SLN+: 3 LNM: 0	Stg=IB1, Op=RH, Sz=23, Inv=11 LVSIy, Hist=Sq	SLN1- nLN	SLN1+ nLN	0SLN nLN	SLN+	SLN1+ nLN	0SLN nLN	0SLN nLN	SLN-	0SLN nLN	0SLN nLN	nLN	0LND	+
CRF 87 SLN+: 2 LNM: 1	Stg=IB1, Op=RH, Sz=24, Inv=16 LVSIy, Hist=Sq	SLN1+ nLN	0SLN nLN	SLN1- nLN	0LT	SLN1+ nLN	0SLN LNM	0SLN nLN	0LT	0SLN nLN	0SLN 0LND	nLN	0LND	+
CRF 102 SLN+: 1 LNM: 0	Stg=IB1, Op=RH, Sz=28, Inv=14 LVSIy, Hist=Ad	SLN1- nLN	0SLN nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	0SLN nLN	SLN+	SLN1- nLN	SLN1- nLN	0LND	0LND	-
CRF 105 SLN+: 2 LNM: 0	Stg=IB1, Op=Tr, Sz=12, Inv=6 LVSIy, Hist=Sq	SLN+ 0LND	SLN- 0LND	0SLN 0LND	SLN-	SLN1- 0LND	0SLN 0LND	0SLN 0LND	SLN+	SLN1- 0LND	SLN1- 0LND	0LND	0LND	+
CRF 107 SLN+: 4 LNM: 0	Stg=IB1, Op=RH, Sz=20, Inv=14 LVSIu, Hist=Ad	0SLN nLN	SLN1+ nLN	SLN1- 0LND	0LT	0SLN nLN	SLN1+ nLN	SLN1- nLN	SLN-	SLN1- nLN	0SLN nLN	LNM	0LND	+

CRF Number of metastatic SLN/LN	Clinical data	Right UPP				Left UPP				Right LPP	Left LPP	Paraaortic		
		External iliac artery	Obturator fossa	Common iliac artery	PULT	External iliac artery	Obturator fossa	Common iliac artery	PULT	Presacral	Presacral	Infra-mesenteric	Supra-mesenteric	Frozen Section +/-
CRF 108 SLN+: 1 LNM: 0	Stg=IB1, Op=RH, Sz=30, Inv=12 LVSly, Hist=Sq	SLN1- nLN	SLN1- nLN	0SLN nLN	SLN+	SLN1- nLN	SLN1- nLN	0SLN nLN	0LT	SLN1- nLN	SLN1- nLN	0LND	0LND	-
CRF 133 SLN+: 2 LNM: 0	Stg=2A, Op=RH, Sz/Inv=missing, LVSIn, Hist=Ad	SLN1- nLN	SLN1+ nLN	0SLN nLN	0LT	SLN1- nLN	SLNm+ nLN	0SLN nLN	SLN-	SLN1- nLN	SLN1- nLN	nLN	0LND	+
CRF 135 SLN+: 3 LNM: 1	Stg=IB1, Op=RH, Sz=25, Inv=9 LVSly, Hist=Sq	SLN1+ nLN	SLN1- nLN	0SLN nLN	0LT	SLN1- nLN	SLN1+ LNM	0SLN nLN	SLN-	SLN1- nLN	SLN1- nLN	nLN	0LND	+
CRF 153 SLN+: 1 LNM: 0	Stg=IB2, Op=RH, Sz=20, Inv=7 LVSly, Hist=Sq	SLN1- nLN	SLN1- nLN	SLNm- nLN	0LT	SLN1- nLN	SLN1- nLN	0SLN nLN	SLN+	SLN1- nLN	SLN1- nLN	0LND	0LND	-
CRF 162 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=38, Inv=14 LVSly, Hist=Ad	SLN1+ (ITC) nLN	SLN1+ (ITC) nLN	nLN	0LT	SLN1- nLN	SLN1- nLN	nLN	0LT	SLN1 - nLN	SLN1- nLN	0LND	0LND	-
CRF 185 SLN+: 1 LNM: 0	Stg=IB2, Op=RH, Sz=9, Inv=5 LVSIn, Hist=Ad	SLN1- nLN	SLN1- nLN	nLN	0LT	SLN1- nLN	SLN1+ (MIM) nLN	nLN	0LT	SLN1- nLN	nLN	0LND	0LND	-
CRF 188 SLN+: 2 LNM: 0	Stg=IB1, Op=Trach, Sz=12, Inv=9 LVSly, Hist=Sq	SLN1- nLN	SLN1- nLN	SLN1- nLN	0LT	SLN1+	SLN1+ (MIM) nLN	SLN1- nLN	0LT	SLN1-	0LT	0LND	0LND	-
CRF 189 SLN+: 1 LNM: 0	Stg=IB2, Op=RH, Sz=31, Inv=12 LVSly, Hist=Sq	SLN1- nLN	SLN1+ nLN	SLN1- nLN	SLN1-	SLN1- nLN	SLN1- nLN	SLN1- nLN	SLN1-	SLN1- nLN	SLN1- nLN	0LND	0LND	-

CRF Number of metastatic SLN/LN	Clinical data	Right UPP				Left UPP				Right LPP	Left LPP	Paraaortic		
		External iliac artery	Obturator fossa	Common iliac artery	PULT	External iliac artery	Obturator fossa	Common iliac artery	PULT	Presacral	Presacral	Infra-mesenteric	Supra-mesenteric	Frozen Section +/-
CRF 192 SLN+: 1 LNM: 0	Stg=Miss, Op=RH Sz=9, Inv=2 LVSIy, Hist=Sq	SLN1- nLN	SLN1- nLN	SLN1- nLN	0LT	SLN1- nLN	SLN1+ nLN	SLN1- nLN	0LT	SLN1-	SLN1-	nLN	0LND	+
CRF 201 SLN+: 1 LNM: 0	Stg=IB3, Op=RH, Sz=35, Inv=11 LVSIn, Hist=Sq	SLN1- nLN	SLN1- nLN	SLN1-	0LT	SLN1- nLN	SLN1- nLN	SLN1+ (ITC) nLN	0LT	SLN1-	SLN1-	0LND	0LND	-
CRF 203 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=35, Inv=15 LVSIn, Hist=Sq	SLN1-	SLN1+ (ITC)	SLN1-	0LT	SLN1-	SLN1-	SLN1+ (ITC)	SLN1-	SLN1-	SLN1-	0LND	0LND	-
CRF 204 SLN+: 3 LNM: 0 Open RH	Stg=IB2, Op=RH, Sz=16, Inv=12 LVSIn, Hist=Ad	SLN1-	SLN1+ (ITC) nLN	nLN	0LT	SLN1+ (ITC) nLN	SLN1- nLN	nLN	0LT	SLN1+ (ITC)	SLN1-	0LND	0LND	-
CRF 205 SLN+: 1 LNM: 0	Stg=IB1, Op=RH, Sz=26, Inv=12 LVSIy, Hist=Sq	SLN1- nLN	SLN1- nLN	SLN1- nLN	0LT	SLN1+ (MIM) nLN	SLN1- nLN	SLN1- nLN	0LT	SLN1-	SLN1-	0LND	0LND	-
CRF 224 SLN+: 2 LNM: 0	Stg=IB1, Op=RH, Sz=37, Inv=11 LVSIy, Hist=NE	SLN1-	SLN1+	SLN1- nLN	0LT	SLN1-	SLN1+ nLN	0SLN nLN	0LT	SLN1-	0SLN	0LND	0LND	+
CRF 228 SLN+: 1 LNM: 0	Stg=IB2, Op=RH, Sz=16, Inv=8 LVSIy, Hist=Ad	SLN1-	SLN-	0LND	0LND	SLN1-	SLN1+ ITC	SLN1-	0LND	SLN1-	SLN1-	0LND	0LND	-

Discussion

This thesis describes a methodological development and evaluation of the sensitivity and negative predictive value of an SLN algorithm based on lymphatic anatomy and a clear definition of SLNs based on ICG as a tracer, utilizing reinjection, in women with early-stage cervical cancer. The included studies were all prospective, and only three experienced surgeons performed the surgical procedures. The SLN-algorithm successfully identified all node-positive women. The included studies provide new insights into the anatomical distribution of nodal metastases, particularly regarding the obturator fossa and the importance of meticulously evaluating this location, as it was the sole position for metastatic SLNs in 40.0% of node-positive women. Furthermore, the importance of treating the PULT as SLN tissue has been emphasized, as isolated LNMs evidently can occur in this anatomic structure. These LNs are juxtauterine LNs, i.e., SLNs.

Choosing an optimal tracer is one of many fundamentals in developing an SLN algorithm, not only because it must facilitate achieving high bilateral mapping rates, and high sensitivity and NPV, but also fit the design of the algorithm. A dye tracer is necessary in the described algorithm, importantly to identify parallel lymphatic channels along a major pathway and for the distinction between SLN type 1 and type 2 is only possible when the lymphatic vessels are readily visualized. Blue dye has been shown to be inferior or at least non-superior to ICG in multiple studies [113-118]. Using ICG as the sole tracer, in study I proven to be non-inferior to combining it with a radiotracer, is not a controversial topic today. The tracer is considered safe, with low toxicity and easy handling perioperatively, compared to Tc^{99m}. This is also recognized in studies by other authors [85, 130]. Another advantage of ICG is the possibility to reinject the tracer ipsilaterally in case no mapping occurs on either hemipelvis. This was first described by our research team in 2017 [82]. After reinjection, the bilateral mapping increased from 94.5% to 98.3% in the 181 women included in study IV. In 2022, Maramai et al. also found a significant increase in bilateral mapping rates using cervical reinjection of ICG [131]. After the first injection in 251 women, bilateral mapping rates were 73.3%, unilateral rates were 22.7%, and 4.0% did not map at all. After reinjection, corresponding numbers were 94.5%, 5.1%, and 0.4%.

As mentioned previously, the PULT can harbour the only LNMs in some women with early-stage cervical cancer, and removing it and treating it as SLN tissue is essential to reduce the risk of false negatives. The utilization of the Sedlis criteria has

led to smaller-volume tumors being selected for surgery and a lower incidence of metastatic lymph nodes. The median tumor size in study IV was 14 mm and of the included women, 1.7% had isolated metastases to the PULT, corresponding to 10.0% of all node-positive women. In the context of the SHAPE and ConCerv trials and the impact these studies have had on radicality in hysterectomy, dissecting and removing the PULT is a significantly smaller intervention than a parametrectomy. The PULT can be dissected while preserving the autonomous nerves intact and considerably reducing the risk of ureter damage [72, 74]. In study II, part of the conclusions were that removing the PULT added little time to the overall surgery and was not associated with any complications. We, the research team, believe it is of paramount importance to incorporate the removal of the PULT as a part of nodal assessment/SLN algorithm, regardless of the extent of radicality of the hysterectomy. Given that 2% of women with isolated metastases within the PULT, the majority of whom had smaller cervical cancers, this obviously poses a risk of undetected spread of disease in patients with smaller tumors. Pavone et al. conducted a survey-based study, published in June 2025, exploring the understanding of in-transit positive lymph nodes, i.e., positive nodes in the PULT and lateral paracervix [132]. Experts in gynecological cancer were identified via a literature search on the Medline/PubMed database. Out of 471 identified corresponding authors, 66 responded. The 29 questions pertained to the PULT and paracervical lymph node involvement, exploring nomenclature, surgical technique, choice of tracer, clinical staging, and adjuvant treatment options. The authors conclude that there is a general awareness among gynecologic oncologic experts regarding the clinical significance of in-transit disease. Furthermore, they call for the FIGO staging committee to review the impact of in-transit disease to broaden the understanding of its impact on clinical practice.

In the foreseeable future, we can expect the incidence of cervical cancer to drop further thanks to increasing HPV vaccination and public goals of eradicating the disease, defining eradication as an annual incidence of fewer than four women in 100,000 [57]. In study III we were able to show that there are no differences between cervical and endometrial cancer regarding the anatomical distribution of SLNs and metastatic SLNs in the predefined typical anatomical positions; in other words, the lymphatic system does not recognize what type of cancer the uterus is carrying. The insight that SLNs and positive SLNs are similarly distributed along the UPP allows for pooling both cancer types in future studies regarding sensitivity, with adequate power within a smaller time frame. As described earlier, the LPP does not contain any typically positioned SLNs. In our studied populations, the bilateral mapping rates were higher in the cervical cancer group than in the endometrial cancer group. A plausible explanation could be the lower median age in the former cohort, and the fact that uterine blood perfusion is greater in premenopausal women, which subsequently leads to a higher lymphatic outflow. This difference was not studied further. Regardless, the LPP can harbor metastatic SLNs. In study IV, 6.0% of all metastases were located here, and exploring the area is imperative in achieving high sensitivity and NPV.

The obturator fossa position stood out as the predominant area for LNMs, and the region must be included in a SLN algorithm. This strongly emphasize that any algorithm suggesting that only one SLN needs to be removed per hemipelvis is insufficient, since the SLNs in the external iliac position are easier to identify and remove, thereby increasing the risk of overlooking the SLNs in the more technically challenging and less visible obturator fossa. In their 2023 study on pelvic node distribution in cervical cancer, Xie et al. came to the same conclusion. They stressed the importance of removing the obturator fossa nodes in any lymphadenectomy procedure [133]. In a recent study on endometrial cancer by Bollino et al, and given the insights from study III in this thesis, a hybrid SLN-algorithm stating removal of non-mapped nodes at typical positions regardless of mapping at other positions added another 5% to the group of node positive women [134]. Given the results and discussion as of above, it is very likely, and we recommend, that a hybrid algorithm should be applied also in cervical cancer.

The long time frames required to achieve adequate power to analyse sensitivity and NPV are reflected in the relatively few prospective studies published. To my knowledge, study IV is one of the largest prospective studies to date. More importantly, the SLN algorithm is based on the uterine lymphatic anatomy, a clear definition of what an SLN is, and an optimally performing tracer. The prospective multicentre SENTICOL trial (2011) included 139 women with early-stage cervical cancer planned for a laparoscopic SLN procedure, using a combination of patent blue and Tc^{99m} as tracer. Before the study began, one surgeon at each included centre (n = 7) had received special training in SLN biopsy, thereby enhancing the internal validity of the study. The respective trained surgeon at each hospital carried out all SLN biopsies included in the trial. The number of performed surgeries varied from 4 to 47 between the centres. A majority of patients (n = 131) had a lymphoscintigraphy preoperatively, which was used as an intraoperative guide in the search for SLNs. The SLNs were defined as blue-stained and/or radioactive, or lymph nodes having a bluish afferent channel, analogous to type 1 and 2 SLNs in the present thesis. The bilateral detection rate of at least one SLN per hemipelvis was 74.8% and the unilateral mapping rate was 97.8%. In three patients (2.2%) there was no mapping. Two patients had false-negative SLNs, corresponding to 8.0% of 25 node-positive women. Sensitivity was 92.0% and NPV 98.2%. The tracers are likely to impact the mapping rates. In light of the results from studies III and IV, there is a risk of understaging when settling for one SLN per hemipelvis, and without a well-defined reference to the lymphatic anatomy. Lymphoscintography is a possible bias in evaluating the methodological effectiveness of the surgical approach [17]. The 2021 SENTIREC trial is a prospective multicentre study including 245 women with early-stage cervical cancer eligible for surgery with robotic-assisted laparoscopy at three different centres in Denmark. All women had additional preoperative PET-CT to improve detection of metastatic LNs. Indocyanine green was used as tracer. Women stages FIGO 2009 IA2 – IB1 with tumors <20 mm underwent SLN-only staging procedures (n = 130), while IB1 with tumors >20 mm and stages IB2 – IIA1

had a SLN + PLND procedure (n = 115). The bilateral detection rate was 82.0%, with 83.1% in the group <20 mm tumor size and 80.9% in the >20 mm group. Thirty-eight of all women (15.5%) had LNMs, and in the >20 mm tumor size group 31 of 115 women (27.0%) were node positive. The sensitivity for the SLN algorithm was 96.3% (95% CI, 81.0-99.9%) and the NPV 98.7% (95% CI, 93.0-100%). Among the 31 women, 20 had positive SLNs and eleven non-SLNs. Of the eleven, the utilized algorithm identified six, and four were identified postoperatively as having parametrial metastatic nodes. One false-negative SLN was reported. Eight of the eleven women had unilateral mapping only, and in all eight, the metastases were detected either in the retrieved SLN or from the contralateral hemilymphadenectomy [20]. The study does not provide details on how SLNs were defined, and it is thinkable that subcategorizing into the likes of SLN type 1 and SLN type 2, could increase the bilateral mapping rates and decrease the number of metastatic non-SLNs reported. The algorithm suggests removal of suspicious lymph nodes, corresponding to SLN-macro. Furthermore, there were postoperative positive parametrial nodes, corresponding to positive nodes in the PULT. It is possible that if the surgeons had utilized the algorithm presented in this thesis, scrutinizing the PULT more thoroughly by treating it as SLN tissue, they would have found more metastatic lymph nodes in the area. The use of PET-CT, for which the surgeons were not blinded, is a potential bias and contributes to the sensitivity and bilateral mapping rates of the SLN algorithm.

The 2020 study by Cibula et al., the SENTIX trial, is a prospective non-randomized multicentre study on SLN-only biopsy with historical oncologic data as controls. Included were 391 women, planned for surgery related to early-stage cervical cancer. Both laparotomy and MIS were allowed, as were different tracers, i.e., blue dye, radiocolloid, and ICG, or combinations of the three. Although the lymphatic anatomy is taken into consideration, there is no existing algorithm, nor any reference to any previous studies on establishing an algorithm. Rather, the authors "designed protocol to mimic current clinical practice, without restrictions on the technique used for SLN detection or the surgical approach" [135]. Bilateral mapping rates were 91% and 62 women (15.9%) had metastatic SLNs. Since it was an SLN-only study, no reports on false negatives were reported. They reported that, of the women 51% and 54%, left and right respectively, had SLNs retrieved from the obturator fossa. Corresponding numbers for the external iliac area were 48% and 46%. Compared to the results from studies III and IV, these numbers are low, which poses a risk of understaging. The SENTIX trial is not evaluating an algorithm, but rather the oncological outcome of SLN biopsy. As such, comparing the study to this thesis is difficult. Although considering the description of a structured process on developing an SLN algorithm presented in this thesis, the SENTIX trial is worth mentioning since it is exploring the last step of the described SLN developmental process without the foundation of methodological studies. One imminent risk of this is the possibility of selection bias when attempting to draw conclusions from data collected

using vastly different methods of surgical approach, retrieval of SLNs, tracers, and combinations of tracers.

Results of the oncological outcome regarding disease-free survival and overall survival from the SENTIX-trial were published in July 2025 [136]. The primary endpoint was the recurrence rate at 24 months after surgery, where non-inferiority was decided at a reference mark of less than 7%. A total of 594 of 731 women with early-stage cervical cancer were included for final analysis in the study, which was conducted at 47 sites distributed over 18 different countries. Excluded were patients with metastatic SLNs and non-bilateral mapping of tracers. In 64% minimally-invasive surgery was utilized, and 36% had a laparotomy. The bilateral mapping rate was 92.3%. The median number of SLNs retrieved was 3 (range 2-4). Positive SLNs were identified in 56 (9%) patients at final ultrastaging. Another 46 had metastatic SLNs on frozen section and were excluded from follow-up; this means they were part of the 731-woman cohort but not included in the final 594-woman cohort. The two-year disease-free survival rate was 93.3%, and the overall survival rate was 97.9%. There was no significant difference between MIS and open surgery in terms of outcomes. Women with tumors larger than 2 cm at final histology were found to have a significantly higher risk of recurrence than those with tumors less than 2 cm. Regarding DFS and tumor size, a hazard ratio of 5.226 (95% CI, 1.970 – 13.868) for tumors >4 cm and 2.562 (95% CI, 1.434 – 4.477) for tumors 2-4 cm, as compared to <2 cm in diameter. Regarding typical positions, in 654 (99.2%) of 659 with bilateral mapping, an SLN was detected in either the external iliac or obturator fossa position. The authors conclude that an SLN-only procedure was non-inferior to patients who underwent a systematic PLND, given that the recurrence rate was 6.1%, i.e., less than the stipulated 7% mark.

As stated above, the SENTIX-trial does not explore the efficacy of a specific algorithm. The surgical approach, choice of tracer, and definitions of SLNs are at the hands of the surgeon. The authors of the SENTIX-tial conclude that SLN-only is non-inferior to a complete PLND procedure. Nevertheless, conclusions drawn from results obtained using such a diverse range of surgical methods should be approached with caution. In Study IV, 16.6% were node-positive with metastases to at least one SLN, and the median tumor size in our study was 14 mm (range 2.0 – 80 mm). In Table 4, tumor sizes for all women with metastases are presented, ranging from seven to 45 mm. An SLN procedure with high sensitivity is required regardless of tumor size. Cibula et al. also stress the importance of SLNs at the obturator fossa position, even though they emphasize both that and the external iliac position. As we have underlined, the obturator fossa in particular often holds the sole metastatic SLN in our experience. Our research team firmly believes that utilizing the algorithm presented in this thesis will have further positive effects on disease-free and overall survival in future studies on oncological outcomes. Scrutinizing the results will also be facilitated by the uniform structure of the underlying surgical algorithm. One final thing worth noticing is that the authors found no

significant difference in outcomes regarding MIS and open surgery, results that are in contrast to the LACC trial [64]. Further investigations on the matter of surgical approach, such as the RACC (ClinicalTrials NCT03719547) and ROCC (ClinicalTrials NCT04831580) trials, are welcome.

Sentinel lymph node algorithms in cervical cancer, the development, implementation, and to what extent they should be utilized are all "hot topics", and results from poorly designed trials risk harming progress in these areas. This thesis presents a well-defined algorithm based on anatomy, a clear definition of SLNs, and integrated studies on tracers and injection techniques to achieve a standardized SLN algorithm. These traits set it apart from the trials mentioned above and also other, retrospective studies. The lack of standardization in the area is a potential hindrance in establishing an SLN algorithm and thereby making it more difficult to compare outcomes and achieve further development. The lack of, and yearning after, consensus is addressed in a Bizzarri et al. study (2025), presenting the result of a questionnaire survey where international experts in gynaecologic oncology were responding. Of 38 approached experts, 25 (65.8%) responded to 26 questions, rating them in steps 'recommended', 'optional', and 'not recommended'. The ambition being to present a surgical guide that may aid surgeons in their clinical work for quality assurance and potentiate clinical trials [21]. Given the differences between the examples above and other previous attempts to present an SLN algorithm, including this thesis, the actual value of the consensus achieved is questionable. A better process is that of careful development of an algorithm, as described in this thesis, through prospective studies on anatomy, surgical methodology, trials on sensitivity and NPV, and finally, implementation and studies on oncological outcomes. In this context, the SENTICOL III trial is highly anticipated, recruiting 900 women with early-stage cervical cancer, randomized to either SLN only or SLN with full PLND. The aim is to demonstrate the non-inferiority of SLN alone compared to more extensive lymphadenectomy for DFS. The planned follow-up time is five years, and the last follow-up is expected to take place in Q2 2026 [137]. Different tracers or combinations of tracers are allowed, and surgical methods can also vary between the 300 included centres. Reinjection of tracers is permitted. The study requires that each involved surgeon follow the Memorial Sloan Kettering Cancer Center algorithm [16]. The lack of coordination of the methodology, as the choice of surgical approach and tracer, is a weakness. Especially when the number of included patients is distributed at many different centres and thereby in relatively low volumes at each centre. On the other hand, positive results from a study of this size and conducted at a broad array of centres, all using the same algorithm, will point towards the results being generalizable. Even more so for a more specifically defined algorithm than the Memorial Sloan Kettering Cancer Center algorithm.

At the 2025 ASCO (American Society of Clinical Oncology) Annual Meeting II, an abstract of the PHENIX trial by Tu et al. was presented [138]. The study includes women with cervical cancer FIGO 2009 stages IA1 with LVSI, IA2, IB1, or IIA1.

Patients were assigned perioperatively to PHENIX-I (SLN-negative) and PHENIX-II (SLN-positive) and then randomized to SLN-only or PLND in 1:1 ratio. In patients with unilateral detection, a side-specific PLND was performed. All women had radical hysterectomies performed. Of the 908 included women, 838 were SLN-negative (PHENIX-I) and 70 were SLN-positive (PHENIX II). The bilateral mapping rate was 82.6%. The randomization process assigned 455 women to the SLN-only cohort (PHENIX-I n=420, PHENIX-II n=35), while 453 women had a PLND performed (PHENIX-I n=418, PHENIX-II n=35). After a median follow-up of 52 months (1-104), recurrences were observed among the PHENIX-I patients in 16 women in the SLN-only group, and in 26 patients in the PLND group. Disease-free survival at three years was 96,8% and 94.5% in the respective groups (HR 0.61, 95% CI 0.33-1.14, p=0.12). The three-year cancer-specific survival rates were 100% for the SLN-only group and 97.8% for the PLND group (HR 0.21, 95% CI 0.06-0.74, p=.007). The authors observed similar trends in the PHENIX-II cohort. In conclusion, the authors suggest that PLND should be abandoned in cervical cancer as it does not demonstrate any survival benefits as compared to SLN-only procedures. Unexpectedly, they found an elevated risk of nodal recurrence and cancer-specific mortality in the PLND patients. Furthermore, they describe significantly shorter surgical times (p<.0001), less blood loss (p<.001), and less morbidity related to surgery (p<.001) in SLN-only surgical procedures as compared to PLND. The results are interesting, as they demonstrate non-inferiority regarding disease-free survival. However, the results are only presented in a congress abstract, and they raise some questions, the most obvious one being why the PLND procedure seems to be outperformed by the SLN-only approach, as both cohorts went through an SLN procedure before being randomized into the SLN-only and PLND groups. Surgical methodology, choice of tracer, and surgical algorithm also remain unclear from the abstract alone. Until the full study is presented in an article and available for scrutiny, a cautious approach to the results is necessary.

The strengths of this thesis are that all underlying studies are prospective studies concentrated to one surgical centre with highly experienced surgeons. This ensures high internal validity, as all involved surgeons adhered meticulously to the SLN algorithm, based on lymphatic anatomy and clear definitions of SLNs. Even though advanced surgery should be conducted by highly skilled surgeons at centres with adequate case-loads, a weakness of the studies are that the results can be difficult to reproduce. Another weakness, concerning the current attitude on MIS in cervical cancer, as a result of the LACC trial, the presented algorithm is not primarily suited for open surgery. Docking the robot to an open wound is, however, possible when performing the SLN staging.

Finally, and importantly, for the sake of women with cervical cancer, adhering to the “ideal SLN development idea” outlined in this thesis, there will be a need for continuous educational efforts and site-based internal control of results.

Conclusions

- Combining ICG with a radiotracer does not increase the bilateral SLN detection rates, compared to using ICG alone. Using ICG only in an SLN algorithm is a feasible alternative in a robotic surgical approach in women with early-stage cervical cancer.
- The PULT can hold isolated metastatic LNs.
- The PULT should be removed and treated as SLN tissue in any SLN algorithm.
- The separate dissection and removal of the PULT does not impose any risk of harming the autonomous nerves, and there is a negligible risk of ureter damage. The procedure adds minimal time and has no known complications associated with it.
- Removing the PULT does not stand in contrast to performing less radical surgery.
- There are no significant differences between cervical and endometrial cancer regarding the anatomical distribution of SLNs and metastatic SLNs. The typical positions of SLNs along the UPP are the external iliac (interiliac) and proximal obturator fossa areas.
- Typical positions along the LPP cannot be defined due to cross-over lymphatics and longitudinal variations of positions of the SLNs.
- Results from SLN studies on women with cervical and endometrial cancer can be merged to achieve adequately powered materials on sensitivity and NPV in women with cervical cancer.
- The present SLN algorithm identified all women with LNMs, with no false negatives. The algorithm's sensitivity and NPV are both 100%.
- Forty-six percent of all positive SLNs were located in the obturator fossa, emphasizing the importance of meticulously exploring the anatomic position in an SLN procedure. An algorithm allowing for retrieving only one SLN per pelvic wall is insufficient and increase the risk of false-negative nodes.
- The described SLN algorithm, based on the uterine lymphatic anatomy, performed by experienced surgeons, can accurately identify pelvic nodal metastases in early-stage cervical cancer. Further studies on sensitivity are not necessary; rather, the algorithm should be implemented and used for benchmarking and for internal quality control. Studies regarding oncological outcome should proceed.

Future aspects

The next step, according to the previously described process, should be to educate and implement the SLN algorithm on a nationwide basis, and closely and continuously monitor surgical outcomes to support the implementation process adequately. Parallel to the clinical implementation, nationwide studies on oncological outcomes, specifically regarding OS and DFS, should be initiated.

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