# Implications and results of a further developed sentinel lymph node algorithm in endometrial cancer

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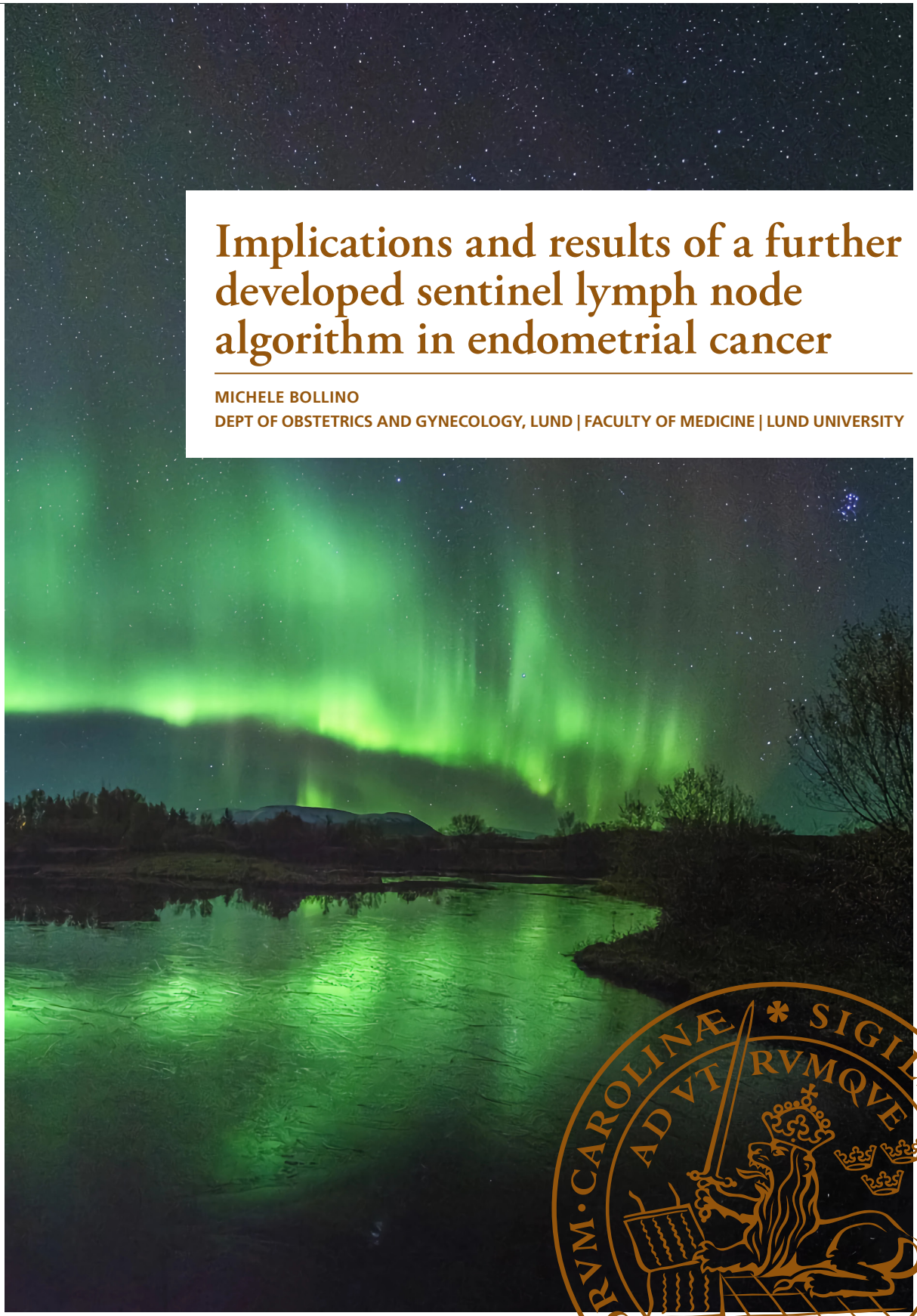

# Implications and results of a further developed sentinel lymph node algorithm in endometrial cancer

Michele Bollino

LUND
UNIVERSITY

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 December 20th at 09.00 AM in the Conference Hall, Department of Obstetrics and Gynecology, Klinikgatan 12, Lund, Sweden.

*Faculty opponent*
Professor Francesco Fanfani
Rome, Italy

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**Implications and results of a further developed sentinel lymph node algorithm in endometrial cancer**


The overall aim was to evaluate and refine a strict anatomically based surgical sentinel node algorithm (SLN) for detection of metastatic pelvic disease in women with uterine confined endometrial cancer (EC).

*Study I:* To evaluate the sensitivity of the SLN algorithm for detecting pelvic lymph node metastases (LNMs) in women with high-risk endometrial cancer (HREC). The SLN-ICG algorithm achieved a sensitivity of 98% and a bilateral mapping rate of 95%.

*Study II:* Investigating the locations of metastatic pelvic SLNs along the upper paracervical lymphatic pathway. 95.7% of women with positive pelvic nodes had at least one metastatic SLN located at a typical position.

*Study III:* Investigating the prevalence and size of pelvic LNMs and their association with risk factors in a cohort of 1045 women with EC. LNM were detected in 1/10 women with presumed low-grade endometrioid uterine stage IA cancer compared to 5/10 of women with high-grade EC or non-endometroid stage IB cancer.

*Study IV:* investigating the incidence of non-mapped isolated pelvic LNMs at pre-defined typical anatomical positions. 4.3% of node positive women had isolated metastases in a “SLN anatomy”.

*Conclusions:* The initially found high sensitivity of the SLN algorithm was further increased by approximately 5% by adding removal of non-mapped nodes at typical positions despite ICG-mapping at other positions (the Hybrid algorithm). In case of complete non-mapping, a side specific lymphadenectomy can be replaced by a selective removal of nodes at those typical positions further reducing the risk of lymphedema while retaining knowledge on nodal status. The high rate of pelvic nodal metastases in presumed low risk EC strongly indicates that detection of SLNs using the proposed SLN-algorithm should be offered to all women with EC.



**Key words:** endometrial cancer, sentinel lymph node algorithm, pelvic metastatic lymph nodes, side specific pelvic lymphadenectomy, typical positions of sentinel lymph nodes, metastasis size risk groups, hybrid algorithm

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*To Maddalena, Maria Elena and Marta.*

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# Thesis at a glance

| Study | Aim | Results | Conclusion |
|---|---|---|---|
| *Study I:* Pelvic sentinel lymph node detection in High-Risk Endometrial Cancer (SHREC-trial), the final step towards a paradigm shift in surgical staging. | To prospectively assess the diagnostic accuracy of a pelvic sentinel lymph node (SLN) algorithm in high-risk endometrial cancer (HREC). | Two hundred fifty-seven women were analysed; 54 had pelvic lymph node metastases (LNMs), and 52 of those were correctly identified by the SLN-ICG algorithm.The SLN-ICG algorithm had a sensitivity of 98% (95% confidence interval [CI] 89-100) and a negative predictive value of 99.5% (95% CI 97-100). The sensitivity of the overall SLN algorithm was 100% (95% CI 92-100) and the negative predictive value was 100% (95% CI 98-100). The bilateral mapping rate was 95%. Two women (1%) had isolated para-aortic metastases. | With a complete sensitivity to detect pelvic LNMs, the described pelvic SLN algorithm can, in the hands of experienced surgeons, exclude overall nodal involvement in 99% and thereby safely replace a full lymphadenectomy in HREC. |
| *Study II:* A selective anatomically based lymph node sampling can replace a side specific pelvic lymphadenectomy in endometrial cancer with failed sentinel node mapping | To evaluate the locations of metastatic pelvic SLNs and the proportion of SLNs outside and within defined typical anatomical positions along the upper paracervical lymphatic pathway (UPP). | A median of two (range 1–12) SLN metastases along the UPP including the parauterine lymphatic tissue (PULT) were found in 162 women. 41 of 162 women (25.3 %) had isolated metastases in the obturator fossa harboring 49.1 % of all SLN metastases. Three women (1.9 %) had isolated PULT metastases. SLN metastases outside typical positions were identified in 28/162 women (17.3 %); isolated metastases were seen in seven women (4.3 %), so 95.7 % of pelvic node positive women had at least one metastatic SLN located at a typical position. | A selective removal of lymph nodes at typical proximal obturator and interiliac positions and the PULT can replace a full side specific pelvic LND when SLN mapping unsuccessful. The obturator fossa is the predominant location for metastatic disease. |

| Study | Aim | Results | Conclusion |
|---|---|---|---|
| *Study III*: Prevalence and size of pelvic sentinel lymph node metastases in endometrial cancer | To assess the association of prevalence and size of pelvic SLN metastases with risk factors in endometrial cancer. | SLN-metastases were present in 174/1045 (16.6 %) women; in 9.8 % of preoperatively presumed low-grade endometroid uterine stage IA (6.4 % of low-grade stage IA at final histology) and in 58.3 % and 47.8 % respectively in women with high-grade endometroid and nonendometroid uterine stage IB cancer. In low-grade endometrial cancer 45/95 (47.4 %) had only isolated tumor cells (ITC) in SLNs compared with 15/78 (19.2 %) in high-grade or non-endometroid cancer ($p < .0001$) | This large population-based study, applying a consequent SLN-algorithm over time, provides important detailed information on the risk for, and size of, SLN metastases within risk groups of endometrial cancer. The 9.8 % risk for metastases in women with presumed low grade uterine stage IA endometrioid endometrial cancer motivates detection of SLNs within this subgroup. The proportion of ITCs in SLNs was significantly lower in higher risk histologies. |
| *Study IV:* Optimizing the sensitivity of a pelvic sentinel node algorithm requires a hybrid algorithm combining Indocyanine Green based mapping and the removal of non-mapped nodes at defined anatomic positions. | To investigate the incidence of non-mapped isolated metastatic pelvic lymph nodes at pre-defined anatomical positions | Unilateral non-mapping of either the obturator or interiliac area occurred in 180 of the 620 women (29%). 114 women (18.4%) were node positive and five of these women (4.3%) had isolated metastases in a “SLN anatomy” suggesting a similar lower sensitivity of the ICG-only algorithm | In an optimized SLN algorithm for endometrial cancer, to avoid undetected nodal metastases in 4.3% of node positive women, if mapping fails in either the proximal obturator or interiliac area, nodes should be removed from those defined anatomic positions, despite mapping at other positions. |

# List of scientific papers

This thesis is based on following original studies, referred to in the text by their Roman numerals. The studies are appended at the end of the thesis. All studies are available as Open access.

I. Persson J, Salehi S, Bollino M, Lönnerfors C, Falconer H, Geppert B. Pelvic Sentinel lymph node detection in High-Risk Endometrial Cancer (SHREC-trial) the final step towards a paradigm shift in surgical staging. Eur J Cancer. 2019 Jul; 116:77–85. doi: 10.1016/j.ejca.2019 04 025. Epub 2019 jun 7. PMID: 31 181 536

II. Bollino M, Geppert B, Lönnerfors C, Persson J. A selective anatomically based lymph node sampling can replace a side specific pelvic lymphadenectomy in endometrial cancer with failed sentinel node mapping. Eur J Cancer. 2024 Jun; 204:114049. doi: 10.1016/j.ejca.2024.114049. Epub 2024 Apr 12. PMID: 38657525

III. Bollino M, Geppert B, Lönnerfors C, Måsbäck A, Kasselaki I, Persson J. Prevalence and size of pelvic sentinel lymph node metastases in endometrial cancer. Eur J Cancer. 2024 Sep; 209:114265. doi: 10.1016/j.ejca.2024.114265. Epub 2024 Aug 7. PMID: 39142212

IV. Bollino M, Geppert B, Reynisson P, Lönnerfors C, Persson J. Optimizing sensitivity of a pelvic sentinel node algorithm re-quires a hybrid algorithm combining ICG based mapping and removal of non-mapped nodes at defined anatomic positions. Cancers (Basel). 2024 Sep 23;16(18):3242. doi: 10.3390/cancers16183242. PMID: 39335213

*List of publications not included in the thesis:*

Publications outside the thesis (not for evaluation)

I. Geppert B, Lönnerfors C, Bollino M, Arechvo A, Persson J. A study on uterine lymphatic anatomy for standardization of pelvic sentinel lymph node detection in endometrial cancer. Gynecol Oncol. 2017 May;145(2):256-261. doi: 10.1016/j.ygyno.2017.02.018. Epub 2017 Feb 10. PMID: 28196672

II. Persson J, Geppert B, Lönnerfors C, Bollino M, Måsbäck A. Description of a reproducible anatomically based surgical algorithm for detection of pelvic sentinel lymph nodes in endometrial cancer. Gynecol Oncol. 2017

Oct;147(1):120-125. doi: 10.1016/j.ygyno.2017.07.131. Epub 2017 Jul 24. PMID: 28751118

III. Geppert B, Lönnerfors C, Bollino M, Persson J. Sentinel lymph node biopsy in endometrial cancer-Feasibility, safety and lymphatic complications. Gynecol Oncol. 2018 Mar;148(3):491-498. doi: 10.1016/j.ygyno.2017.12.017. Epub 2017 Dec 20. PMID: 29273307

IV. Bollino M, Geppert B, Lönnerfors C, Falconer H, Salehi S, Persson J. Pelvic sentinel lymph node biopsy in endometrial cancer-a simplified algorithm based on histology and lymphatic anatomy. Int J Gynecol Cancer. 2020 Mar;30(3):339-345. doi: 10.1136/ijgc-2019-000935. Epub 2020 Feb 18. PMID: 32075897

V. 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. Gynecol Oncol. 2022 Jun;165(3):466-471. doi: 10.1016/j.ygyno.2022.03.027. Epub 2022 Apr 16. PMID: 35437170

***Author's contribution to the papers***

In 2016, I joined the research group led by my supervisor Prof. Jan Persson. In November 2017, I was registered as a PhD student at Lund University. Papers I-IV involved contributions from several researchers, with me being the main responsible for Papers II-IV. My contributions in Papers I-IV, using CRediT author statement, are listed below:

*Paper I:* Data curation, Formal analysis, Investigation, Validation, Visualization, Writing the original draft, Reviewing and Editing the manuscript.

*Paper II:* Conceptualization, Methodology, Formal analysis, Investigation, Validation, Data Curation, Writing - Original Draft, Project administration, Visualization, Writing - Review and Editing the manuscript, Funding acquisition.

*Paper III:* Conceptualization, Methodology, Formal analysis, Investigation, Validation, Data Curation, Writing - Original Draft, Writing - Review and Editing the manuscript, Project administration, Funding acquisition.

*Paper IV:* Conceptualization, Methodology, Formal analysis, Investigation, Validation, Data Curation, Writing - Original Draft, Writing - Review and Editing the manuscript, Project administration, Funding acquisition.

# Populärvetenskaplig sammanfattning

Livmodercancer, den vanligaste gynekologiska cancerformen i västvärlden, drabbar årligen 1400 kvinnor i Sverige. Prognosen är överlag god och borttagande av livmoder och äggstockar är oftast botande då vaginala blödningar tidigt i förloppet leder till diagnos innan tumören blivit spridd. Vid spridning sker detta först till lymfkörtlar i bäckenet vilket oupptäckt leder till återfall. Risken för spridning till lymfkörtlarna beror på celltyp och hur djupt tumören, som startar i livmoderns inre slemhinna, (endometriet) hunnit växa in i livmoderns muskelvägg. Tidigare har spridning till lymfkörtlarna (körtelmetastaser) bara kunnat diagnosticeras genom mikroskopisk undersökning av ett stort antal borttagna lymfkörtlar. Då omfattande lymfkörtelkirurgi inte sällan leder till bestående lymfsvullnad i benen har denna kirurgi tidigare reserverats för kvinnor med större risk för lymfkörtelspridning. En genväg till information om körtelspridning, där endast de körtlar som har störst risk för metastaser tas bort (portvaktskörtlar, Sentinel nodes, SLNs) vore därför önskvärd så att körtelmetastasering även kan identifieras hos kvinnor med en lägre risk. Utvärdering av SLN-tekniker som både har en hög känslighet att hitta sjuka lymfkörtlar (sensitivitet), och som fungerar tekniskt i en tillräckligt stor omfattning har pågått internationellt i flera år. Ingen teknik, som innefattar insprutning av ett spårämne som tas upp i lymfsystemet i livmodern och sen sprids till lymfkörtlarna, hade vid detta avhandlingsarbetes start visat sig uppfylla båda dessa kriterier tillräcklig bra. I en tidigare avhandling i Lund har samma forskargrupp studerat lymfavflödets anatomi vilket legat till grund för en ny, mera exakt definition av vilka körtlar som är SLNs, liksom legat till grund för en ny detaljerad beskrivning hur kirurgin skall utföras för att säkrast hitta dessa körtlar. Ett vid tidpunkten nytt spårämne, Indocyanin grönt (ICG), har använts och för att öka lyckandegraden (hur ofta spårämnet återfinns i lymfkörtlarna) infördes en andra insprutning om den första inte gav önskat resultat.

Den första studien i avhandlingen (SHREC studien) beräknades för att statistisk bevisa en tillräckligt hög känslighet att hitta körtelmetastaser. Studien utfördes på de kvinnor där alla lymfkörtlar enligt dåvarande vårdprogram skulle tas bort. På så sätt ökades inte risken för lymfkomplikationer och kvinnorna kunde vara sina egna kontroller för SLN-konceptets känslighet att hitta körtelmetastaser. Studien, som efter publikation 2019 ledde till en ändring av vårdprogrammet i Sverige så att detektion av SLNs rekommenderades till alla kvinnor med livmodercancer, ett paradigmskifte, redovisade den högsta detektionsgraden (95%) och känsligheten (98%, med statistiskt intervall 89-100%) som publicerats till dags dato.

Hos en andel kvinnor (i andra studier kring 75%) tas inte ICG upp i lymfsystemet. I denna situation rekommenderas internationellt att alla lymfkörtlar tas bort, ett problem

i synnerhet hos kvinnor med lägre risk för körtelmetastaser där rekommendationen tidigare varit att avstå från lymfkörteldiagnostik.

I en parallell andra grund-studie, där endast SLNs tagits bort, först på lågriskkvinnor, efter ändring i vårdprogrammet, på alla kvinnor med livmodercancer, angav kirurgen det anatomiska läget av SLNs på en anatomikarta. På så sätt kunde vi definiera typiska positioner för SLNs och visa att risken för isolerade körtelmetastaser utanför dessa positioner var så låg att en full körtelutrymning kunde ersättas av borttagande av dessa" typiska belägna" körtlar, ett stort steg framåt för att minimera risk för lymfsvullnad.

Ett intressant, och samtidigt problematiskt,"bi-fynd" i SHREC-studien, vilket styrkts av vidare insamlat material, var att kvinnor med metastaser i lymfkörtlarna uppvisade ICG upptag i lägre omfattning vilket samvarierade med ett lägre upptag i den anatomiska position som hade högst risk för metastaser. Det väckte tanken att känsligheten att hitta sjuka lymfkörtlar kunde ökas genom en kombination av ICG upptag och borttagande av körtlar på typiska högriskpositioner även om de inte uppvisade ICG-upptag, en" Hybrid-algoritm". Teorin visade sig stämma då 5% kvinnor med körtelmetastaser missats, utan" Hybridalgoritmen" (studie IV). Resultaten styrker dessutom slutsatsen i studie II.
Slutligen kunde vi, i den största befolkningsbaserade prospektiva studien hittills på väl över 1000 kvinnor, (studie III) visa att risken för körtelmetastaser (i form a metastaser i SLNs) är större än tidigare visats, i synnerhet hos kvinnor med lågrisk EC där körteldiagnostik tidigare, och i många länder fortfarande, inte anses nödvändig. Detta styrker ytterligare indikationen att använda SLN-metoden på alla kvinnor med livmodercancer.

Sammanfattningsvis visar avhandlingen vikten av att använda en distinkt anatomiskt baserad SLN-algoritm för att uppnå högt ICG-upptag och hög känslighet, att algoritmen ytterligare har kunnat förfinas för att minimera risk för körtelkomplikationer. Vidare, från en redan hög nivå, att en ännu högre känslighet att identifiera körtelmetastaser kan uppnås. Avhandlingen presenterar dessutom tillförlitliga data på risken för körtelmetastasering för alla celltyper som förekommer vid livmodercancer uppdelat på olika omfattning av tumörväxt i livmodern.

Forskning leder alltid till nya frågeställningar vara några exempel nedan:

-Studie III visade att andelen kvinnor med små eller mycket små områden med tumörceller i lymfkörtlar var relativt stor och skiljde sig mellan olika celltyper. Diagnos av dessa små tumörområden kräver en detaljerad patologisk undersökning, resursmässigt möjligt endast på ett litet antal lymfkörtlar (SLNs). Denna grupp kvinnor har med tidigare diagnostik på alla lymfkörtlar inte identifierats. Det

prognostiska värdet av små metastaser, och behovet av onkologiskbehandling, är därför okänt.

- livmodercancer uppvisar olika biologiska markörer som nyligen börjat analyseras rutinmässigt. Markörerna kan både vara kopplade till en ökad eller minskad risk för återfall i sjukdomen. Dessa markörers samvariation med körtelmetastasering, den hittills starkaste riskfaktorn för återfall, är otillräckligt studerat.

- Tumören kan vid patologens mikroskopiska undersökning bedömas växa in i lymfkärl eller kapillärer (LVSI) vilket anses öka risken för återfall. Denna patologi-bedömning är inte exakt och vidare studier av LVSI som isolerad riskfaktor kräver en säker diagnostik av körtelmetastasering.

# Riassunto di divulgazione scientifica

Il carcinoma dell'endometrio colpisce ogni anno 1400 donne in Svezia. La prognosi è generalmente buona. I linfonodi pelvici sono tra le prime stazioni di diffusione. Il rischio di diffusione ai linfonodi dipende da diversi fattori tra cui l'istotipo e l'infiltrazione miometriale. In passato, la stadiazione chirurgica richiedeva che le donne fossero sotopposte a linfoadenectomia, un intervento chirurgico non privo di complicanze. Per tale motivo la linfoadenectomia è stata riservata solo alle donne a maggior rischio di metastasi linfonodali. Il linfonodo sentinella (SLN) si presenta come alternativa alla linfoadenectomia permettendo una stadiazione chirurgica ed evitando tutte le complicanze associate alla linfoadenectomia.

Il primo studio della tesi (SHREC tiral) ha riportato una la sensibilità del 98%, con intervallo di confidenza del 89-100% nell'individuare le metastasi linfonodali. Dopo la pubblicazione del I studio, le linee guida svedesi hanno raccomandato il SLN in tutte le donne con carcinoma dell'endometrio. In alucni casi l'ICG non viene assorbito dal sistema linfatico. Di consegenza non è sempre possibile osservare i SLN. In questi casi, le linee guida internazionali raccomandano la linfoadenectomia mono o bilateramente. Questo potrebbe essere un controsenso soprattutto nelle donne a basso rischio di metastasi linfonodali; donne in cui fino a qualche anno fa era raccomandata solo l'asportazione dell'utero.

In un secondo studio parallelo indicando la posizone anatomica di tutti i SLN è stato possibile studiare quali sono le posizioni tipiche in cui è possibile rilevare i SLN metastatici più frequentemente. Il rischio di metastasi linfonodali isolate al di fuori delle posizioni tipiche era così basso che una linfoadenectomia mono o bilaterale pelvica poteva essere sostituita dall'asportazione dei linfonodi in "sedi tipiche". Questo è stato un ulteriore passo avanti per ridurre al minimo il rischio di complicanze linfatiche.

Un interessante e allo stesso tempo problematico "risultato secondario" dello studio SHREC è stato che le donne con metastasi linfonodali mostravano una minore captazione di ICG. Ciò ha fatto nascere l'idea che la possibilià di trovare linfonodi metastatici potesse essere aumentata usando un "algoritmo ibrido" che prevedeva l'asportazione di SLN-ICG positivi in combinazione con l' asportazione di linfonodi in "posizioni tipiche" anche se ICG negativi. La teoria si è dimostrata corretta, in quanto il 5% delle donne con metastasi linfonodali non sarebbere stato individuato senza l'"algoritmo ibrido" (studio 4). I risultati supportano anche le conclusioni dello studio II. Infine nello studio III si è visto che il rischio di metastasi nei SLN è maggiore di quanto precedentemente dimostrato, in particolare nelle donne con EC a basso rischio. In conclusione, la tesi sottolinea l'importanza di utilizzare un algoritmo anatomico per

ottenere un'elevata detection dei SLN; che l'algoritmo può essere ulteriormente perfezionato per ridurre al minimo il rischio di complicazioni linfatiche e che il SLN dovrebbe essere proposto a tutte le donne con tumore dell'endometrio, indipendentemente dall' istotipo.

# Abstract

*Background*

Surgery is the primary therapeutic approach in early-stage endometrial cancer (EC). Lymph node status serve as a prognostic factor and guides adjuvant treatment. Historically, the evaluation of lymph nodes entailed a complete pelvic and paraaortic lymphadenectomy. However, the associated risk of lymphatic complications limited lymph node assessment to women with presumed high-risk tumors, even though preoperative risk group allocation errors occur in approximately 20% of patients. Sentinel node (SLN) detection aims to minimize surgical morbidity while retaining the knowledge of lymph node status.

The overall aims of this thesis were to evaluate an anatomically based surgical algorithm for detection of metastatic pelvic SLNs irrespective of preoperative allocated risk group in women with presumed uterine confined EC and to investigate location, prevalence and size of metastatic SLNs related to risk factors. Further, whether removal of nodes from designated locations is representative of nodal status in case of non-mapping.

*Aims of the studies*

*Study I:* To evaluate the diagnostic accuracy of a surgically and anatomically defined SLN-ICG algorithm, as well as the overall SLN algorithm, for detecting pelvic lymph node metastases (LNMs) in women with high-risk endometrial cancer (HREC) when performed by selected high-volume robotic surgeons.

*Study II:* To investigate the overall locations of metastatic pelvic sentinel lymph nodes (SLNs) and the proportions of isolated metastatic SLNs located inside and outside the defined typical anatomical positions along the upper paracervical lymphatic pathway (UPP), to evaluate whether selective sampling could be a safe alternative to side-specific lymph node dissection (LND) in cases of non-mapping.

*Study III:* To provide prospective data from a large population-based cohort of consecutive women with endometrial cancer (EC), focusing on the prevalence and size of pelvic LNM and their association with known risk factors, such as the depth of myometrial invasion and histological subtypes.

*Study IV:* To investigate the incidence of non-mapped isolated pelvic LNMs at pre-defined anatomical positions despite ICG-mapping at other positions.

*Material and methods*

An anatomically based SLN-algorithm including cervical injection of indocyanine green (ICG), exploring parallel pelvic lymphatic pathways, allowing for reinjection in case of non-mapping and including a separate removal of the parauterine lymphovascular tissue (PULT) was adhered to in all the following studies. All women underwent robotic surgery using the da Vinci Surgical system.

*Study I:* From June 2014 to May 2018, 257 women with presumed FIGO stage I-II high-risk EC (HREC) underwent robotic surgical staging at two academic centres by five accredited surgeons. Removal of SLNs was followed by a pelvic and infrarenal para-aortic lymphadenectomy.

*Study II:* SLN metastases along the UPP (including the parauterine lymphovascular tissue, PULT) were identified in 162 women with EC of all risk groups. The proximal third of the obturator fossa and the interiliac area were classified as typical positions for SLNs. The proportions of metastatic SLNs, both overall and isolated, in typical and atypical locations, were investigated.

*Study III:* 1101 consecutive women with uterine-confined EC or EIN who underwent robotic SLN detection between June 2014 and January 2024 were included. The prevalence and size of SLN metastases were evaluated in relation to pre- and postoperative histologic types and estimates of myometrial invasion.

*Study IV:* Between June 2019 and January 2024, 620 consecutive women with uterine-confined EC undergoing a refined SLN procedure were included. In cases where ICG mapping did not occur, nodes corresponding to the “typical positions” in the obturator and interiliac areas, were removed and classified as "SLN-anatomy and the incidence of isolated LNMs in “SLNs-anatomy” was evaluated.

*Results*

*Study I:* The SLN-ICG algorithm correctly identified 52 of 54 women with LNMs. In two women, one with false-negative ICG-SLNs and one where non-mapping occurred, pelvic LNMs were detected by the overall SLN algorithm including macroscopically cancer-suspect nodes. The SLN-ICG algorithm demonstrated a sensitivity of 98% (95% confidence interval [CI]: 89–100) and a negative predictive value of 99.5% (95% CI: 97–100). The overall SLN algorithm achieved a sensitivity of 100% (95% CI: 92–100) and a negative predictive value of 100% (95% CI: 98–100). The bilateral mapping following reinjection rate was 95%.

*Study II:* 95.7% of women with positive pelvic nodes had at least one metastatic SLN located at a typical position. Isolated LNMs in the obturator fossa were detected in 25.3% accounting for 49.1% of all SLN metastases. Isolated PULT metastases were

observed in three women (1.9%). SLN metastases located outside typical positions were detected in 28 women (17.3%); seven of which (4.3%) had isolated metastases

*Study III:* SLN metastases were found in 174 of 1045 women (16.6%) who had EC at final histology. 9.8% of women with preoperatively presumed low-grade endometrioid uterine stage IA cancer, 58.3% of women with high-grade endometrioid cancer and 47.8% of women with non-endometrioid uterine stage IB cancer had SLN metastases. In women with low-grade EC 47.4% of women with SLN metastases had only isolated tumor cells (ITC) in their SLNs, compared with 19.2% of women with high-grade or non-endometrioid cancer ($p< .0001$).

*Study IV:* A non-mapping of either the obturator or interiliac area occurred in 180/620 (29%). In all, 114 women (18.4%) were node positive; five of which (4.3%) had isolated metastases in a "SLN anatomy". Hence, the ICG- based sensitivity can be further increase in the range of 5% by a hybrid algorithm adding non-mapped nodes at typical high-risk positions.

In *study I*, the SLN algorithm detected all pelvic node positive patients in women with high-risk EC. In all women with EC regardless of risk group allocation, pelvic SLNs were typically located in the interiliac area and the obturator fossa, the latter being the predominant location for metastatic disease. The incidence and size of lymph node metastases were associated with the risk factors depth invasion and high-risk histologies where LNMs were five times more common in women with high-grade endometrioid cancer or non-endometroid uterine stage IB cancer compared to women with low-grade uterine stage IA cancer, although the latter occurred in 9.8%. In case of non-mapping at a typical position, select removal of corresponding nodes in the obturator and interiliac area and the PULT should be performed to avoid undetected nodal metastases.

*Conclusions*

SLN detection using the proposed algorithm should be offered to all women with a uterine confined EC regardless of preoperative risk group allocation. SLN metastases are located at typical positions in 95.7% of women. In case of non-mapping, nodes at the typical positions including the PULT should be removed to avoid a full side specific pelvic LND while retaining knowledge on nodal status. In case of a partial side-specific non-mapping, removal of non-mapped node at those typical positions (the Hybrid algorithm) can, despite an initial high level and a high mapping rate, further increase sensitivity.

# Abbreviations

AJCC = American Joint Committee on Cancer
CSI = Cervical stroma invasion
dMMR = Mismatch repair deficient
EC = Endometrial cancer
ESGO = European society of gynecologic oncology
ESMO = European society of medical oncology
ESTRO = European society of therapeutic radiotherapy and oncology
FIGO = International federation of gynaecology and obstetrics
GOG = Gynecologic Oncology Group
HREC= High-risk endometrial cancer
ICG = Indocyanine green
IHC = Immunohistochemistry
IMA = Inferior mesenteric artery
IPP = Infundibolopelvic ligament
IRPALND = Infrarenal paraaortic lymphadenectomy
ITC = Isolated tumour cells
LACE = Survival effect of paraaortic lymphadenectomy in endometrial cancer
LAP2 = Laparoscopic approach to carcinoma of the endometrium
LND = Lymphadenectomy
LNM = Lymph node metastases
LPP = Lower paracervical pathway
LVSI = Lymphovascular space invasion
MAM = Macrometastasis
MI = Myometrial Invasion
MIM = Micro metastases
MIS = Minimally invasive Surgery
MSI = Microsatellite instability
p53 abn = p53 abnormal
PALND = Paraaortic lymph node dissection/lymphadenectomy
PLND = Pelvic lymph node dissection/lymphadenectomy
POLE = DNA polymerase epsilon gene
PORTEC = Post-operative radio therapy in endometrial cancer
PULT = Parauterine lymphovascular tissue
SEPAL = Survival effect of paraaortic lymphadenectomy in endometrial cancer
SLN = Sentinel lymph node
SLNB = Sentinel lymph node biopsy
UPP = Upper paracervical pathway

# 1 Introduction

## 1.1 Background

### 1.1.1 Epidemiology of endometrial cancer

Endometrial cancer (EC) is the most prevalent gynecologic malignancy in developed countries, with an estimated 125000 new cases diagnosed in Europe in 2022 [1]. In Sweden, EC ranks as the sixth most common cancer, with approximately 1,400 new cases reported annually corresponding to an annual incidence rate of 30.2 per 100000 women [2, 3].

Several risk factors contribute to the development of EC, including obesity, unopposed estrogen exposure, diabetes mellitus, nulliparity, late menopause and advanced age [4-13]. The median age at diagnosis in Sweden is 69 years. Additionally, Lynch syndrome, characterized by mutations in DNA mismatch repair genes, elevates the risk, with affected individuals having a 42-60% risk of developing EC by the age of 70 [14-16].

The use of hormonal contraceptives such as combined oral contraceptives for at least five years or a progesterone-releasing intrauterine device has been linked to a decreased risk of EC [11, 17-19].

### 1.1.2 Symptoms and diagnosis

The primary symptom of EC is postmenopausal vaginal bleeding, which often leads to early detection of localized disease [20]. Additional symptoms may include menorrhagia, hematometra, pyometra, or abnormal discharge [21]. Diagnosis involves gynecological examination and transvaginal ultrasonography. In postmenopausal women where the endometrium is either unmeasurable or measures ≥5 mm, an endometrial biopsy is recommended unless hydrosonography indicates a polypoid lesion indicating hysteroscopy [22-24].

### 1.1.3 Traditional histologic classification

According to the 5th edition of WHO Classification of tumors; Female Genital Tumors, EC include several epithelial subtypes such as low grade (grade 1 and 2) or high grade (grade 3) endometrioid carcinoma, serous carcinoma, clear cell carcinoma, mixed carcinoma, undifferentiated carcinoma, carcinosarcoma, and other unusual types, such as mesonephric-like and gastrointestinal mucinous type carcinomas [25].

Bokhman's study from 1983 classified EC into Type I and Type II where the former include low-grade endometrioid tumors with positive hormone receptor expression associated with obesity and a favourable prognosis, and the latter non-endometrioid tumors or high-grade tumors characterized by hormone receptor loss with a poorer prognosis [26, 27].

### 1.1.4 Molecular classification and recent development

The need for more sophisticated classifications that integrate clinical, pathological, and molecular data has been emphasized by recent research studies and genomic analyses of EC [21, 28-30]. In a publication from 2013, recent genomic analyses by the Cancer Genome Atlas (TCGA) Research Network identified four distinct genomic subgroups; POLE ultra-mutated, microsatellite instability hypermutated, copy number low and copy number high, each with distinct prognostic implications [30].

More easily assessable molecular biomarkers have been developed as a result of translating these genomic abnormalities. These four molecular subgroups of EC have different clinical and prognostic outcomes (Table1).

POLE-mutated tumors are associated with a good prognosis, Mismatch Repair Deficient/ Microsatellite Instable (dMMR/MSI) and No Specific Molecular Profile (NSMP/p53wt mutations) indicate an intermediate prognosis, and p53 abnormalities correlate with a poorer prognosis. Among these, endometrioid adenocarcinomas constitute approximately 75% of cases, serous carcinomas account for 5-11% and clear cell carcinomas represent 1-5% of all uterine tumors [27, 29].

**Table 1**. Characteristics of Molecular and Clinicopathological Features in EC Subtypes.

| | **POLEmut (i.e. POLE EDM)** | **dMMR (i.e. MSI)** | **NSMP (i.e. p53-wt)** | **p53aberrant (i.e. p53-abn, p53-mut)** |
|---|---|---|---|---|
| Prevalence, % | 5-15 | 25-30 | 30-40 | 5-15 |
| Most frequently associated histological features | Endometrioid, often high grade | Endometrioid, often high grade | Mostly low grade<br>Squamous differentiation<br>ER/PgR diffuse | All histological subtypes<br>Mostly high grade |
| Associated clinical features | Lower BMI<br>Early stage | Higher BMI<br>Lynch syndrome | Higher BMI | Lower BMI<br>Advanced stage |
| Prognosis | Excellent | Intermediate | Intermediate | Poor |
| Diagnostic test | Genetic panel targeted hotspot analysis | Genetic panel MMR-IHC | No specific molecular profile | Genetic panel p53-IHC |

BMI, body mass index; dMMR, mismatch repair deficient; ER, estrogen receptor; EDM, exonuclease domain mutation; IHC, immunohistochemistry; MMR-IHC, mismatch repair immunohistochemistry; MSI, microsatellite instability; NSMP, no specific molecular profile; p53-abn, p53-abnormal; p53-mut, p53-mutant; p53-wt, p53-wild type; ER, estroge receptor; PgR, progesterone receptor; POLE, polymerase epsilon; POLEmut, polymerase epsilon-ultramutated. Data available from Sweedish guidelines.Adapted from ESMO Clinical Practice Guideline [31].

### 1.1.5 Preoperative risk stratification

In the past, numerous guidelines have been proposed to distinguish between women with a low risk of lymph-node metastases (LNM) ("low-risk women") and those with a high risk ("high-risk women") and the subsequent possible benefit of lymph node assessment [31-37].

According to ESGO-ESTRO-ESP guidelines for the management of women with EC, risk factors for LNM include: higher tumor grade, non-endometrioid histology, deep myometrial invasion (MI), FIGO stage ≥ IB and lymphovascular space invasion (LVSI) [38]. LVSI, however is rarely available on preoperative histology specimens why some subgroups cannot be defined before surgery.

Preoperative imaging, either expert vaginal ultrasonography or magnetic resonance imaging (MRI) is recommended to evaluate myometrial and cervical involvement, although these assessments are sometimes inaccurate [21, 38, 39]. In more than 20% of women with EC, the final pathology report will lead to a change in the preoperative risk group, primarily due to inconsistencies in preoperative evaluation of MI [40, 41].

Preoperative imaging for assessing MI has proven incorrect in approximately 16-26% of women using transvaginal ultrasonography and in 18% using MRI [42, 43].

In all, 15-29% of women initially diagnosed with FIGO grade 1 endometrioid EC were upgraded on final pathology and 21% of women initially considered low-risk were upstaged due to MI [40, 41, 44]. Furthermore, interobserver variability in pathological diagnosis underscore the need for molecular tools to enhance diagnostic accuracy [45].

To avoid the consequences of inaccurate preoperative risk group categorization, a SLN concept should be incorporated in both high-risk and low-risk groups women with EC. Consequently, preoperative assessment of MI may become redundant, streamlining the process and lowering costs.

### 1.1.6 Postoperative risk assessment

A postoperative risk assessment is conducted to recognize women with a higher likelihood of relapse and to guide postoperative treatment. The concept of "high risk" in relation to recurrence and survival in EC lacks a universal definition.

The ESMO-ESGO-ESTRO consensus guidelines categorize EC into four risk groups [35]. Similarly, a recent consensus document published by ESGO-ESTRO-ESP outlines diverse risk categories, incorporating molecular risk stratification [38].

Swedish national guidelines recommend adjuvant treatment to all stage IIIC EC, all non-endometrioid EC, FIGO grade 3 endometrioid EC with MI or cervical stromal invasion (CSI) and all women with p53 abnormal cancers regardless of other risk factors unless POLE mutated (Figure1) [46]. A brief summary of risk classifications is tabulated below (table 2).

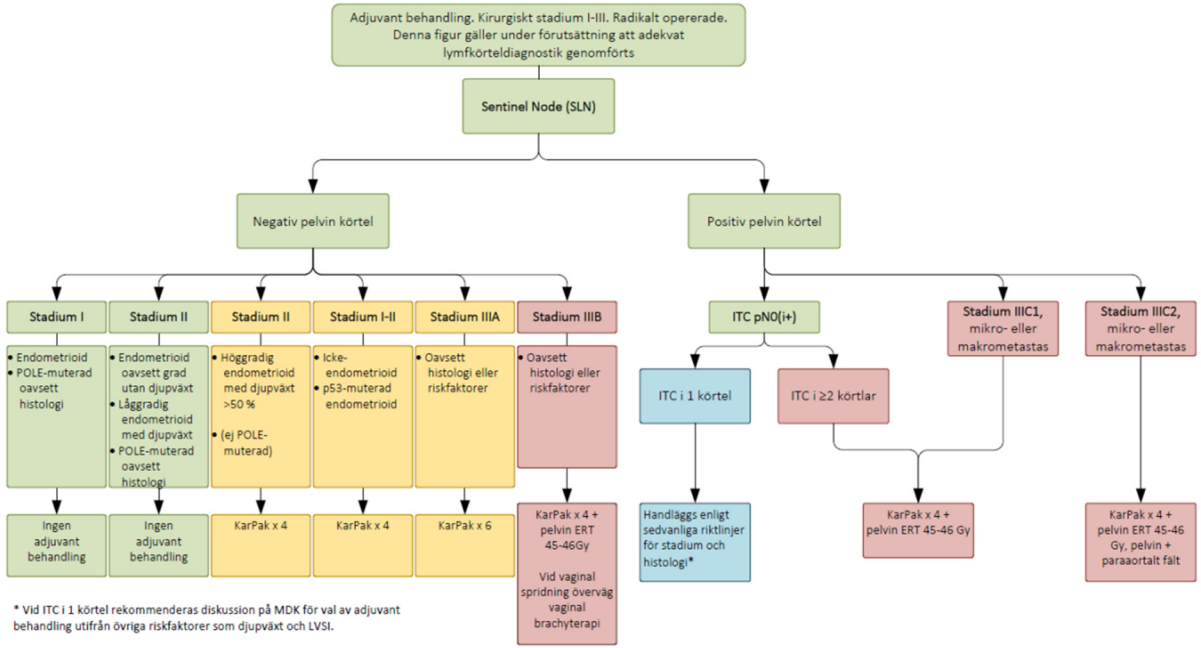



**Figure 1**: Adjuvant treatments in stages I-III according to the Swedish guidelines [46]. The figure assumes that sufficient lymph node diagnostics have been carried out. For women who have not undergone lymph node evaluation and present with stage IB-IIIA, p53-abnormal, or non-endometrioid histology, reoperation for lymph node diagnosis should be discussed at the multidisciplinary conference (MDK). Recently an amendment state that all women with p53 mutation in stage I-II ( p53 abn), shall be offered adjuvant treatment regardless others risk facto

**Table 2.** Overview of the risk classification according to ESMO-ESGO-ESTRO and ESGO-ESTRO-ESP.

| | **ESMO-ESGO-ESTRO 2009 [35]** | **European ESGO/ESTRO/ESP molecular class not known[38]** | **European ESGO/ESTRO/ESP with molecular classification[38]** |
|---|---|---|---|
| **Low-risk** | -EEC G1-2, Stage IA and no LVSI | -EEC G1-2, Stage IA and no LVSI | -Stage I–II POLEmutated, no residual disease<br>-EEC G1-2, Stage IA, MMRd/NSMP and no LVSI |
| **Intermediate-risk** | -EEC G1-2, Stage IB and no LVSI | -EEC G1-2, Stage IB and no LVSI<br>-EEC G3 Stage IA and no LVSI<br>-NEC and no MI | -EEC G1-2, Stage IB MMRd/NSMP and no LVSI<br>-EEC G3, Stage IA MMRd/NSMP and no LVSI<br>-Stage IA p53abn and/or NEC, no MI |
| **High-intermediate risk** | -EEC G3 Stage IA<br>-EEC G1-2, LVSI positive regardless of MI | -EEC Stage I with LVSI<br>-EEC G3, Stage IB<br>-Stage II | -EEC Stage I MMRd/NSMP and LVSI<br>-EEC G3 Stage IB MMRd/NSMP<br>-EEC Stage II MMRd/NSMP |
| **High-risk** | -EEC G3, with MI<br>-stage II<br>-NEC | -Stage III–IVA with no residual disease<br>-NEC Stage I–IVA with MI and no residual disease | -EEC Stage III–IVA MMRd/NSMP no residual disease<br>-EEC Stage I–IVA p53abn, with MI, no residual disease<br>-NEC Stage I–IVA NSMP/MMRd, with MI, with no residual Disease |

Abbreviations: EEC= endometrioid EC, NEC= non-endometrioid EC, G1= FIGO grade 1, high differentiated EEC, G2= FIGO grade 2, intermediate differentiated EEC, G3= FIGO grade 3, low differentiated EEC, CSI= cervical stroma invasion, LVSI= lymph vascular space invasion, POLEmutated= polymerase epsilon mutated, dMMR= mismatch repair deficient, NSMP= non-specific molecular profile, ESGO= European Society of Gynelogical Oncology, ESMO= European Society For Medical Oncology

### 1.1.7 FIGO staging, 2009 and 2023

Staging of EC has evolved since the FIGO Committee on Gynaecologic Oncology introduced surgical staging in 1988 [34, 47, 48]. The FIGO 2009 classification updated stages I and II and further subdivided stage IIIC to better reflect survival disparities between women with and without paraaortic LNM [49]. A further update was included in the FIGO 2023 staging system [50]. This system includes refined criteria for LVSI, type of adnexal involvement, presence of micro (MIM)- or macrometastases (MAM) in pelvic and/or para-aortic lymph nodes. Unlogically, the presence of ITCs in lymph nodes, representing a definite proof of LVSI leading to tumor spread is not included in the staging. Moreover, the updated FIGO 2023 system integrates molecular classifications, such as POLE-mutated and p53abnormal [51, 52]. This inclusion of molecular parameters expanded the number of (sub)stages from 10 to 23 potentially enhancing the staging system's prognostic and predictive capabilities [50]. For Stage I-II POLE-mutated tumors, the administration of adjuvant therapy can potentially be reduced. Women with tumors displaying abnormal p53 expression may have the greatest advantage of chemotherapy in conjunction with radiotherapy [52]. For previous and current staging according to FIGO, see appendices 11.1 and 11.2.

### 1.1.8 Adjuvant treatment

The combination of carboplatin and paclitaxel is considered first-line adjuvant treatment in select women with EC. Numerous studies have examined a possible survival benefit of postoperative chemotherapy in early-stage EC. Regardless of histology, women with stage I-II and POLE mutations (5-15%) are not expected to benefit from adjuvant chemotherapy [53-56]. Women with p53-mutated tumors have a poorer prognosis, regardless of other risk criteria probably benefit the most from adjuvant chemotherapy [52]. Intermediate prognoses are seen in women with dMMR (deficient mismatch repair) and NSMP (no specific molecular profile). In the NSMP group, there is a trend toward better outcomes with the combination of chemotherapy and radiotherapy, while no notable differences were observed in dMMR women [52]. These findings underscore the potential of molecular classification to reduce both over- and under-treatment.

In advanced-stage EC (stage III-IV) the evidence supporting the benefit of adjuvant chemotherapy is stronger. In Sweden, the combination of chemotherapy and radiotherapy is generally reserved for women with lymphatic spread (stage IIIC) or involvement of the parametria or the vagina (stage IIIB). These women usually receive four cycles of carboplatin and paclitaxel, followed by 45-46 Gy of pelvic radiotherapy. SLN diagnostics including ultrastaging provide more detailed information about lymph

node involvement, identifying MAM, MIM and ITC, which offer more precision than traditional lymph node dissection. Women with MAM and MIM are staged and treated as stage IIIC, while ITC does not affect staging but is noted as N0(i+).

There is no solid evidence on how to treat women with ITCs in lymph nodes as the only potential risk factor. Some studies suggest a good prognosis, though many women in these studies received postoperative treatment [57-60]. Women with a low-grade endometrioid stage IA and ITC in lymph nodes not receiving adjuvant treatment, had a higher risk for recurrence compared to those who were lymph node-negative [61]. According to Swedish recommendations, women with ITC in one lymph node should be treated based on other risk factors, whereas women with ITCs in two or more lymph nodes are treated as stage IIIC, awaiting further evidence (Figure 1 above).

### 1.1.9 Prognosis

The 5-year survival rate in EC is more than 80% and the 10-year survival rate is 78% [34, 62, 63]. Over 80% of women are detected at stage I [35]. The prognosis is influenced by the stage at diagnosis and histologic subtype [64]. (Figure 2 and Figure 3).

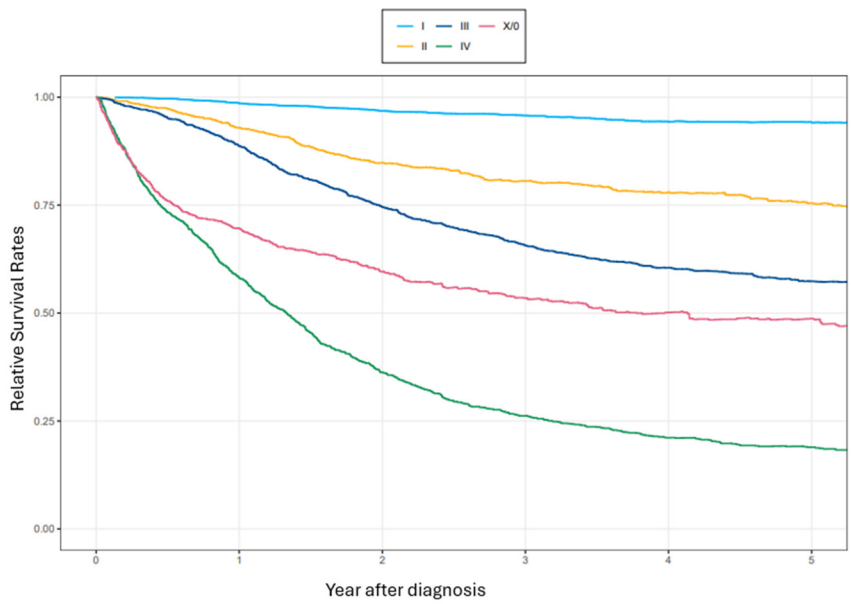



**Figure 2** Relative Survival Rates in Corpus Cancer by FIGO Stage, Based on Diagnoses from 2012-2023 [Data publicly available from Swedish Quality Registry of Gynecologic Cancer][46]. Blue: Stage I, yellow: Stage II, dark blue: Stage III, green: Stage IV; pink: stage X/0

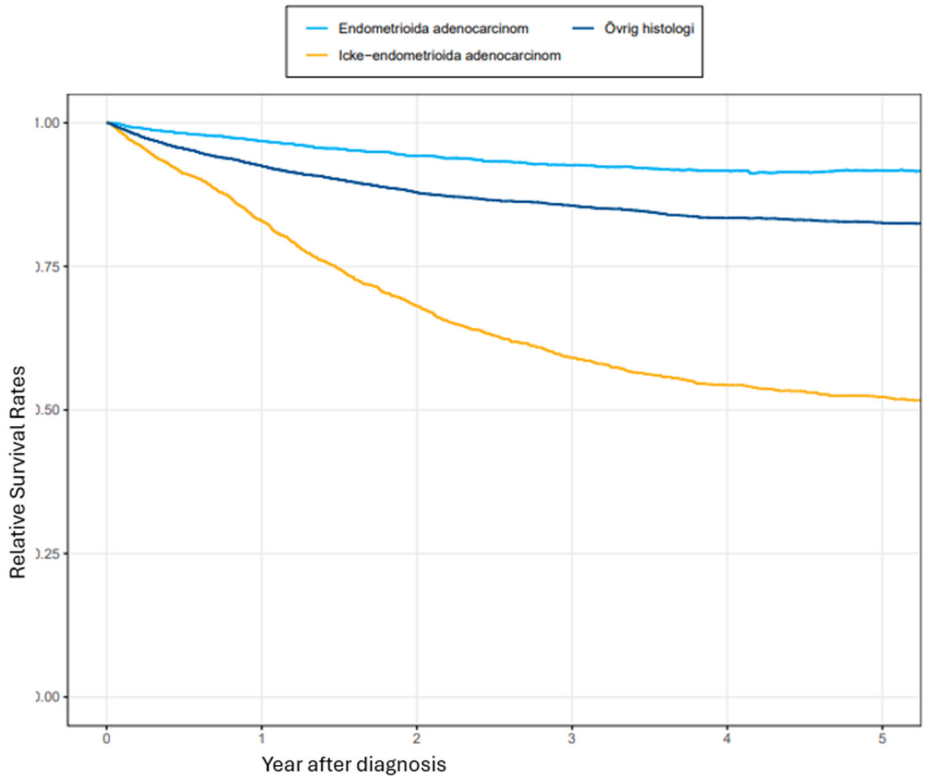



**Figure 3** Relative Survival Rate for Corpus Cancer by Histology, Based on Diagnoses from 2012-2023. 2023 [Data publicly available from Swedish Quality Registry of Gynecologic Cancer][46]. Blue:endometrioid adenocarcinoma, yellow: non endometrioid adenocarcinoma; dark blue: other hystologi.

# 2 Surgical treatment of endometrial cancer

In Sweden, over 90% of women with EC undergo hysterectomy and bilateral salpingo-oophorectomy as first line treatment [65]. In young women with a non-invasive uterine confined grade I hormone receptor positive endometrioid EC, uterine-preserving hormonal treatment may be considered [38, 66]. Traditional EC preoperative risk grouping was based on the assumption that morbidity of lymph node dissection (LND) was greater than the benefit of detecting metastases, present in a presumed small fraction of low-risk women.

The detection of SLNs in EC may offer advantages as an alternative to preoperative risk grouping and to a full pelvic lymphadenectomy (PLND) and infrarenal paraaortic lymphadenectomy (IRPALND) in women with high-risk tumors. SLN mapping eliminates under/overestimation of preoperative riskgroups by erroneous MI evaluation and inconsistencies in pre- and postoperative histology. In addition, SLN detection will identify node positivity in women without other risk factors. Moreover, the identification of low-volume metastases through ultrastaging and immunohistochemistry (IHC) in SLNs may reduce the proportion of women with isolated paraaortic metastases by an improved diagnose of pelvic lymph node metastases. Although the primary scientific support for SLN has focused on high-risk EC, its applicability could extend to low-risk groups although sensitivity data cannot be directly applied. Consequently, the 2020 Swedish guidelines recommend the SHREC SLN algorithm for all women with EC considered fit for adjuvant treatment [46, 67].

## 2.1 Minimally invasive and Robot assisted surgery

The introduction of laparoscopic and later robot assisted laparoscopic surgery changed the management of EC, offering similar survival outcomes to laparotomy but with fewer postoperative complications particularly in obese women [68-72]. The oncologic

safety of minimally invasive surgery (MIS) including lymphadenectomy for EC has been studied by two Phase III trials. The GOG-LAP2 study demonstrated that laparoscopic hysterectomy with pelvic lymphadenectomy (PLND) and para-aortic lymph node dissection (PALND) to the inferior mesenteric artery provided similar survival outcomes compared to laparotomy in women with early-stage EC [70]. Although intraoperative complications were similar and the conversion rate was 25%, the laparoscopy group experienced shorter hospital stay and fewer postoperative complications [70]. The findings supported minimally invasive surgery (MIS) as the optimal surgical approach for EC. The LACE trial further supported the oncologic safety findings of the GOG-LAP2 trial, in addition to healthcare costs for laparoscopy [73]. However, LND was not mandatory in the LACE trial, and if performed, only PLND was required while PALND was optional [73]. However, type II cancers were excluded from the LACE trial and accounted for less than 20% of cases in the GOG-LAP2 trial. Additionally, LACE only included women with stage I disease, while LAP2 included those with stage I-IIA disease (FIGO 2009). In a retrospective single centre cohort focusing solely on 283 women with type II EC, Monterossi et al. reported fewer complications and comparable survival rates when comparing MIS with open surgery. However, better overall survival was observed with open surgery in stage III disease [74]. A recent review by Scaletta et al. on high grade or type II tumors treated with MIS (laparoscopic or robotic) or laparotomy concluded that MIS provided better perioperative and postoperative outcomes and comparable oncological outcomes, with no differences across stages [75] Jørgensen et al. reported that after Denmark implemented a national policy for MIS, overall survival rates improved for women with early-stage EC [76]. The introduction of robotic surgery for early-stage cases was linked to better survival outcomes, unaffected by variables such as age, body mass index (BMI), American Society of Anesthesiologists (ASA) score, existing comorbidities, smoking, socioeconomic factors, and histopathological risk. Swedish guidelines recommend MIS as the first choice for women with EC [46].

## 2.2 Lymphadenectomy

Lymphadenectomy was recommended for the first time for staging of EC in 1987, supported by the American Gynecologic Oncology Group (GOG 33 study) [47, 77]. Based on this study, three preoperative risk categories for PLND were established, guiding the decision to perform lymphadenectomy:

1) Low risk (<5% risk of pelvic node metastases): intra-mucosal endometrioid cancers of all grades and grade 1 endometrioid cancers with MI, excluding non-endometrioid histologies.

2) Moderate risk (5-10% risk of pelvic node metastases): grade 2 endometrioid cancers with any MI and grade 3 endometrioid cancers with less than 66% MI.

3) High risk (>10% risk of pelvic node metastases): grade 3 endometrioid cancers with more than 66% MI and non-endometrioid histologies.

Lymphadenectomy was thus adopted into routine clinical use, although prospective trials had not yet validated its efficacy. From 2010 Swedish guidelines recommended a pelvic and paraaortic lymphadenectomy utilizing slightly modified EC risk criteria as defined at that time.

### 2.2.1 Extent of lymphadenectomy

According to the American Gynecologic Oncology Group (GOG) and the U.S. National Comprehensive Cancer Network (NCCN), a comprehensive PLND involves removing nodal tissue from several key areas. These includes the distal half of the common iliac arteries, the anterior and medial area of the proximal half of the external iliac vessels, and the distal half of the obturator fossa, located anterior to the obturator nerve [78, 79]. A PALND is defined as removing nodal tissue over the distal inferior vena cava and aorta, extending from the level of the left venal rein to the mid right common iliac artery, and removing nodal tissue between the aorta and left ureter, stretching from the mid inferior mesenteric artery to the mid left common iliac artery [79, 80]. Notably, there was no consensus regarding the required number of harvested paraaortic lymph nodes to consider the lymphadenectomy as adequate [81]. In Europe, lymphadenectomy in EC is generally defined as removal of pelvic and para-aortic lymph nodes extending to the level of the left renal vein [35].

### 2.2.2 Outcome of pelvic lymphadenectomy

Nodal status guide adjuvant treatment and predict prognosis. Two randomized controlled trials (RCTs) have investigated the impact on survival outcomes by adding PLND in EC, finding no significant superiority over cases without lymphadenectomy [82, 83]. The results are hampered by bias such as inconsistent application of adjuvant treatments and insufficient removal of lymph nodes [82, 83]. Benedetti-Panici et al. demonstrated that PLND did not provide overall survival or disease-free survival benefit in early-stage EC. The study lacked strict criteria for adjuvant treatment [82].

The multinational MRC-ASTEC trial found no therapeutic benefit of systematic PLND. The study included women with low-risk disease, reported a low pelvic lymph node count and had a low proportion of LNM (9%). Additionally, women with postoperative high or intermediate risk factors alone were further randomized to receive external beam radiotherapy or no additional treatment after surgery, an additional bias [83, 84].

Despite this, some retrospective studies suggest that lymphadenectomy may improve survival, particularly in high-risk women, indicating that it could be beneficial in selected individuals [85-87]. The Japanese SEPAL study retrospectively compared women with EC confined to the uterus who underwent either PLND or combined PLND and PALND. The study showed a survival benefit for women who underwent extensive surgical staging [87]. Additionally, Chan et al. conducted a retrospective review of over 12,000 women with endometrioid EC and found that women with high and intermediate risk EC who underwent lymphadenectomy had better survival outcomes [88]. However, there is also retrospective data presenting opposite results [89].

Current studies are investigating if removal of SLNs, can provide similar staging benefits with fewer complications [90-92]. This ongoing research aims to refine lymph node assessment strategies to maximize women benefits while minimizing risks:

1. The ongoing *SNEC trial* aims to investigate the non-inferiority of SLN concept alone compared to a full pelvic and PALND in women with intermediate-high-risk EC in preoperatively early-stage, with recurrence-free survival as the primary outcome measure [90].

2. The ongoing *ENDO-3 trial* aims to investigate the non-inferiority of refraining from SLN assessment, with disease-free survival as the primary outcome measure in apparent early-stage EC [91].

3. The ongoing *ECLAT trial* examines the efficacy of comprehensive PLND and IRPALND versus no lymphadenectomy on overall survival in preoperatively high-risk women with stage I or II EC [92].

In the last two trials, one randomization arm excludes lymph node assessment. A recurring issue from earlier randomized studies is the lack of adjuvant treatment standardization based on lymph node status, which may affect how the results are interpreted.

In conclusion, the therapeutic value of PLND and PALND remain unclear.

### 2.2.3 Morbidity associated with pelvic lymphadenectomy

Morbidity following PLND in women with EC includes lower extremity lymphoedema, lymphocele formation, truncal/genital lymphoedema, and chylous ascites, each with distinct challenges and impacts [36, 37, 93-97].

*Lower extremity lymphoedema* is reported in between 5% and 38% following a complete lymphadenectomy reflecting the lack of standardized methods for diagnosis and evaluation [93, 98, 99]. Lower extremity lymphoedema is a chronic, progressive condition that impairs health-related quality of life and increases healthcare costs. Risk factors for developing lower extremity lymphoedema is related to the number of lymph nodes removed, obesity and the use of radiotherapy. Symptoms can appear at any time after treatment, but typically develop within six months after surgery [93, 98-104].

*Lymphocele* formation is reported to occur in between 1.4% to 15.4% following lymphadenectomy [37]. Lymphoceles may lead to pain, infection, leg edema, deep vein thrombosis, and obstructive uropathy. Treatment involves conservative approaches, such as percutaneous drainage, antibiotics or surgical fenestration or removal, the latter effective in most cases where conservative treatment fails [105].

*Truncal and genital lymphoedema* can occur after PLND and PALND. This condition is often self-limiting with expectant management [106].

*Chylous ascites*, i.e the accumulation of milky, triglyceride-rich fluid in the peritoneal cavity, occurs in 2% to 9% of women following PALND [107, 108]. This condition is typically managed by a combination of a low-fat diet with medium-chain triglycerides to reduce lymphatic flow and octreotide (somatostatin) [109]. Surgical interventions guided by lymphoscintigraphy may be necessary [107, 108].

# 3 The Sentinel Lymph Node concept

## 3.1 Overview of a sentinel lymph node concept

The concept of SLN removal originates from Virchow's observations in the 19th century that lymph nodes filter cancer cells from the lymphatic system [110]. In the late 19th and early 20th centuries, cadaver studies were conducted on the anatomy of the lymphatic system [111-114]. In 1923, The British surgeon Braithwaite introduced the term "sentinel node" for the first lymph node affected by cancer [115, 116]. Hence, the nodes closest to the tumor are called the sentinel lymph nodes, a word derived from the Italian words "Sentina" (attention) and from the latin "Sentinare" meaning to perceive or observe or avoid a danger with care, later used as a word for a guard or a doorkeeper, a Sentinel.

Tumor spread to regional lymph nodes is important clinical information in most cancer forms, both for guiding treatment and for assessing prognosis and follow up measures. The last decades, the possibility to diagnose LNM with limited surgery therefore has gained increasing interest and has been implemented in surgery for several cancers such as breast cancer, vulvar cancer, cervical cancer, malignant melanoma and penile cancer [117-120].

The SLN concept is based on injection of a tracer (a dye or a radioactive substance alone or in combination) for further transportation via the lymphatic system to the lymph node(s) closest to the primary tumor postulating that cancer cells do not bypass a lymph node and that lymph nodes within a lymphatic vessel are serially connected. Hence, a non-metastatic SLN is an indicator that no further spread of cancer along that particular lymphatic pathway has occurred. This is however a simplification as parallel lymphatics within a major pathway may occur. This also implies that *in vivo* studies of the lymphatic system, ideally utilizing the tracer planned for clinical use, should precede the clinical use of the SLN concept for a certain type of cancer and its localization.

As a rule, the injection of tracer is performed peritumorally to ensure that nodes detected by the chosen tracer are representative for the lymphatic drainage of the tumor. The choice of tracer or a choice to combine tracers, depends on its ability to map the

nodes and practical surgical circumstances. For instance, in vulvar cancer, usually two tracers are utilized, a radiotracer and a dye, as there is a need to define the place for the skin incision in the groins by the radiotracer with signals penetrating intact skin while the dye adds to finding the SLN after the wound is opened.

The situation in EC differs as the primary tumor is concealed within the uterine cavity and the border between the cancer and adjacent non-cancerous endometrium may be indistinct. Hence, a peritumoral injection is difficult to perform accurately why standardization is problematic. Therefore, various attempts by hysteroscopic injection have not gained popularity as the cancer in addition frequently bleeds, obscuring vision. Other options are for instance a blind transcervical injection into the fundus of the uterus (i.e with a Kobac needle to reduce the risk for a transmural injection) or a transabdominal fundal injection, hence possibly not peritumoral [121-125]. Neither of these latter alternatives have been used extensively due to the risk of leaking of tracer into the abdominal cavity and/or due to technical complexity or difficult standardization and low reproducibility. A fundal injection does however have the potential to generate display of tracer along lymphatics within the Infundibolo pelvic ligament (IPP) draining directly to paraaortic infrarenal lymph nodes, rarely achieved by a cervical injection [125].

An important insight in EC was that a cervical injection of tracer, hence not peritumoral, resulted in representative display of tracer to SLN [126]. This catalyzed the implementation of SLN detection in EC and resulted in an abundance of studies, the majority retrospective with an unclear algorithm and definition of SLNs and utilizing a variety of tracers alone or in combination.

Definition of "paraaortic SLNs" deserves a comment; given the assumption that lymph draining the uterus runs cranially via pelvic lymphatics always with lymph nodes closer to the uterus (per definition the SLNs), lower paraaortic dyed nodes are secondary echelon nodes, not true SLNs. Therefore, a SLN concept in endometrial (and cervical) cancer should be based on detection of pelvic SLNs only. Furthermore, a need for a paraaortic dissection to complete a SLN algorithm would be a major step away from the intention to minimize surgical trauma, one of the ideas with detection of SLNs.

Another important difference in endometrial (and cervical) cancer, compared with for instance vulvar cancer, is that the lymphatic vessels draining to the regional lymph nodes by a defined surgical approach can be visualized throughout is entire course from the uterus in case a dye is used [125, 127]. In this way, SLN can be more distinctively defined. A radiotracer does not allow this more precise definition [128]. The fluorescent dye Indocyanine green (ICG) has emerged as the superior tracer [129-131]. ICG will emit infrared (invisible) light when illuminated with a 806 Nm wave length

light (near infrared light) and require adapted hardware and a software transforming the infrared light to visibility, i,e a colour against a contrasting background. Several manufacturers have adapted equipment for the use of ICG. One application is integrated in the Da Vinci surgical robots (the Firefly mode, Intuitive surgical, Sunnyvale, CA), in addition allowing a fast switch between normal light and the Near Infra-red mode. This helps determining whether a green area is a lymph node or a dilated lymph vessel. In this way, so called "empty packages", green tissue perceived as a SLN by the surgeon, but with no lymph node found on histology, can be minimized, in particular in situations with a strong uptake of ICG. ICG also allows reinjection in case of non-mapping and rarely results in neo-allergic reactions [132, 133]. ICG do however contain Iodine why women with an allergy should be offered another dye as tracer.

## 3.2 Ideal development of a sentinel lymph node concept

An ideal development of a SLN algorithm in uterine cancer should start with *in vivo* studies of the uterine lymphatic drainage to guide along which lymphatic pathways SLN should be defined. Secondly, a reproducible surgical algorithm should be developed with a clear definition of a SLN; the mapped node closest to the uterus in each of relevant pathways / sub pathways. As metastatic lymph nodes may dye less well, the algorithm should include macroscopically cancer suspect nodes and non-mapped nodes with a clear dyed lymphatic vessel ending in that node.

Studies of lymphatic anatomy and a description of a standardized surgical algorithm including a strict definition of a SLN has been published in a previous thesis at our institution [125, 127].

Following these two developmental steps, prospective adequately powered studies on sensitivity and negative predictive value of the SLN algorithm to detect /rule out pelvic nodal metastatic disease should follow. Ideally, these initial studies should be performed in a setting where a few well-educated surgeons are involved to enable a high internal validity to demonstrate the potential of the algorithm for other centres to aim for during implementation of the algorithm. In those initial studies the quality of the completory PLND and PALND is important. A completory PALND, ideally to the level of the left renal vein (infrarenal area), provides information on the risk for isolated paraaortic metastases (so called skip metastases) and the proportion of women with concurrent paraaortic nodal metastases in case of metastatic pelvic SLNs.

If then the SLN concept with the defined algorithm demonstrates a high enough sensitivity, efforts to implement the SLN concept on a wider basis, i.e nationally should follow. In that phase, repeat educational efforts are needed. Furthermore, it is imperative to consistently evaluate the results such as mapping rates, the number of removed SLN as an indirect sign of adherence to protocol and that all pathways have been explored. We believe that a comparison of the proportions of SLN metastases within well postoperatively defined risk groups is the best way to evaluate the accuracy of a SLN only procedure, for instance for quality control during and implementation phase and later. It is important to bear in mind that a SLN concept is no stronger that its weakest link, those links are, an inadequate understanding and knowledge of lymphatic anatomy, of the SLN concept as whole and inadequate surgical training and experience.

Finally, the impact of detection of SLNs on clinical outcomes should follow.

Detection of a reduced number of lymph nodes at higher risk for metastatic disease (SLNs) allow for a more detailed pathological evaluation by an increased number of sections of the lymph node (ultrastaging), for resource reasons difficult to accomplish if all pelvic nodes are removed. This enables a better detection of smaller volume metastases (MIM and ITC). This is a strength but will result in new information where prognostic data is missing indicating a need for further studies. Figure 4 highlights the prerequisites for an ideal development of a SLN concept and its implementation.

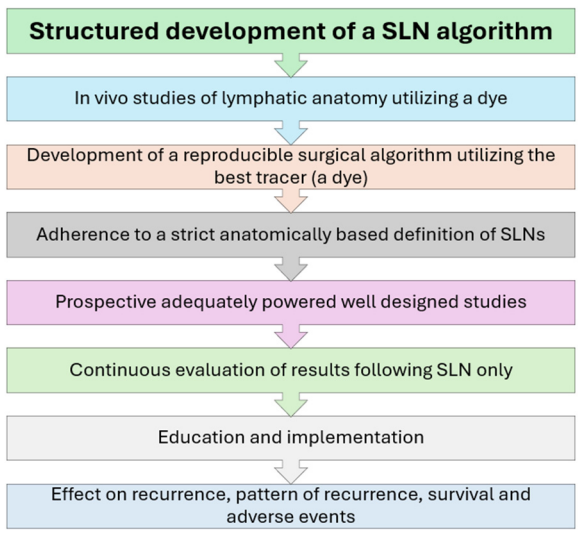



**Figure 4.** Ideal development of a SLN algorithm.

## 3.3 Uterine lymphatic anatomy, previous studies

Knowledge of the uterine lymphatic anatomy is pivotal for understanding cancer spread. Early investigations of the lymphatic system relied on cadaver dissection in women using various substances such as quicksilver and Prussian blue, to trace lymphatic pathways [111, 113, 134, 135]. These studies identified three lymphatic pathways:

1) External iliac pedicle, pre-ureteral pedicle, or primary pedicle (later defined by Geppert et al. as the upper paracervical pathway (UPP)) which follows the uterine artery and drains into the external iliac and obturator lymph nodes further draining to lower para-aortic nodes [125].

2) Posterior pedicle, hypogastric pedicle or retro-ureteral pedicle (later defined by Geppert et al. as the lower paracervical pathway (LPP)) aligned with the uterine vein, extending into the hypogastric or presacral lymph nodes further draining to lower para-aortic nodes [125].

3) The Infundibulo-Pelvic Pathway (IPP), draining directly to the infrarenal para-aortic lymph nodes [111-113, 125, 134, 135].

## 3.4 Uterine lymphatic anatomy, recent studies

An *in vivo* study on uterine lymphatic drainage was initially performed at our institution utilizing ICG as tracer during robot assisted surgery [125]. The study basically confirmed previous cadaver studies and provided information a cervical injection of ICG displays relevant pathways, an important find. Importantly, ICG allows for display of both lymphatic vessels and lymph nodes. ICG as tracer was initially chosen due to our access to the Da Vinci FireFly application late 2011for the developmental studies as of above and the initial good experience. ICG was later proven the superior tracer in the randomized phase III FILM (Fluorescence Imaging for Lymphatic Mapping) study [131].

We identified three separate major pathways without observed inter-pathway communications beyond the parauterine lymphovascular tissue (a tissue further studied, and finally named PULT) [125, 136].

The pathways were named:

1) The Upper Paracervical Pathway (**UPP**) which runs via the PULT usually with parallel sub-pathways to lymph nodes in the obturator fossa, and to external iliac nodes continuing lateral to the common iliac artery to the lower paraaortic/precaval areas, then further cranially along the aorta (Figure 5).

2) The Lower Paracervical Pathway (**LPP**) which runs medial to the hypogastric artery and vein, then presacrally medial to the common iliac artery over the sacral promontory then further to the lower paraaortic/precaval areas as of above. In contrary to the UPP, crossover between sides were observed, usually from the left to the right side, mainly just below the aortic bifurcation over the bifurcation of the vena cava (Figure 5).

3) The Infundibulo-Pelvic Pathway (**IPP**) which runs along the IPP to nodes in the infrarenal paraaortic area. This lymphatics in this pathway rarely displays following a cervical injection of ICG and is not used for definition of SLNs. Figure 6 demonstrates that lymph drains directly to the infrarenal area when there is an uptake of ICG in the IP ligaments (Figures 5 and 6).

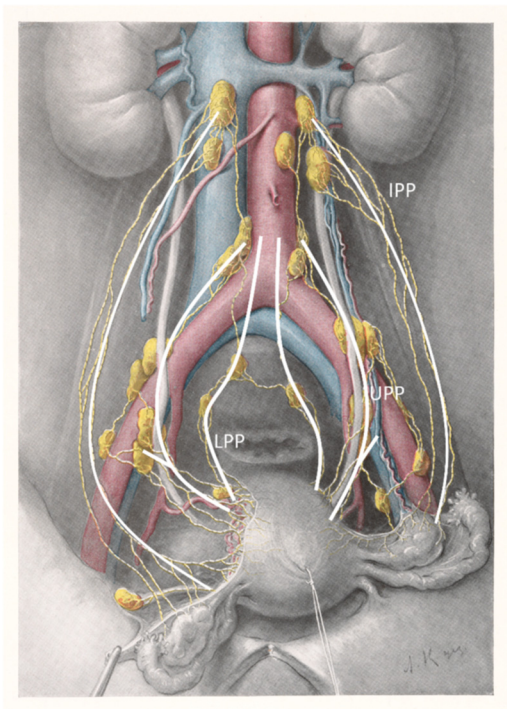



**Figure 5** Uterine lymphatic anatomy according to Döderlein and Krönig in 1912 modified by Geppert et al. [62]. Reproduced with kind permission from Thieme Medical Publishers, Germany.

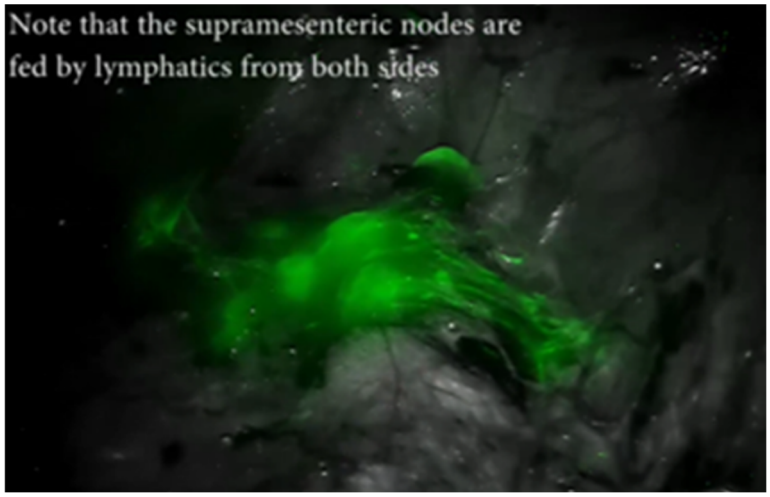



**Figure 6**. ICG display in the supramesenteric/infrarenal paraaortic area following ICG-uptake in the lp-ligament. Note the absence of ICG in the inframesenteric area.

The **PULT**. Studying the display of ICG it became obvious that if nodes were present in the PULT, those were logically to be considered SLNs. Therefore, despite lack of recommendations in guidelines for EC, we removed this tissue and processed it as a whole as SLN unless a distinct node was identified, in that case separately sent for histology.

## 3.5 Development of a strict surgical algorithm

A reproducible surgical algorithm was developed to standardize the performance of detection of SLNs between surgeons in studies, of major importance to achieve reproducible results and possibly for other studies to apply [125, 127]. The algorithm states that the avascular presacral, pararectal and paravesical planes shall be opened widely without disrupting lymphatic vessels (Figure 7, A). This is achieved by changing between the Near-Infrared light (NIR) mode and white light to avoid leaking of ICG and important for defining what is a true SLN and which ICG dyed nodes are secondary echelon nodes, a definition based on findings of afferent lymph vessels. In case a detection of SLNs along the LPP is indicated, this space shall be opened first to minimize leaking ICG that may obscure detection of SLN along the UPP, to be opened later.

SLNs were defined as followed:

**SLN type 1** as the juxtauterine ICG mapped lymph nodes defined by an afferent lymphatic vessel allowing for sub pathways within a major pathway, frequently observed along the UPP. Picture A below demonstrates how the pararectal and paravesical planes have been developed leaving the afferent lymphatics intact for definition of, in this case, the interiliac lymph node as one of SLNs along the UPP, whereas the ICG-mapped node lateral of the external iliac artery is defined as an echelon node non-SLN due to the lack of a separate afferent lymphatic vessel. Any obturator ICG positive SLNs are looked for after the external iliac SLN is removed (not shown).

**SLN type 2** is an ICG non-mapped node defined by an afferent lymphatic vessel in the absence of other ICG mapped nodes along that (sub) pathway.

**SLN" macro"** is defined as any macroscopically cancer suspect node regardless of, but with information on, ICG uptake.

Preparation of ICG for injection were as follows. A vial of 25 mg ICG powder (Pulsion Medical Systems, POCG0025SE, Feldkirchen, Germany) was diluted in 10 mL of sterile water to create a 2.5mg/mL solution and distributed to mL syringes with a 0.6 × 38 mm 23G × 1½ needle. 0.25 mL (0.625 mg) ICG was injected submucosally in the cervix at 2–4–8–10 O'clock.

The algorithm further introduced the concept of an ipsilateral re-injection of ICG in case of non-mapping. Reinjection was performed after a 10 minute observation time.

Usually, display of ICG uptake can be seen transperitoneally (Figure 7, B) unless obesity, having in mind that the limited penetrance of fluorescent light. Hence, in case of no ICG display, spaces were opened before decision of a reinjection.

A modification was introduced in the SLN-only study following completion of the SHREC study. To avoid overdose of ICG along the UPP, no reinjection was performed due to non-mapping of the LPP as this frequently coincided with an ipsilateral mapping of the UPP.

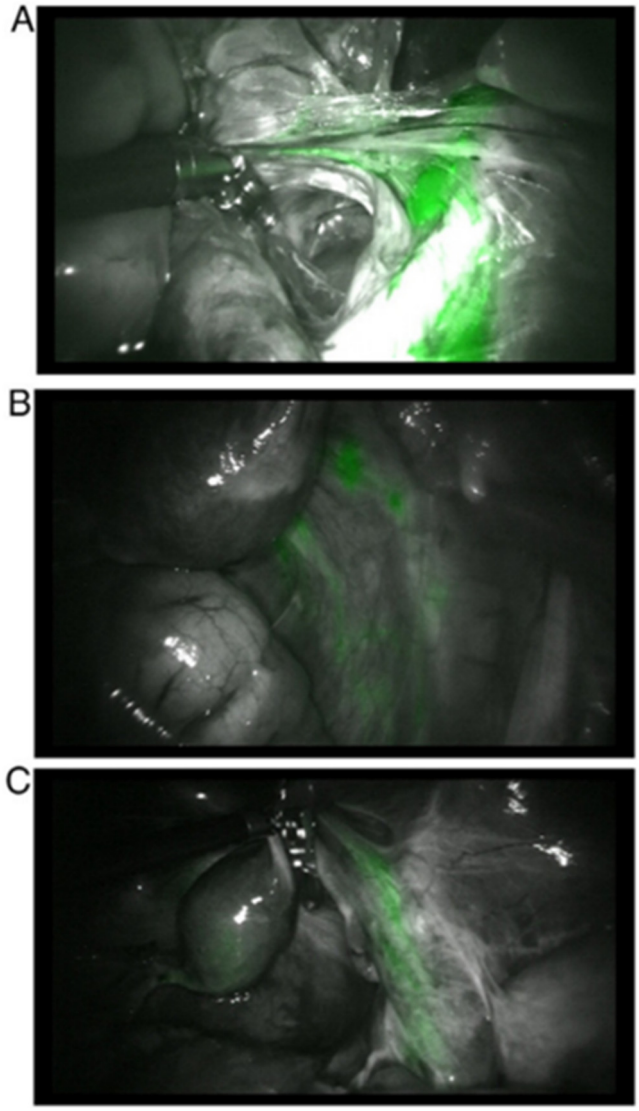



**Figure 7** (A) Upper paracervical pathway (B) Upper and lower paracervical pathway seen transperitoneally (C) The infundibolo-pelvic pathway. With permission.

The positions and types of SLN were depicted during surgery on an anatomical chart. A detailed specimen list was used for the pathologist with corresponding premade glued labels for the specimen jars (Figures 8 and 9 below). For surgical protocol see appendix 11.3.

**Anatomical plan for localization of sentinel lymph nodes/ list of nodal specimens.**

Injection site of ICG ☐ cervix

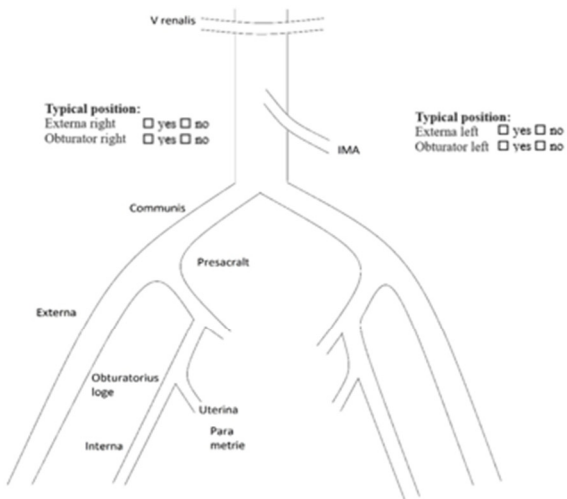
Reinjection cervix: ☐ yes ☐ no

Display after first injection

| | UPP | LPP | IP-ligament |
|---|---|---|---|
| Right | | | |
| Left | | | |

Display after second injection

| | UPP | LPP | IP-ligament |
|---|---|---|---|
| Right | | | |
| Left | | | |




Mark position and type of SLN on anatomical chart with number corresponding to position and number at list and on separate jars for each SLN.

○ = ICG positive juxtauterine Sentinel node (SLN1)
☐ = ICG neg juxtauterine lymph node with afferent lympahtic vessel (SLN 2)
Δ= SLN anatomy
X = Tumor suspect lymph nodes regardless of mapping (SLN makro) but with information on ICG positivity or not.

**Figure 8** Anatomical chart used to depict positions of SLNs, lymphatic uterine pathways and need of reinjection. UPP= upper paracervical pathway, LPP= lower paracervical pathway, IPP= infundibolopelvic pathway, ICG= indocyanine green.

**Preparatöversikt Endometrie- & Cervixcancer Plats för pat-id datum** Om preparat saknas från station stryks raden i listan. Burknumrering behålls för övriga prep.

| KK Burk nr | Körtelposition | Patol burk nr | Dosa nr | Antal bitar | Mikro Antal körtlar | Varav med metast |
|---|---|---|---|---|---|---|
| 1 | Uterus, höger ovarium & tuba, vänster ovarium & tuba | | | | | |
| 2 | Lgl Iliaca Externa höger | | | | | |
| 4 | Lgl Obturatorius höger | | | | | |
| 6 | Lgl Iliaca Communis höger | | | | | |
| 8 | Lgl Presacralt höger | | | | | |
| 10 | Lgl Iliaca Externa vänster | | | | | |
| 12 | Lgl Obturatorius vänster | | | | | |
| 14 | Lgl Iliaca Communis vänster | | | | | |
| 16 | Lgl Presacralt vänster | | | | | |
| 18 | Lgl Paraaortalt nedom IMA | | | | | |
| 20 | Lgl Paraaortal ovan IMA | | | | | |
| 21 | Oment | | | | | |
| 22 | SLN Parametrium höger | | | | | |
| 23 | SLN Parametrium vänster | | | | | |
| 24 | SLN typ 1 presacralt höger | | | | | |
| 25 | SLN typ 1 presacralt vänster | | | | | |
| 26 | SLN typ 1 iliaca externa höger | | | | | |
| 27 | SLN typ 1 obturatorius höger | | | | | |
| 28 | SLN typ 1 iliaca externa vänster | | | | | |
| 29 | SLN typ 1 obturatorius vänster | | | | | |
| 30 | SLN typ 1 | | | | | |
| 31 | SLN typ 1 | | | | | |
| 32 | SLN typ 2 | | | | | |
| 33 | SLN typ 2 | | | | | |
| 34 | SLN makro ICG pos ICG neg | | | | | |
| 35 | SLN makro ICG pos ICG neg | | | | | |
| 36 | SLN anatom iliaca externa höger | | | | | |
| 37 | SLN anatom Iliaca externa vänster | | | | | |
| 38 | SLN anatom obturatorius höger | | | | | |
| 39 | SLN anatom obturatorius vänster | | | | | |
| 40 | SLN anatom communis höger | | | | | |
| 41 | SLN anatom communis vänster | | | | | |
| 42 | SLN anatom | | | | | |
| 43 | SLN anatom | | | | | |
| 44 | | | | | | |

Numbers 30-35 will be used for describing locations outside the most common sites and for SLN type 2 and SLN macro as appropriate. The locations will be written by hand on list and labels for jars.

**Figure 9** List of nodal specimens.

## 3.6 Insights and pitfalls

There are some important insights and logic pitfalls when evaluating sensitivity to detect nodal metastases of a SLN concept. Most prospective SLN studies are performed on women where national guidelines recommend a PLND and IRPALND, i,e in women where the risk for nodal metastases are considered higher. These criteria are not consistent between countries or over time and may in addition to non-endometrioid cancers, include high grade endometrioid cancers and low grade endometrioid cancer with a presumed MI or cervical stroma invasion (CSI). The reason for including these women in prospective studies on sensitivity of a SLN concept is ethically, rather than methodologically, motivated, as a full backup/ completory PLND and IRPALND cannot be justified in women with a presumed lower risk for metastases due to known side effects.

**First,** presuming that women at a higher risk of metastases more often, compared to women at low risk for metastases, have multiple pelvic nodal metastases. This increases the likelihood of finding a metastatic" SLN" by chance, in particular when a less strict algorithm is applied. Therefore, a certain SLN algorithm in high-risk women risk overestimation of sensitivity and data cannot be directly applied in women with a lower risk for nodal metastases. This given, there is no incentive to select subgroups of women at an estimated higher risk for metastases for studies evaluating sensitivity/negative predictive value of a certain chosen algorithm, rather the opposite.

**Second**, over the years many studies applied the Barlin algorithm, a breakthrough in 2012 when it was published, as it pin-pointed the need for a full lymphadenectomy to identify node positivity in case of non-mapping or in the absence of macroscopically tumor suspect nodes [126]. At that time, there was methodologic inconsistencies in the surgical performance of the SLN procedure, in definitions of a SLN and by the use of various tracers resulting in lower bilateral mapping rates. Robotic surgery was still in an developmental phase. The logic paradox is that the lower the tracer based mapping rate, the higher the perceived sensitivity of the overall Barlin algorithm as the finding of a nodal metastases following a full lymphadenectomy will also be the control to which sensitivity is compared, i.e always a SLN- "hit". Hence, sensitivity should be looked upon parallel with tracer-based mapping rates. With further development of the SLN surgical algorithm, the use of robot assisted surgery with an unsurpassed visualization and the use of ICG for mapping and applying reinjection of ICG a bilateral mapping rates in the range of 95% can be achieved, for cervical cancer even higher likely, due to younger women [137].

**Third**, the quality of the backup lymphadenectomy in terms of harvested nodes and harvested nodal compartments is if importance despite no consensus of the number of nodes required for an adequate back-up lymphadenectomy, neither which compartments should be explored, i.e whether the presacral pelvic nodes should be removed.

**Fourth**, the median number of SLNs per woman is an indicator of the meticulousness of the SLN algorithm, the surgical quality and which nodal compartments are explored. Furthermore, not all studies report whether the number of SLNs perceived by the surgeon or perceived by the pathologist, the latter resulting in a 30-50% higher number [67]. A median of two-three SLNs, in particular if estimated by the pathologist indicate that all relevant pathways have not been explored.

**Fifth**, sensitivity to detect pelvic nodal metastatic disease can also be evaluated by subgroup-size comparisons of the proportions of (SLN-) metastases with other studies where a similar pathologic processing with ultrastaging and IHC has been performed and a similar subgrouping is applied. Given adequate data to compare with, this evaluation is likely the best to use for an overall evaluation of efficacy as it takes all aspects of above into consideration and in particular represents the only mode for comparison when no backup lymphadenectomy has been performed, i.e in SLN-only studies.

**Finally**, it should be kept in mind that ultrastaging and IHC rarely is performed on completory PLND /PALND nodes why a non-metastatic SLN still may coincide with a pelvic and/or paraaortic non-SLN with undetected small volume metastases. Theses nodes, however, would have remained undetected without the SLN concept.

## 3.7 Ultrastaging of sentinel lymph node

Pathology assessment of SLNs employs ultrastaging to detect low-volume metastases. The definition of LNM was based on the American Joint Committee on Cancer (AJCC) staging system for axillary nodes in breast cancer [138].

Specifically:

• Macro-metastases = tumor greater than 2.0 mm in diameter.

• Micro-metastases = tumor cell aggregates between 0.2 and 2.0 mm in diameter.

• Isolated tumor cells =individual tumor cells or aggregates that are less than 0.2mm in diameter, usually detected by IHC.

• Tumor absent – no tumor cells identified in H&E (or immunohistochemically, if applicable) stained sections

Ultrastaging of SLNs in EC increase detection of MIMs and ITCs, frequently missed in routine evaluations.

## 3.8 Sentinel lymph node in endometrial cancer

The potential of the SLN technique in EC has been acknowledged. However, a substantial number of studies do not account for lymphatic anatomy or lack a clear definition of a SLN.

This risk of missing nodal metastases by a SLN-algorithm was explored in a study by Barlin et al. on early-stage EC [126]. False negative rates significantly decreased when applying an overall algorithm involving removal of enlarged nodes and a side specific PLND in case of failed mapping. However, the algorithm does not include a clear definition of a SLN.

The European SENTI-ENDO trial (2011) is an early basically well-designed prospective study evaluating the accuracy of SLN biopsy using cervical injection of radiotracer (Technetium 99) and patent blue dye [128]. Detection of SLNs was performed by open or laparoscopic surgery why the result may not be applicable to studies utilizing ICG as tracer and robot assisted surgery. There is limited information on the definition of a SLN. The study was published before onset of study I on this thesis [128].

The FIRES (2017) study was the first adequately powered prospective study evaluating SLN mapping by ICG and robotic surgery [139]. The bilateral mapping was 52% and reinjection of ICG was not performed. There is limited information on the exact definition of a SLN. The study demonstrated a sensitivity of 97.2% and a false-negative rate of 2.8%. This calculation however, incorporated isolated metastatic paraaortic ICG positive nodes as "SLNs", from a strict SLN definition perspective questionable. The low bilateral mapping rates may be attributed to the inclusion of institutions and surgeons with varying initial experience in SLN mapping [139].

Holloway et al. (2017) compared the use of blue dye alone or in combination with ICG in a proportion of 20/180 in a single institution prospective study, achieving a bilateral mapping rate of 84% and a sensitivity of 97.5% (95% CI 85-100%) based on 42 node positive women. Paraaortic SLNs were defined [140].

In the SENTOR (2021) prospective trial, sensitivity analysis was based on 27 node positive women with intermediate and high-grade EC [141]. The bilateral mapping rate of ICG was 77.6% and one false negative case occurred. The study involved three centres and 14 surgeons. The results support previous studies on the reliability of a SLN algorithm by the use of ICG and robotic surgery [141].

The SENTIREC-endo study (2024) combined a preoperative PET-CT with detection of SLN by ICG during robotic surgery [142]. Five algorithms including PET-CT were used to assess sensitivity and false negative rates based on 42 node positive women. The publication contains no information on definitions of SLNs. The study, performed mainly at three academic institutions, achieved an ICG-based sensitivity of 86% and a bilateral mapping rate of 73% [142].

Details of included studies are available in table 3.

In all, the studies confirm the overall feasibility of detection of SLNs on endometrial cancer. The studies demonstrate that sensitivity and mapping rates achieved in a multi-centre setting under an implementation phase may have a potential of improvement in terms of mapping rates and sensitivity.

**Table 3**. Summary of significant studies on detection of SLN in EC. Prospective studies with <15 node positive women for evaluation of sensitivity and retrospective studies excluded.

| Study | N. of centres/ Surgeons/Surgical approach | % node positivity/ N+women for sens. analysis | % women with high-risk histol. | % PALND to LRV | Tracer | Bilateral mapping | Re-injection of tracer |
|---|---|---|---|---|---|---|---|
| **Ballester et al.[128] SENTI-ENDO 2011** | -9<br>-ND<br>-Open/Lap | -17% (19/111)<br>-19 N+ Women | 13% | 12% PALND Level ND | Tc-99<br>Blue dye | 62% | no |
| **Barlin et [126] 2012** | -1<br>-10 surg<br>-Open/ Lap/Robot | -11.8%<br>-47 N+ women | 21.1% | % PALND ND | Blue dye<br>Tc-99<br>40women<br>Mix cx/ fundal | 51% | No |
| **Rossi et al.[139] FIRES 2017** | -10<br>-18 surg<br>-Robot | -12%<br>-37 N+ women | 28% | 58% PALND Level ND | ICG | 52% | no |
| **Soliman et al.[143] 2017** | -Single Center<br>-14 surg<br>-Open/ Lap/Robot | -22.8%<br>-23 N+ women | 56% | 46% PALND to LRV | Tc-99<br>Blue dye<br>ICG | 58% | no |
| **Holloway et al.[140] 2017** | -1<br>-5 surg<br>-Robot | -21%<br>-42 N+ women | 27% | 47% PALND Level ND | Blue dye<br>+- ICG | 84% Blue dye +ICG | no |
| **Persson et al.[67] SHREC 2019** | -2<br>-5 surg<br>-Robot | -21%<br>-54 N+ women | 49.2% | 81% PALND to LRV | ICG | 95% reinj<br>82% single injetcion | yes |
| **Cusimano et al. [141] SENTOR 2021** | -3<br>-14 surg | -17%<br>-27 N+ women | 80.8% | 80% PALND Level ND | ICG | 77.6% | no |
| **Bjørnholt et al. 2024 SENTIREC-endo [142]** | -3<br>-Surg ND<br>-Robot | -24,7%<br>-42 N+ women | 100% | 60,5% PALND to LRV | ICG | 73 % | no |

| Clear definition of SLN | Paraaortic SLNs defined | Number of SLNs as perceived by surgeon/ Histology | -Sensitivity -94% CI -False neg pelvic SLN | Pelvic lymph node count | Paraaortic lymph node count | Power analysis for sensitivity analyses |
|---|---|---|---|---|---|---|
| no | yes | -1 (0-6)<br>-3 (1-9) | -Sensitivity 84%<br>-95% CI 62–95 %<br>-2/19 (10,5%) | 14(1-50) | 9 (4-26)<br>12 women | Yes<br>Sample size 144 women with ≥1 SLN |
| no | Yes 2 isolatedPA “SLN”+ in analysis) | -ND<br>-3 (0-15) | -85 %<br>-CI NR<br>-7/47 (15%) by tracer | 8 (0-59) | NR | ND<br>Review of database |
| yes | Yes 3 isolated included in analysis | -ND<br>-2 (0-20) | -94%<br>-95% CI 70-100<br>-4/37 (11.1%) | 19 (1-61) Total nodes removed | NR | Yes<br>Interim analysis at 36 N+ women |
| no | no | -2 (1-9)<br>-ND | -95%<br>95% CI 75-100<br>-1/23 (4.3%) | 17(4-36) | 6 (0-28) | Yes analysis at 25 N+ women |
| yes | Algorithm included pelvic and paraarotic nodes | -2 (0-4)<br>-ND | -97.5%<br>-95% CI 85-100 %<br>-2;5% unclear denominator | 18 (11-25) | 9 (4-15) | Yes 200 women for tracer comparison<br>Not for sensitivity analyses |
| yes | no | -4 (1-7)<br>-6 (1-22) | -100%<br>-95% CI 89-100<br>-0/54 0% | 29 (8-75) | 12 (2-51) | Yes interim analysis at 50 N+ women |
| yes | yes | -3 (2-5)<br>-ND | -96.3%<br>-95%CI 81-100%,<br>-3 /26 (11.1%) by tracer | 16 (12-20) | 5 (3-9) | Yes Interim analysis at 27 N+ women |
| no | Yes<br>No isolated Included in analysis | -ND<br>-3 (2-4) | -86% by tracer alone<br>-95% CI 74–100<br>-6/24 (14.2%) by tracer alone<br>Preoperative CT PET | 15 (12-20) | 10 (6-15) | Yes<br>Based on NPV 150 women estimated 20% node positivity |

## 3.9 Evaluating and comparing sensitivity

The ideal developmental steps of a SLN concept have been described above. The steps needed for improvement of a SLN concept must be seen from an individual institution's, or even from an individual surgeon's perspective.

It is, with given sensitivity data from existing well designed prospective studies, both unrealistic and unethical for an institution to entertain a separate adequately powered study on sensitivity (requiring backup PLND). Still, it is of great importance, even for institutions with previous experience, to continuously evaluate their results.

There are several indirect measures of accuracy that can be used for quality control:

1. **The incidence of metastatic SLNs** within defined subgroups is the best overall measure of performance of the SLN detection. Quality studies on SLN where ultrastaging has been performed on SLN should be the comparison. Inferior results call for evaluation of individual factors as of below.

2**. Bilateral mapping rates;** bilateral mapping seems to be lower in studies where many surgeons are involve indicating an effect of surgical experience and/or injection techniques. With reinjection of ICG a bilateral mapping rate of up to 95 % is proven feasible. A lower mapping rate indicates a potential of improvement. ICG should be injected slowly with a thin needle and at least a 5-10 minute interval between injection and exploration of the spaces is beneficial. A thorough exploration of all nodal spaces is particularly important in obese women.

3. **The number of retrieved SLNs** (as perceived by the surgeon); adherence to and understanding of a lymphatic anatomy based SLN concept will lead to at least four SLNs per women if SLNs along the UPP is aimed for, another two SLNs if SLNs along the LPP is included. A lower number indicates that SLNs along sub-pathways within first and foremost the UPP are not consistently looked for. While external iliac SLNs are easily removed, SLNs in the obturator fossa, where most SLN metastases located, is technically more challenging to remove.

3. **The proportion of "empty pockets"** i.e ICG positive tissue perceived by the surgeon as a SLN but where the pathologist fails to find a lymph node. This may be a sign of ICG overdose or an inadequate surgical technique.

5. **Overall feasibility,** i.e the proportion of women with a successful SLN procedure is more of a measure of overall surgical experience and results from experienced centres may be aimed for.

**6. Complications** associated with detection of SLN as such. Of particular interest are injuries to the obturator nerve and major vessels.

Most of recent prospective studies on SLN detection in EC is performed with robot assisted laparoscopy with the use of ICG as tracer. A general good experience with robot assisted surgery is implicated. A review from 2016 comparing surgical approaches did not find a difference in bilateral mapping rates between open surgery, laparoscopy and robot assisted surgery [129]. The average bilateral mapping rate in that study was as low as 50% and a variety of tracers alone or in combination was utilized, Therefore the results are likely non-valid given the development of SLN algorithms and in robot assisted surgery and the demonstrated high mapping rates [129]. The discussion as of above and beneath refers to SLN detection by the use of ICG as tracer during robot assisted surgery.

## 3.10 Oncological outcomes of sentinel lymph node mapping

In a retrospective study by Raimond et al., involving 304 women with low- or intermediate-risk EC, SLN mapping identified metastatic nodes in 16.2% of women, significantly more than the 5.1% detection rate with LND ($p$=0.03). No difference in recurrence-free survival between the two groups was found, partly due to a lack of standardized adjuvant treatments and level of power of the study [144].

Comparisons between SLN mapping and LND were also explored in historical cohorts from the Mayo Clinic and the MSKCC [145, 146]. As expected, SLN mapping led to a lower median number of lymph nodes removed compared to LND but increased the detection rate of pelvic metastases (5.1% vs. 2.6%, $p$=0.03). However, para-aortic metastasis detection and 3-year disease-free survival rates were similar between groups (94.9% in the SLN cohort and 96.8% in the LND cohort, $p$=0.35) [145].

Capozzi et al. conducted a retrospective analysis of the long-term survival in high-risk women who underwent SLN detection alone versus a systematic PLND. The findings suggest that a systematic lymphadenectomy offers no survival advantage compared with SLN mapping alone for high-risk EC over an extended observational period [147].

A European study analyzed 171 high-risk EC women, finding similar 5-year recurrence-free survival rates between SLN (79.2%) and LND cohorts (81.6%) [148].

Overall, in high-risk EC the oncologic outcomes of SLN mapping versus LND remains unclear. However, in a non-inferiority situation the proven higher detection rate of

pelvic nodal involvement following SLN detection, and the lower incidence of long-term complications promote detection of SLN.

## 3.11 Morbidity after sentinel lymph node biopsy

Papadia et al. has examined intraoperative outcomes, showing that SLN biopsy is associated with shorter operative times and reduced estimated blood loss compared to PLND and PALND [149].

Geppert et al. analyzed data from 188 women with EC, showcasing the high feasibility, no intraoperative complications, and the low risk of lymphatic complications following SLNB in low-risk EC women [150].

Casarin et al. assessed the lymphatic-specific morbidity, focusing on lower extremity lymphedema, associated with laparoscopic management of early-stage EC using the SLN algorithm. Their findings indicated a significant reduction in lymphatic complications with SLN mapping compared to systematic lymph node dissection [151].

Bjørnholt et al. investigated the risk of lymphedema following SLN mapping in women with low-grade EC. Moreover, they examined the risk factors for lymphedema. The results showed that women with low-grade EC are at low risk of developing lymphedema after SLN mapping. Increased lymphedema 12 months after surgery was predicted by baseline leg swelling and body mass index [152].

In summary, present studies indicate a low risk for lymphatic complications following SLN biopsy.

## 3.12 Integration into clinical practice

SLNB has been increasingly implemented in clinical guidelines reflecting its growing acceptance. For example:

• *NCCN Guidelines*: Initially recommending SLN mapping as an alternative to LND for select women, the NCCN now supports a SLN biopsy in all risk groups of uterine confined EC [79].

• *European Guidelines*: The ESGO quality indicators for EC surgery recommend SLN biopsy for high and intermediate risk EC and to be considered for low risk EC [153].

• The *Swedish National Guidelines* (NCCCG) states detection of SLN for women with presumed uterine confined EC of all risk groups [46].

These national endorsements provide the basis for evaluating recurrence and survival following detection of SLNs where new patient cohorts are identified, such as low grade/ risk EC women with known nodal status. A summary of the characteristics and recommendations of various guidelines are shown in table 4.

**Table 4.** comparison summary of the characteristics and recommendations of NCCCG (sweden), NCCN, ESGO, BGCS, JSGO guidelines.

| Parameter | NCCCG (Swe)[46] | NCCN[79] | ESGO[153] | BGCS[154] | JSGO[155] |
|---|---|---|---|---|---|
| **Indications:** | | | | | |
| -Stages I–II (uterineconfined) (low/intermediate risk) | Recommended | May be considered | Can be considered | Can be considered | An option. Omission of full LND is suggested |
| -High grade (grade 3,clear cell/serous/carcinosarcoma)(intermediate–high/high risk) | Recommended | Potential alternative to full LND | Acceptable alternative to full LND in stagesI–II | Can be considered | Not mentioned |
| **Techniques—dyes:** | | | | | |
| Colorimetric mapping | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned |
| Radionuclear method:Tc99 | Not mentionated | Most commonly used | Not mentioned | Not mentioned | Not mentioned |
| Near infraredmethod: ICG dye | Highest detection rate | Very high detection rate | Highest detection rate | Highest detection rate | Not mentioned |
| **Injection site** | Cervix. | Cervix- superficial and deep | Cervix | Cervix | Not mentioned |
| **Failed mapping** | Selective sampling an option | Side-specific LND | Side-specific LND in intermediate–high−/high-risk women | Full LND | Not mentioned |
| **Ultrastaging** | Increases the detection of ITCs and micrometastasis | Important for detection of low volume metastasis | Is recommended | Should be used | Not mentioned |
| **Positive pelvic SLN** | Para-aortic staging can be considered | PALND(at attending discretion) | Para-aortic staging can be considered (imaging or surgery) | Not mentioned | Not mentioned |
| **Frozen section of SLN** | Not mentioned | Only if suspicious | Not mentioned | Not mentioned | Not mentioned |
| **No. references** | 26 | 32 | 72 | - | 8 |

Abbreviations: BGCS, British Gynecological Cancer Society; ESGO, European Society of Gynecological Oncology; JSGO, Japan Society of Gynecologic Oncology; LND, lymphadenectomy; LND, lymphadenectomy; NCCN, NationalComprehensive Cancer Network; NCCCG: National Clinical Cancer Care Guidelines (Sweden); SLN, sentinel lymph node; Tc99, technetium 99. Adapted from Dick et al. with kind permission of Wiley [156].

# 4 Rationale and scientific gaps

**Gap 1**. At the time for designing study I in the thesis (the SHREC-study) no adequately powered prospective studies evaluating sensitivity of detection of SLNs by the use of ICG in EC were available. Existing studies demonstrated low bilateral mapping rates and a general lack of clear definitions of a SLN.

**Gap 2.** There were few data on the risk for pelvic SLN metastases by different anatomic positions.

Two research questions arose during first years of data retrieval:

A. Can a full LND be safely replaced by a selective anatomically based nodal sampling in case of non-mapping? This would greatly diminish lymphatic complications and facilitate an implementation of SLN biopsy in low-risk EC.

B. Can a selective anatomically based nodal sampling add to sensitivity of the SLN concept?

**Gap 3.** No population based prospective data on the risk for SLN metastases utilizing ultrastaging and immunohistochemistry from a sufficiently large cohort of women with EC was available.

# 5 Aims

## 5.1 Summary of overall/general aims of the thesis

The first overall aim of this thesis was to evaluate a new developed anatomically based SLN algorithm with a clear definition of SLN on women with preoperatively presumed high risk uterine confined EC as defined by the national Swedish guidelines at onset of the study (study I, the SHREC study). The guidelines recommended a PLND and IRPALND why the addition of an initial detection and removal of pelvic SLN was considered ethically justified and the completory lymphadenectomy as of above enabled women to act as their own controls.

The new algorithm is a part of a previous thesis at our institution (ISBN: 978-91-7619-570-3 Dr Barbara Geppert) containing the published studies on lymphatic anatomy (UPP and LPP respectively described) and the defined surgical algorithm with a strict definition of SLNs as the juxtauterine mapped nodes within pathways allowing for sub-pathways [125, 127]. To ensure a high internal validity only five surgeons were involved, of which two surgeons outside our institution had a site visit in Lund followed by a local audit by the surgeon responsible for the protocol to ensure adherence to the protocol.

In parallel to the study as of above, applying the same strict algorithm, detection of SLN only (i.e with no back-up PLND but a IRPALND upfront or as a restaging in selected cases) was for a start performed on preoperatively presumed FIGO grade 1-2 (low grade) EC without deep MI, after finalizing study I, on all subtypes of presumed uterine confined EC.

A continuous evaluation of results was performed, and three main additional research questions were addressed (see rationale and gaps) within the existing protocols. The addressing of those research questions was made possible by the prospective intraoperative mapping of positions and types of all SLNs utilizing designated anatomical charts (see above) and ultrastaging and IHC on all SLNs over the years.

**First**, as a full side specific PLND is recommended by the ESGO in case of non-mapping of SLNs, this will potentially lead to a resistance to include SLN detection as

routine in presumed low risk EC, furthermore, inflict an increased risk for lymphatic complications in a proportion of women in the range of 10-30% depending on mapping rates [153]. In case the proportion of women with isolated SLN metastases outside defined typical positions was found low, an anatomically based sampling of nodes at those defined positions could replace a side specific lymphadenectomy and reduce side effects. This was the basis of study II.

**Second**, as prospective population-based data on the risk for pelvic SLN metastases within defined subgroups of EC is lacking the large material achieved by early 2024 enabled us to evaluate not only the proportion of SLN metastases within groups, but also evaluate the proportions of MIMs and ITCs among SLN-positive women. Furthermore, to evaluate the proportions of SLN positive women based on preoperative subgrouping and compare with subgroupings based on final histology. This was the basis of study III.

**Third**, an intriguing result from study I (the SHREC study) was that overall bilateral mapping with ICG was significantly lower in pelvic node positive women, furthermore that mapping in the obturator fossa, a frequent site for nodal metastases, was lower. These finds implicate that sensitivity may be improved by removing nodes at defined typical positions despite mapping at other positions. To answer the question, an amendment to the SLN-algorithm was initiated in 2019 introducing the entity of "SLN anatomy", i.e typically positioned non-mapped nodes per positions above. In all, an attempt to further increase sensitivity and refine the SLN algorithm. This was the basis of study IV.

## 5.2 Specific aims

Study I

• To assess sensitivity and false negative rates of the pelvic SLN concept based on a defined surgical algorithm and with a clear definition of SLN based on described uterine lymphatic anatomy with the use of ICG as tracer in women with high-risk early-stage EC as defined in national guidelines.

• To evaluate if reinjection of tracer increases the bilateral ICG based SLN mapping rates and the mapping rates per defined major lymphatic pathways.

• To evaluate adverse events related the detection of SLN and the occurrence of "isolated" paraaortic LNM adhering to the utilized SLN algorithm.

Study II

• To evaluate the positions of metastatic pelvic SLN and the proportions of SLNs outside and within defined typical anatomical positions along the UPP.

Study III

• To assess the prevalence and size of pelvic SLN-metastases within pre- and postoperatively defined EC risk groups.

Study IV

• To investigate the incidence of isolated non-mapped metastatic pelvic lymph nodes at pre-defined typical anatomic positions.

# 6 Participants and methods

## 6.1 Study populations

Consecutive consenting women with EC suitable for inclusion based on the inclusion criteria for the SHREC study and the SLN-only study respectively.

### 6.1.1 SHREC trial

The study (ClinicalTrials.gov identifier NCT02690259) recruited consecutive consenting women with preoperatively presumed uterine confined high-risk EC as defined by the Swedish national guidelines at onset of the study.

### 6.1.2 SNL only trial

The "SLN-only study" (ClinicalTrials.gov identifier NCT03838055) initially recruited consecutive women with presumed uterine confined low risk EC, following completion of the SHREC study, women with EC of all risk groups and from June 2019 women with EIN.

## 6.2 Treatment description

### 6.2.1 Pelvic sentinel lymph node algorithm

The exact surgical algorithm, definitions a types of SLNs, principles for injection and reinjection of ICG as well as the protocols utilized for nodal positions and types are described in detail in paragraph 3.5 and in a previous publication [125, 127, 137]. An overview of the SLN algorithm is shown in figure 10; the exact boundaries for the completory PLND is shown in table 5.

**Table 5.** Defining the Anatomical Boundaries of Lymph Node Compartments in the Female Pelvis.

| Lymph node compartment | Proximal limit | Lateral limit | Distal limit | Medial limit |
|---|---|---|---|---|
| **External iliac area** | Bifurcation of external and internal iliac artery | Genitofemoral nerve | Cloquet's lymph node | External iliac vein |
| **Obturator fossa** | Internal iliac vein | Ileopsoas muscle | Os pubis, obturator nerve | Obliterated umbilical artery |
| **Common iliac** | Aortic bifurcation | Genitofemoral nerve | Bifurcation of external and internal iliac artery | Common iliac artery |
| **Presacral** | Aortic bifurcation | Common iliac artery | Promontory | Hypogastric nerve (as distinction between right and left) |
| **Lower paraaortic (inframesenteric)** | Inferior mesenteric artery | Ureter | Aortic bifurcation | |
| **Higher paraaortic (infrarenal)** | Left renal vein | Ureter | Inferior mesenteric artery | |

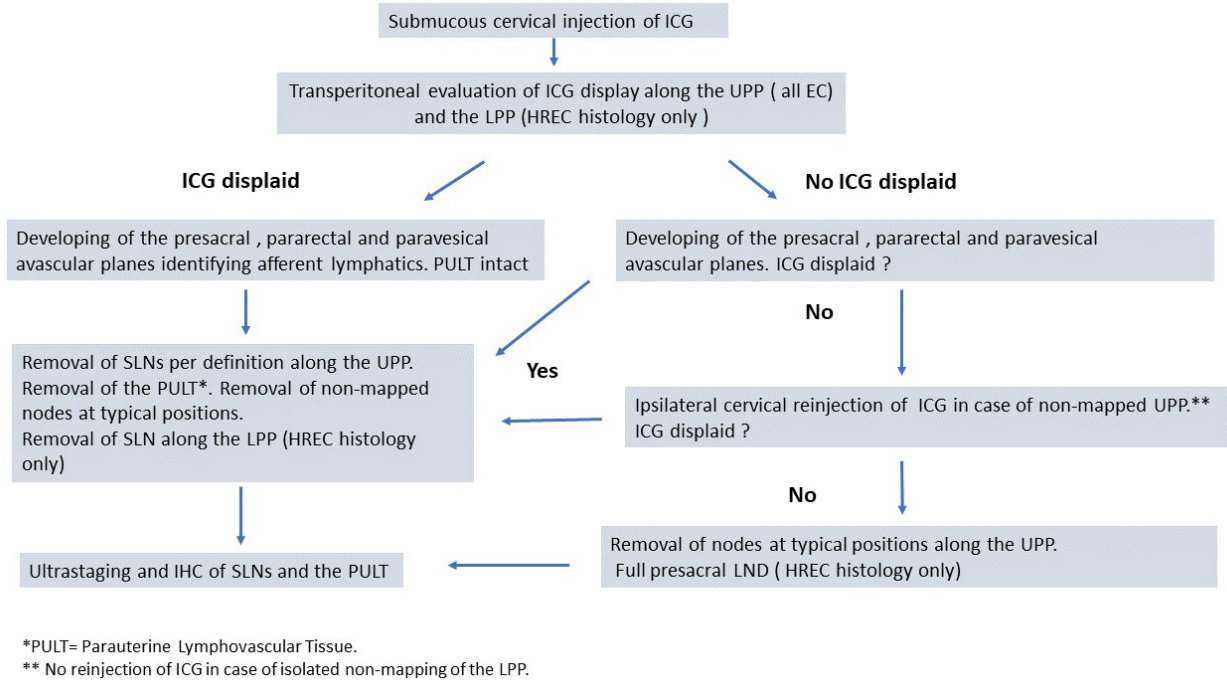



**Figure 10** Surgical algorithm in EC women

### 6.2.2 Pelvic lymphadenectomy

The completory PLND in study I was defined as the removal of all pelvic lymph nodes as described in paragraph 2.2.1.

### 6.2.3 Infrarenal para-aortic lymphadenectomy

The infrarenal para-aortic lymph node dissection (IRPALND) was defined as the removal of all visible paraaortic and paracaval lymphnodes to the level of the left renal vein from the level of the aortic bifurcation.

### 6.2.4 Hysterectomy with a bilateral salpingo-oophorectomy

A robot assisted bilateral salpingo-oophorectomy and a Querleu-Morrow type B1-2 hysterectomy were performed after LND. An infracolic omentectomy was performed in women with a non-endometrioid histology.

### 6.2.5 Histopathologic evaluation of the sentinel nodes

All SLN tissues were embedded and bisected if the minimum thickness exceeded three mm. Ultrastaging using haematoxylin and eosin staining (H&E) was performed in three to five sections at three different levels, 200 µm apart, if the maximum diameter of the sentinel node tissue exceeded one mm. IHC with staining for pan-cytokeratin and cytokeratin MNF 116 was performed. Non-SLNs with a thickness less than three mm were embedded entirely, and for nodes exceeding three mm, at least half the node was embedded. Non-SLNs were stained with H&E but were not subjected to IHC. Metastatic disease was classified according to a modification of the American Joint Committee on Cancer staging definitions for axillary nodes in breast cancer (macrometastases = tumour greater than 2.0 mm in diameter, micrometastases = tumour cell aggregates between 0.2 and 2.0 mm in diameter, isolated tumour cells = individual tumour cells or aggregates that are less than 0.2 mm in diameter and less than 200 cells).

The pathologists were not blinded to the results of SLNs and non-SLNs when performing their assessment.

### 6.2.6 Data management

Data from standardized study protocols were continuously entered to a secured database and assigned a unique CRF-number to ensure anonymity. Women were identified by their study number. During interim- and final analysis, the dataset was monitored by the study coordinators and the principal investigator.

### 6.2.7 Follow-up

Per protocol, peri- and postoperative complications were registered. Postoperative complications were classified by Clavien-Dindo criteria system [157]. Per protocol, clinical follow-ups were conducted every six months for at least two years to monitor surgical and lymphatic complications and recurrencies, the latter outside the aims of this thesis.

## 6.3 Statistical analysis plan

*Study I*

The analysis of sensitivity and negative predictive value was evaluated per patient with regard to the SLN-ICG- and the overall SLN algorithms. As at least a full PLND was performed after the removal of SLNs, each woman served as her own control in terms of pelvic nodal status. All women who underwent the planned procedure according to protocol were included in the analyses of the primary outcome. All women injected with ICG were included in the safety assessment. As it has the highest probability of early termination under the efficacy hypothesis, we used the Fleming two-stage design for determination of the sample size, interim analysis and decision to stop accrual based on sensitivity [158]. The null hypothesis that sensitivity was 85% was tested against a one-sided alternative with a desired sensitivity of at least 92.5%. The interim analysis was planned after 50 ICG-mapped women with pelvic LNMs were identified. Enrolment was paused, and interim analyses were performed accordingly. Enrolled women awaiting final histology at this point were included. If the number of patients with pelvic LNM correctly identified by at least one ICG-mapped SLN was equal to or lower than 43, the study would be stopped for futility. If the number of patients identified was equal to or higher than 48, the hypothesis of inefficacy could be rejected with no further enrolment. If the total number of successes was between the lower and the upper cut-off points, the trial would continue by including an additional 69 patients with pelvic LNMs. The hypothesis of inefficacy would be rejected if 107 or more patients with pelvic LNMs were correctly identified by an ICG-mapped SLN. This

design yielded a type 1 error of 0.05 and a power of 0.8 when the true sensitivity is 92.5%.

Exact 95% confidence intervals (CIs) and sensitivity and negative predictive values are reported and estimated by proportions. Descriptive data are presented with numbers and percentage or median and range.

The *SLN-only study* (the basis for studies 2-4)

As no back-up LND was performed, we initially powered the study from a non-inferiority perspective to detect nodal metastases compared with available published results (data on the risk for pelvic nodal metastases without ultrastaging and immunohistochemistry). Evaluating the results it became clear that detection rates of pelvic SLN metastases was higher and the study was continued to answer other research questions, some of which not included in this thesis. As no distinct sample size analysis could be performed, this study was planned to go on until at least 1500 women were included as some subparts not included in this thesis will need a larger number of included women than other subparts. The study can be considered hypothesis generating in several aspects. The study also acts as a continuous internal quality assessment as recommended by the Swedish guidelines. The study complies with the national treatment protocol for endometrial cancer.

*Study II-IV*

No sample size was performed. We examined:

-The distribution of metastatic pelvic SLN, focusing on the relative proportions of SLNs located within and outside predetermined anatomical positions along the UPP.

-The relationship between the prevalence and size of pelvic SLN metastases and risk factors in EC, as well as the incidence of non-mapped isolated metastatic pelvic lymph nodes at predefined anatomical positions.

Statistical methods included chi-square tests or Fisher's exact tests. A $p$-value of $<0.01$ was considered significant and analyses were conducted according to the intention-to-treat principle.

## 6.4 Ethical considerations

All studies were approved by the appropriate Institutional or National ethics committees (DNR 2013/163, DNR 2018/541 and DNR 2023-01299-01). All

participants gave their informed written consent. As the national guidelines recommended a PLD and PALND in women with high-risk EC, no additional risk for perioperative or lymphatic complications was estimated in study one, but a longer operative time due do the addition of the SLN-algorithm. This was considered acceptable. For the SLN-only study in low-risk EC the SLN algorithm prolonged surgical time and could add peri- and postoperative complications attributable to the SLN biopsy as such. This was weighed against the potential benefit of a diagnose of otherwise undetected pelvic nodal metastases.

The diagnose of a single ITC did not alter adjuvant treatment but the awareness of its presence might add anxiety for those women. Women with ≥ 2 nodes with ITC were offered adjuvant treatment in the absence of other risk factors. This principle was decided by the national guidelines committee outside the protocols but represents a consequence of the implementation of SNLB.

ICG was administered to all women. ICG contains iodine and can cause allergic reactions. In women denying such allergy, reactions still can occur, but the risk is considered very low [159].

## 6.5 Methodological consideration

### 6.5.1 Histopathological assessment of sentinel and non-sentinel lymph node

Ultrastaging was utilized for the histopathologic evaluation of the SLNs, while non-SLNs were assessed using conventional sectioning. This may have introduced a systematic bias in the SHREC study. Ideally, ultrastaging of pelvic and paraaortic non-SLNs would have rendered a more accurate evaluation of sensitivity. For resource reasons this was not possible to achieve.

### 6.5.2 External validity

Notably, the surgeries were performed by only five accredited surgeons, initially at two tertiary referral centres with high volumes, for the majority of included surgeries at a single centre. This may limit generalizability of results.

### 6.5.3 Confounders, strengths and weaknesses

**Confounders:**

- Older women may have less prominent lymphatic drainage due to a less vascularized uterus. Obesity can affect the visibility of ICG and accessibility of lymph nodes during surgery. Data were not stratified for these factors.

- Despite efforts to ensure initial surgical competence, it is likely that surgical skill and experience in the execution of the SLN-algorithm increased over the 10 years of these studies. Data were not adjusted for this potential bias.

- Anatomical variability. Individual differences in lymphatic anatomy could affect the likelihood of SLN detection and the distribution of metastatic nodes.

**Strengths:**

- Prospective design and large sample size: the prospective nature of study III ensures systematic data collection and reduces bias, improving the reliability of the results. The study includes a large population-based cohort providing robust data for analysis and increasing the generalizability of the findings.

- Detailed Methodology: The use of a well-defined, anatomically based SLN-algorithm and ultrastaging and IHC for SLN examination enhances the accuracy of metastasis detection.

- Experienced Surgeons: Procedures are performed or supervised by experienced surgeons, ensuring high internal validity and consistency in surgical techniques.

**Weaknesses:**

- Study II-IV are conducted at a single institution which may limit the generalizability of the results to other settings and populations.

- Until June 2018 it was not mandatory for the pathologist to report ITC. This have likely resulted in an underestimation of the overall risk of metastases and proportion of SLNs with ITCs.

- The results in study III are based on a restricted regional northern European population and may not be directly applicable to other demographic areas. We believe that this limitation is minor.

# 7 Results

## 7.1 Study I

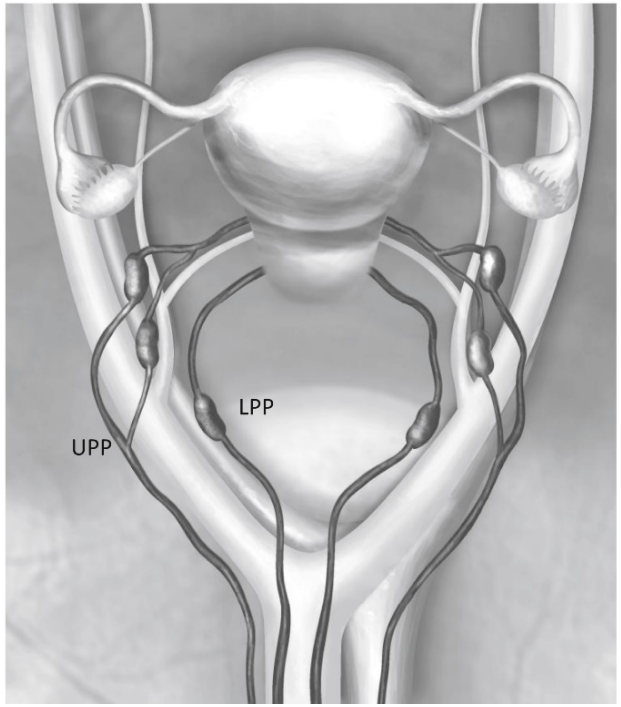
Between June 2014 and May 2018, a total of 355 women were assessed for eligibility in the study. Of these, 275 women were enrolled. All participants were classified as having high-risk EC (HREC) based on preoperative assessments.The overall pelvic SLN algorithm had a sensitivity of 100% (95% CI 92-100) and a negative predictive value of 100% (95% CI 98-100). The ICG based algorithm had a the sensitivity of 98% (95% CI 89-100) and a negative predictive value of 100% (95% CI 97-100). The overall mapping rate, defined as the detection of at least one ICG-positive SLN, was 95% before reinjection and 98% after reinjection, while the bilateral mapping rate was 83% and 95%, respectively. Of the 257 women analyzed per protocol, 54 had pelvic metastases (21%) and two isolated paraaortic LNM (1%). All 54 pelvic node positive women were identified by the overall SLN algorithm (including SLN macro) whereas 52/54 were correctly identified by the SLN-ICG algorithm. All but one pelvic node positive woman were identified by a SLN along the UPP. One woman had an isolated pre-sacral SLN metastasis. No cases of isolated metastatic PULT nodes were observed. The anatomic location, distribution and frequency of LNM and SLNs are presented in Figure 11 and Table 6.

**Left obturator area:**
SLN-ICG[1]: 63%
Metastatic SLNs[2]: 25%
Any metastatic LN[2]: 34%

**Left external iliac area:**
SLN-ICG[1]: 82%
Metastatic SLNs[2]: 41%
Any metastatic LN[2]: 45%

**Left presacral area:**
SLN-ICG[1]: 52%
Metastatic SLNs[2]: 5%
Any metastatic LN[2]: 13%

**Left common iliac area:**
SLN-ICG[1]: 13%
Metastatic SLNs[2]: 2%
Any metastatic LN[2]: 7%




**Right Obturator area:**
SLN-ICG[1]: 62%
Metastatic SLNs[2]: 36%
Any metastatic LN[2]: 43%

**Right external iliac area:**
SLN-ICG[1]: 80%
Metastatic SLNs[2]: 25%
Any metastatic LN[2]: 29%

**Right presacral area:**
SLN-ICG[1]: 59%
Metastatic SLNs[2]: 9%
Any metastatic LN[2]: 13%

**Right common iliac area:**
SLN-ICG[1]: 13%
Metastatic SLNs[2]: 4%
Any metastatic LN[2]: 16%

**Figure 11** Distribution and typical localisations of pelvic SLNs defined by ICG and proportion of metastatic SLNs per pelvic lymphatic compartment/uterine lymphatic pathway in high-risk EC. [1]Percentages refer to the total number of women. [2]Percentages refer to the total number of node-positive women; more than one ICG positive or metastatic SLN was possible per women [67].

**Table 6** Incidence and anatomical localization of metastatic lymph nodes among 257 women with preoperatively High-Risk EC [67].

| **Number of women** | **Endometrioid histology (*n* = 166)** | | **Non-endometrioid histology (*n* = 91)** | |
|---|---|---|---|---|
| **Metastatic lymph nodes (all positions)** | 31 (18.7%) | | 25 (27.5%) | |
| **Upper paracervical pathway** | 31 (18.7% [a] /100% [b]) | | 22 (24.2% [a] /88% [b]) | |
| **Lower paracervical pathway** | 3 (1.8% [a] /9.7% [b]) | | 8 [c] (8.8% [a] /32% [b]) | |
| **Paraaortic** | 10 (7.4%[d]/40%[e]) | | 13 (15.9%[d]/54.2%[e]) | |
| **Isolated paraaortic** | 0 (0%[d]/0%[e]) | | 2 (2.4%[d]/8.3%[e]) | |
| **In SLNs only** | 18 (10.8% [a] /58.1% [b]) | | 11 (12.1% [a]/44% [b]) | |
| **Parametria** | 5 (3.1%[a] /16.1% [b]) | | 3 (3.3% [a] /12% [b]) | |
| **Identified by SLN's with micrometastases or isolated tumor cells only** | 11 (6.6% [a]/35.5% [b]) | | 8 (8.8%[1]/32% [b]) | |
| **Micrometastase in 1 SLN** | 2 (6.4% [b]) | | 3 (12% [b]) | |
| **Micrometastase in >1 SLN** | 3 (9.7% [b]) | | 1 (4% [b]) | |
| **Isolated tumor cells in 1 SLN** | 4 (12.9% [b]) | | 2 (8% [b]) | |
| **Isolated tumor cells in >1 SLN** | 2 (6.4% [b]) | | 2 (8% [b]) | |
| | **Paraaortic metastases** | **Pelvic metastases in Non-SLN** | **Paraaortic metastases** | **Pelvic metastases in Non-SLN** |
| **Micrometastase in 1 SLN** | 0/2 | 0/2 | 1/3 | 0/3 |
| **Micrometastases in >1 SLN** | 1/3 | 1/3 | 0/1 | 0/1 |
| **Isolated tumor cells in 1 SLN** | 0/2 [d] | 0/4 | 0/2 | 0/2 |
| **Isolated tumor cells in >1 SLN** | 0/2 | 0/2 | 1/2 | 0/2 |

[a] refers to all women with that histology
[b] refers to all node positive women with that histology
[c] one isolated
[d] refers to all node positive women with a PALND with that histology
[e] refers to all women with a PALND with that histology

No complications attributable to the injection of ICG or the SLN procedure as such were noted in procotol. Eighty-five (32%) women had a postoperative complication within 30 days after surgery. According to the Clavien-Dindo classification, 64 (24%) had grade I-II, 19 (7%) had grade III and two (1%) had grade IV complication. Nine women (3%) experienced a serious adverse event, three during surgery and six after surgery. The readmission and reoperation rate within 30 days postoperatively was 3% and 7%, respectively.

## 7.2 Study II

Data from 1,060 women were retrieved for the analysis. Confirming the find in the SHREC study, pelvic node-positive women had significantly more often an incomplete ICG mapping compared with node negative women. (20/162, 12.3%) compared to those without metastases (37/898, 4.1%; $p$<.001). Among 162 women with SLN metastases along the UPP, the external iliac region had a higher mapping rate compared to the obturator fossa (92.3% vs. 76.3%; $p$<.001) and a higher incidence of atypically positioned SLNs (14.9% vs. 4.9% $p$<.01). However, the obturator fossa contained isolated SLN-metastases in 25.3% of women and accounted for 49.1% of all SLN metastases. The PULT harboured metastatic nodes in 22/162 node positive women (13.6%) of which three (1.9%) were isolated. Among women with non-endometrioid histology, isolated metastases along the LPP were found in two of 53 (3.8%) node-positive women. Details and locations of all metastatic SLNs, including isolated ones, are outlined in Figure 12 and Table 7.

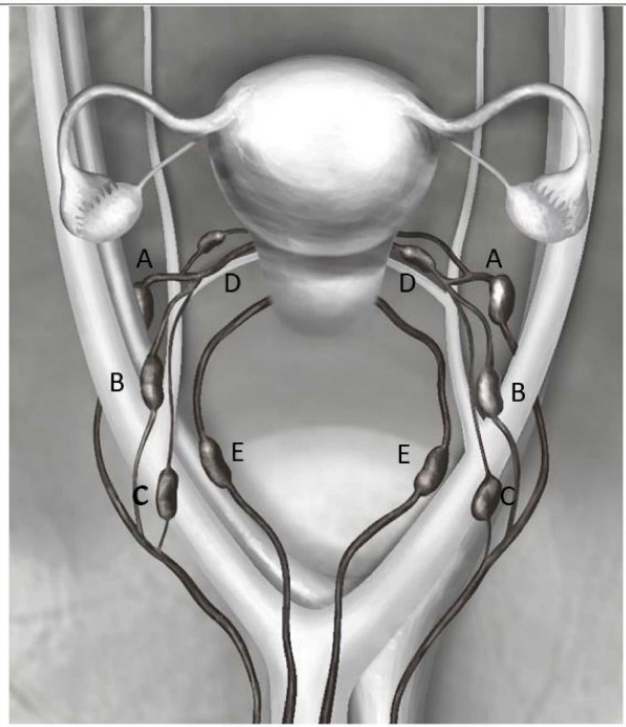
| LEFT | | RIGHT |
|---|---|---|
| **Obturator fossa (A)**<br>83/342 (24.3%) |  | **Obturator fossa (A)**<br>85/342 (24.8%) |
| **Interiliac area (B)**<br>54/342 (15.8%) | | **Interiliac area (B)**<br>39/342 (11.4%) |
| **Lateral external/ distal common iliac (C)**<br>8/342 (2.3%) | | **Lateral external/ distal common iliac (C)**<br>7/342 (2.1%) |
| **PULT (D)**<br>15/342 (4.4%) | | **PULT (D)**<br>9/342 (2.6%) |
| **Presacral area (E)**<br>24/218 (11%) | | **Presacral area (E)**<br>18/218 (8,3%) |

**Figure12** Anatomic distributions, and proportions per compartments, of all metastatic SLNs in EC [160].

**Table 7** Schematic overview of typical and atypical localizations of metastatic pelvic SLNs per compartments [160].

| | **External iliac area** | | **Obturator fossa** | | **PULT** | **Presacral area** |
|---|---|---|---|---|---|---|
| **Data as *n* (%) as appropriate** | **Typical position** | **Atypical position** | **Typical position** | **Atypical position** | | |
| **Proportion of all metastatic SLNs** | 93/342 (27.2%) | 15/342 (4.4%) | 154/342 (45%) | 14/342 (4.1%) | 24/342 (7%) | 42/342 (12.3%) |
| **Proportion of women with metastatic SLN per position** | 79/162 (48.7%) | 15/162 (9.2%) | 114/162 (70.4%) | 13/162 (8%) | 22/162 (13.6%) | 20/53* (38%) |
| **Proportion women with isolated metastatic SLN per position** | 24/162 (14.8%) | 3/162 (1.8%) | 37/162 (22.8%) | 4/162 (2.5%) | 3/162 (1.9%) | 2/53* (3.8%) |
| Compartments presented independently from each other, implying that each women can have SLN's in multiple compartments.<br>* women with non-endometrioid histology EC | | | | | | |

## 7.3 Study III

Data from 1,101 women were included in the analysis. The risk of metastases, based on pre- and postoperative risk group classifications, is outlined in Table 8. Two or more metastatic SLNs (median 2, range 1-12) were found in 94 out of 174 (54.0%) women, with the proportion ranging from 35% in women with ITCs only, to 73% in those with MAM. SLN metastases of any size were detected in 49 out of 501 (9.8%) women with preoperatively presumed low-grade endometrioid uterine stage IA cancer, compared with 6.4% in women with low-grade endometrioid cancer uterine stage IA as defined by final histology. A deep (≥50%) MI identified in final histology was associated with a 28.7% risk of metastatic pelvic SLNs in low-grade endometrioid EC, compared with a 58.3% risk in women with high-grade endometrioid EC and a 47.8% risk in those with non-endometrioid EC (Table 8). In low-grade EC, 45 out of 95 (47.4%) women had only ITCs in SLNs, compared with 15 out of 78 (19.2%) women with high-grade or non-endometrioid cancer ($p<.0001$) (Table 9, Figure 13).

**Table 8**. Risk of SLN metastases (including ITC) among pre-and postoperative histologic types and MI estimates [161].

| **Preoperative** | **EIN** | **Low grade endometrioid** | **High grade endometrioid** | **Serous adenocarcinoma** | **Carcinoscarcoma** | **Clear cell carcinoma** | **Other** |
|---|---|---|---|---|---|---|---|
| **Proportion of all women** [a] | 72/1077 (6.7%) [c] | 724/1077(67.3%) | 75/1077 (6.9%) | 125/1077 (11.6%) | 43/1077 (4.0%) | 31/1077 (2.9%) | 7/1077(0.6%) |
| **SLN+ all** [b] | 4/72 (5.6%) | 91/724 (12,7%) | 21/75 (28%) | 38/125 (30.4%) | 9/43 (20.9%) | 10/31 (32.3%) | 1/7(14.3%) |
| **SLN + MI < 50%** [a] | 4/72 (5.6%) | 49/501 (9.8%) | 10/33 (30.5%) | 18/59 (30.5%) | 4/23 (17.4%) | 4/15 (26.6%) | 1/4 (25%) |
| **SLN + MI >50%** [a] | - | 42/223 (18.8%) | 11/42 (26.2%) | 20/66 (30.3%) | 5/20 (25%) | 6 /16 (37.5%) | -/3 (0%) |
| **Postoperative** | **EIN** | **Low grade endometrioid** | **High grade endometrioid** | **Serous adenocarcinoma** | **Carcinoscarcoma** | **Clear cell carcinoma** | **Other** |
| **Proportion of all women** | 56/686 (8.2%) [c-d] | 759/ 1101 (68.9%) | 79/1101 (7.2%) | 122/1101 (11.1%) | 46/1101 (4.2%) | 32/1101 (2.9%) | 7/1101(0.6%) |
| **SLN+ all** [b] | - | 95/759 (12.5%) | 23/79 (29.1%) | 35/122 (28.7%) | 10/46 (21.7%) | 10/32 (31.2%) | 1/7 (14.3%) |
| **SLN + MI < 50%** | - | 35/550 (6.4%) | 2/43 (4.6%) | 17/89 (19.1%) | 3/27 (11.1%) | 4/20 (20.0%) | -/4 (0%) |
| **SLN + MI >50%** | - | 60/209 (28.7%) | 21/36 (58.3%) | 18/33 (54.5%) | 7/19 (36.8%) | 6 /12 (50%) | 1/3 (33.3%) |
| **SLN+ cx stromal invasion** | - | 17/58 (29.3%) | 8/8 (100%) | 8/16 (50%) | 2/9 (22.2%) | 3/3 (100%) | 1/1 (100%) |

[a] Preoperative myometrial depth invasion unknown in 24 cases (due to obesity, myomas, vaginal stenosis).
[b] Referring to the percentage of women having at least one metastatic SLN subdivided by histology and MI estimates
[c] Proportion of EIN after the definition was implemented in June 2019, 72/686 (10.5%)
[d] 23 women with preoperative low grade EC were deemed as EIN at final histology.

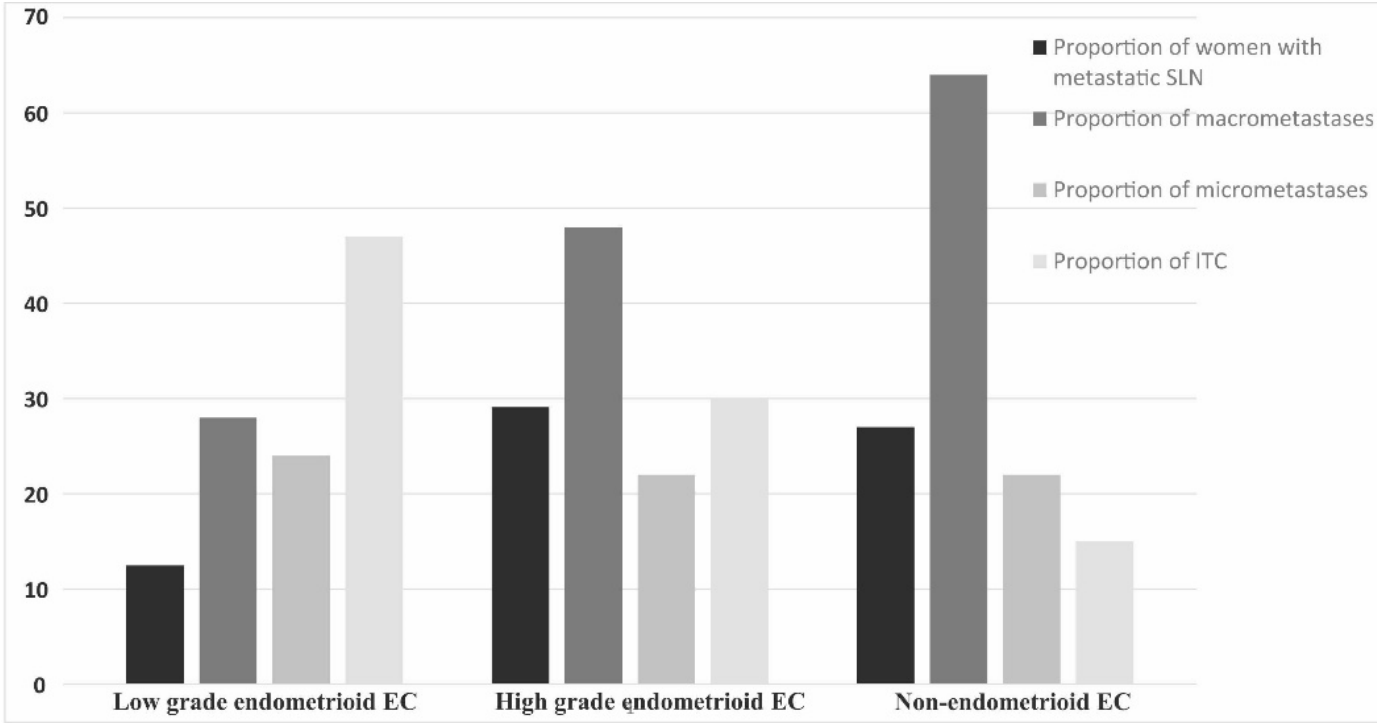



**Figure 13** Overall risk (%) for metastatic SLN and the proportions of MAM, MIM and ITC as largest metastase size in women with EC [161].

**Table 9** Size of largest SLN metastases ITC, MIM and MAM by final histology and extent of MI /CSI in women with EC [161].

| | Low grade endometrioid | High grade endometrioid | Serous adenocarcinoma | Carcinoscarcoma | Clear cell carcinoma | Other |
|---|---|---|---|---|---|---|
| **Proportion of women with EC at final histology** | 759/1045 [a] (72.6%) | 79/1045 (7.6%) | 122/1045 (11.7%) | 46/1045 (4.4%) | 32/1045 (3.1%) | 7/1045 (0.7%) |
| **Proportion of women with positive SLN** | 95/759 (12.5%) | 23/79 (29.1%) | 35/122 (28.7%) | 10/46 (21.7%) | 10/32 (31.2%) | 1/7 (14.3%) |
| **Size of largest SLN metastases:** [b] | | | | | | |
| **- Macrometastases** | 27/95 (28.4%) | 11/23 (47.9%) | 22/35 (62.9%) | 5/10 (50%) | 8/10 (80%) | 1/1 (100%) |
| **- Micrometastases** | 23/95 (24.2%) | 5/23 (21.7%) | 8/35 (22.8%) | 3/10 (30%) | 1/10 (10%) | |
| **- Isolated tumor cells** | 45/95 (47.4%) | 7/23 (30.4%) | 5/35 (14.3%) | 2/10 (20%) | 1/10 (10%) | |
| **LVSI:** | | | | | | |
| **-Yes** | 53/95 (55.8%) | 12/23 (52.2%) | 20/35 (57.1%) | 7/10 (70%) | 4/10 (40%) | 1/1 (100%) |
| **-No** | 35/95 (36.8%) | 7/23 (30.4%) | 10/35 (28.6%) | 3/10 (30%) | 3/10 (30%) | - |
| **-Unknown** | 7/95 (7.4%) | 4/23 (17.4%) | 5/35 (14.3%) | - | 3/10 (30%) | - |
| **LNM [c] in > one SLN (MI [d] <50%)** | 21/35 (60%) | -/2 | 10/17 (58.8%) | 1/3 (33.3%) | 3/4 (75%) | - |
| **LNM in > one SLN (MI >50%)** | 29/60 (48.3%) | 11/21 (52.4%) | 10/18 (55.6%) | 6/7 (85.7%) | 2/6 (33.3%) | 1/1 (100%) |
| **LNM in > one SLN [e] (cx stromal invasion)** | 10/17 (58.8%) | 8/8 (100%) | 5/8 (62.5%) | 2/2 (100%) | 1/3 (33.3%) | 1/1 (100%) |

[a] Women with EIN at final histology excluded from analysis.
[b] Referring to the percentage of women having at least one metastatic SLN.
[c] Lymph node metastasis.
[d] Myometrial invasion.
[e] Separate from degree of myometrial invasion.

## 7.4 Study IV

Data from 620 women were included in the analysis. Among all women with metastatic SLNs, per definition, 55 had at least one "SLN anatomy" removed due to non-mapping. A total of 114 (18.4%) women had pelvic SLN metastases. Among these, five women (4.3%) had isolated metastases in a “SLN-anatomy”. Of those, three were found in the proximal obturator fossa and two in the external interiliac area (table 10).

**Tabel 10** Schematic overview of all, and isolated metastatic SLNs defined by ICG and metastatic “SLN anatomy” per anatomic positions [162].

| | **Upper paracervical pathway** | | |
|---|---|---|---|
| | **External iliac area** | **Obturator area** | **Parauterine lymphovascular tissue** |
| **SLN ICG** | 45/114 (39.5%) | 84/114 (73.7%) | 15/114 (13.2%) |
| **SLN ICG isolated** | 14/114 (12.3%) | 25/114 (21.9%) | 2/114 (1.7%) |
| **”SLN Anatomy”** | 10/114 (8.8%) | 14/114 (12.3%) | - |
| **”SLN Anatomy” isolated** | 2/114 (1.7%) | 3/114 (2.6%) | - |
| Compartments presented independently from each other, implying that each women can have SLN’s in multiple compartments. | | | |

In summary, by identifying and removing "SLNs anatomy" and the PULT , an additional 4.3% and 2% respectively of true node positive women were identified indicating a similar lack of sensitivity by the use of ICG-mapping only.

# 8 Discussion

The developed SLN algorithm utilized in **Study I** (the SHREC study) demonstrated a 95% bilateral mapping rate and a sensitivity of 98% and 100% respectively by ICG only and by the complete algorithm adding macroscopically suspect nodes. Importantly, sensitivity was based on pelvic SLNs only. By the higher number of node positive women, so far, the SHREC study is the largest study contributing to a narrower confidence than in other studies. The study emphasizes the importance of adhering to a strict surgical anatomically algorithm with a strict definition of SLN and to define SLNs within sub-pathways, i.e clearly indicating that detection of only one SLN per hemipelvis is inaccurate. The algorithm also introduced reinjection of tracer with lead to an 13% increase in bilateral mapping. The pelvic and paraaortic backup LND resulted in larger number of nodes than in comparable studies, important to evaluate the quality of the sensitivity-analysis. The proportion of women with a paraaortic LND to the level of the left renal vein was higher than in other studies (80.9%) with an additional 3.5% to the level of the inferior mesenteric artery. This enabled us to evaluate the risk concurrent and isolated paraaortic metastases.

The results of the study lead to a prompt change in the Swedish guidelines recommending SLN biopsy in all subgroups of EC by the use of the described algorithm.

A weakness but also a strength is that the study was performed by a few trained surgeons at two academic institutions, hence the results may not be generalizable. We do believe that adhering to the algorithm and an understanding of lymphatic drainage of the uterus and definition of SLNs will help implementing the SLN concept in institutions on their early phase. Another weakness is that, despite selection of surgeons with previous experience and individual supervision in the execution of the SLN algorithm a further increased experience likely has occurred. A probable consequence of an increasing surgical experience in performing SLN over time (including SLN detection in cervical cancer, well over 1300 SLN-procedures at the end of the study period) is an increased quality in the performance of SLN, in particular in women with complicating surgical factors such as morbid obesity and tortuous vessels. This may be one reason for

the high rates of SLN metastases within all subgroups in the SLN-only study. No stratification for year or surgery was performed which is a weakness.

As data and experience was continuously gathered, some insights from the SHREC study, initially clouded by the overall result, in particular the higher risk for nodal metastases in the obturator fossa, at the same time significantly less frequently mapped by ICG raised the obvious question; can sensitivity be improved by removing non-mapped nodes as typical high risk positions and/or , can a selective removal of nodes at those positions be a replacement for a side specific LND in case of non-mapping.

Given that the 95% CI of the SLN-ICG algorithm in the SHREC study was 89-100% the true sensitivity may be substantially lower than the 98% found in the study, in particular in low-risk EC where an even more sharpened algorithm may be necessary.

Based on these circumstances and theories we performed the **study II and IV** as of below. Both studies were based on an intraoperative depiction of positions and types of SLNs utilizing the same standardized chart over time and with ultrasectioning and IHC on all SLNs.

The results in **study II**, where we demonstrated that a side specific LND can be replaced by a removal of node at defined typical anatomic positions is further supported by the results of **study IV**, as non-mapped nodes at those typical positions automatically will be removed. In all, **study II** and **IV** fill important gaps; there will be no need for at side specific LND in case of non-mapping, consequently fewer lymphatic complications. Second, a hybrid SLN algorithm as described in **study IV** will further increase sensitivity of the SLN concept, of importance for the individual woman with EC and of importance to minimize misclassifications in terms of nodal disease in future coming on the relationship between pelvic nodal metastases and biomarkers and for follow up of first and foremost low-grade EC with and without pelvic nodal involvement.

**Study III** is the largest prospective population-based study on the risk for nodal metastases and may serve for power analyses in future studies as of above. The study provides detailed data on the risk for pelvic SLN metastases identified in a substantially higher proportion within all risk groups than in previous large studies on the risk estimated by standard histology. Of interest is the significant difference in the proportions of small volume metastases co-varying with the "aggressiveness" of histologic subtypes indicating a narrower window of opportunity to detect early pelvic nodal disease in HREC histologies, similarly implying that women with low-risk EC and ITCs in SLNs may require a longer follow up to detect any risk increase for recurrence compared with SNL negative women.

The FIGO 2023 staging manual includes a more strictly defined LVSI as a risk factor. The need for a stricter classification reflects the subjectivity of this parameter, only available after a histology of the full specimen. In our study 37.5% of a SLN positive women were LVSI negative. As the presence of ITC in a lymph node is an absolute proof of a LVSI leading to lymphatic spread there is a logic inconsequence of not incorporating presence of ITC in a lymph node in the classification system. More data is also needed to evaluate the impact of presence of ITCs in several SLNs.

# 9 Conclusions

The utilized clearly defined surgical and SLN-definition algorithm introducing reinjection of ICG resulted in a higher sensitivity with a narrower confidence interval and a higher mapping rate compared with available studies as of today.

We have further demonstrated that:

-a side specific lymphadenectomy can be omitted in favour of a selective sampling of nodes at defined typical high-risk positions, an important development to reduce lymphatic complications.

-removal of nodes as the same positions, albeit ICG negative, despite mapping at other positions, the "hybrid algorithm" have the potential to further increase sensitivity in the range of 5%.

-the presence of metastases and isolated metastases in the PULT, a tissue with well-defined borders, can be removed with minimal risk for complications.

We have provided detailed data on the risk for SLN metastases from a large population-based cohort of women of all pre- and postoperative risk groups. A close to 10% risk for SLN metastases among women preoperatively estimated having a <50% MI low grade EC is an additional incitement to offer women of this risk group detection of SLNs.

In all, the thesis represents a logic development and refinement of the SLN algorithm based on a large prospective study on consecutive women with EC.

# 10 Future perspectives

The thesis implies a need for future studies:

1. There is insufficient data on LVSI, utilizing newer more strict criteria, and its relation to nodal status from well-designed high sensitivity SLN studies: such study may give information on to which extent LVSI is an independent risk factor for recurrence.
2. The impact of small volume metastases on the risk for paraaortic metastases in particular in women with multiple SLNs with ITC would bring further knowledge on the risk associated with ITC. In such study ultrasectioning and IHC on paraaortic nodes should be performed.
3. The risk of recurrence in women with low grade EC and ITC in SLNs need a prospective multicentre well-designed study utilizing identical adjuvant treatment protocols. A sample size analysis is necessary to determine whether this study can be a RTC or a prospective cohort study with matched historical control.
4. The relation between SLN status and biomarkers needs a prospective evaluation in a (multicentre) setting utilizing a high sensitivity SLN algorithm.
5. Recurrence and survival in women with low grade SLN negative and SLN positive endometrioid EC need a thorough well powered evaluation.

# 11 Appendices

## 11.1 Previous FIGO staging endometrial cancer

| 1950-61 | 1st FIGO staging of endometrial cancer--Clinical |
|---|---|
| Stage 0 | Cases which the pathologist considers most likely to be of a carcinomatous<br>nature though it is impossible to arrive at a definite microscopic diagnosis |
| Stage I | The growth is confined to the uterus<br>Group 1. Operation advisable<br>Group 2. Bad operative risks |
| Stage II | The growth has spread outside the uterus |

| 1962-71 | 2nd FIGO staging for endometrial cancer--Clinical |
|---|---|
| Stage 0 | Histological findings suspicious of malignancy but not proven. |
| Stage I | The carcinoma is confined to the corpus. |
| Stage II | The carcinoma has involved the corpus and cervix. |
| Stage III | The carcinoma has extended outside the uterus but not outside the true pelvis. |
| Stage IV | The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum |

| 1971-88 | 3rd FIGO staging for endometrial cancer--Clinical |
|---|---|
| Stage 0 | Carcinoma in-situ. Histological findings suspicious of malignancy. |
| Stage I<br>Ia<br>Ib | The carcinoma is confined to the corpus.<br>The length of the uterine cavity is 8 cm. or less.<br>The length of the uterine cavity is greater than 8 cm. |
| Stage II | The carcinoma has involved the corpus and cervix. |
| Stage III | The carcinoma has extended outside the uterus but not outside the true pelvis. |
| Stage IV | The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum |
| | It is desirable that Stage I cases be sub grouped with regard to the histological<br>type of the adenocarcinoma as follows:<br>G1. Highly differentiated adenomatous carcinoma<br>G2. Differentiated adenomatous carcinoma with partly solid area.<br>G3. Predominantly solid or entirely undifferentiated carcinoma |

| 1988–2009 | 4th FIGO staging for endometrial cancer-- Surgical |
|---|---|
| Stage I<br>IA<br>IB<br>IC | Tumour limited to the uterus.<br>Tumour limited to the endometrium.<br>Invasion <50% of the myometrium<br>Invasion ≥50% of the myometrium |
| Stage II<br>IIA<br>IIB | Extension to the cervix but not beyond the uterus.<br>Endocervical glandular involvement only.<br>Cervical stromal invasion. |
| Stage III<br>IIIA<br>IIIB<br>IIIC | Extension outside of the uterus/cervix with/without regional metastasis.<br>Tumour invades serosa or adnexa or positive peritoneal cytology.<br>Vaginal metastasis.<br>Metastasis to pelvic and/or paraaortic lymph nodes |
| Stage IV | The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum |

| 2009-2023 | 5th FIGO staging for endometrial cancer[49] |
|---|---|
| Stage I[a]<br>IA[a]<br>IB[a] | Tumor confined to the corpus uteri<br>No or less than half myometrial invasion<br>Invasion equal to or more than half of the myometrium |
| Stage II[a] | Tumor invades cervical stroma, but does not extend beyond the uterus[b] |
| Stage III[a]<br>IIIA[a]<br>IIIB[a]<br>IIIC[a]<br>IIIC1[a]<br>IIIC2[a] | Local and/or regional spread of the tumor<br>Tumor invades the serosa of the corpus uteri and/or adnexae[c]<br>Vaginal and/or parametrial involvement[c]<br>Metastases to pelvic and/or para-aortic lymph nodes[c]<br>Positive pelvic nodes<br>Positive para-aortic lymph nodes with or without positive pelvic lymph nodes |
| Stage IV[a]<br><br>IVA[a]<br>IVB[a] | Tumor invades bladder and/or bowel mucosa, and/or distant metastases<br>Tumor invasion of bladder and/or bowel mucosa<br>Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes |
| [a]Either G1, G2, or G3.<br>[b]Endocervical glandular involvement only should be considered as Stage I and no onger as Stage II.<br>[c]Positive cytology has to be reported separately without changing the stage | |

# 11.2 Current FIGO staging endometrial cancer (2023)

| 2023 | 6th FIGO staging for endometrial cancer[a,b] [50] |
|---|---|
| Stage I | Confined to the uterine corpus and ovary[c] |
| IA | Disease limited to the endometrium OR non-aggressive histological type i.e. low-grade endometrioid, with invasion of less than half of myometrium with no or focal lymphovascular space involvement (LVSI) OR good prognosis disease |
| | *IA1* Non-aggressive histological type limited to an endometrial polyp OR confined to the endometrium |
| | *IA2* Non-aggressive histological types involving less than half of the myometrium with no or focal LVSI |
| | *IA3* Low-grade endometrioid carcinomas limited to the uterus and ovary[c] |
| IB | Non-aggressive histological types with invasion of half or more of the myometrium, and with no or focal LVSI [d] |
| IC | Aggressive histological typese[e] limited to a polyp or confined to the endometrium |
| Stage II | Invasion of cervical stroma without extrauterine extension OR with substantial LVSI OR aggressive histological types with myometrial invasion |
| IIA | Invasion of the cervical stroma of non-aggressive histological types |
| IIB | Substantial LVSI[d] of non-aggressive histological types |
| IIC | Aggressive histological typese[e] with any myometrial involvement |
| Stage III | Local and/or regional spread of the tumour of any histological subtype |
| IIIA | Invasion of uterine serosa, adnexa, or both by direct extension or metastasis |
| | *IIIA1* Spread to ovary or fallopian tube (except when meeting stage IA3 criteria) [,c] |
| | *IIIA2* Involvement of uterine subserosa or spread through the uterine serosa |
| IIIB | Metastasis or direct spread to the vagina and/or to the parametria or pelvic peritoneum |
| | *IIIB1* Metastasis or direct spread to the vagina and/or the parametria |
| | *IIIB2* Metastasis to the pelvic peritoneum |
| IIIC | Metastasis to the pelvic or para-aortic lymph nodes or both[f] |
| | *IIIC1* Metastasis to the pelvic lymph nodes |
| | IIIC1i Micrometastasis |
| | IIIC1ii Macrometastasis |
| | *IIIC2* Metastasis to para-aortic lymph nodes up to the renal vessels, with or without metastasis to the pelvic lymph nodes |
| | IIIC2i Micrometastasis |
| | IIIC2ii Macrometastasis |
| Stage IV | Spread to the bladder mucosa and/or intestinal mucosa and/or distance metastasis |
| IVA | Invasion of the bladder mucosa and/or the intestinal/bowel mucosa |
| IVB | Abdominal peritoneal metastasis beyond the pelvis |
| IVC | Distant metastasis, including metastasis to any extra-or intra-abdominal lymph nodes above the renal vessels, lungs, liver, brain, or bone |
| **FIGO endometrial cancer stage with molecular classification** | |
| **Stage description** | **Molecular findings in patients with early endometrial cancer (Stages I and II after surgical staging)** |

| | |
|---|---|
| Stage IAmPOLEm | POLEm endometrial carcinoma, confined to the uterine corpus or with cervical extension, regardless of the degree of LVSI or histological type |
| Stage IICmp53abn | p53abn endometrial carcinoma confined to the uterine corpus with any myometrial invasion, with or without cervical invasion, and regardless of the degree of LVSI or histological type |

Adapted from [1]
Abbreviations: EEC, endometrioid carcinoma; LVSI, lymphovascular space involvement; abn, abnormal; POLEm, polymerase epsilon mutated.
[a]Endometrial cancer is surgically staged and pathologically examined.
[b]In early endometrial cancer, the standard surgery is a total hysterectomy with bilateral salpingo-oophorectomy via a minimally invasive laparoscopic approach. Lymph node staging should be performed in patients with intermediate-high/high-risk patients. Sentinel lymph node (SLN) biopsy is an adequate alternative to systematic lymphadenectomy for staging proposes. SLN biopsy can also be considered in low−/low- intermediate-risk patients to rule out occult lymph node metastases and to identify disease truly confined to the uterus.
[c]Low-grade EECs involving both the endometrium and the ovary are considered to have a good prognosis, and no adjuvant treatment is recommended if all the below criteria are met. Disease limited to low-grade endometrioid carcinomas involving the endometrium and ovaries (Stage IA3) must be distinguished from the extensive spread of the endometrial carcinoma to the ovary (Stage IIIA1), by the following criteria: (1) no more than superficial myometrial invasion is present (< 50%); (2) absence of extensive/substantial LVSI; (3) absence of additional metastases; and (4) the ovarian tumour is unilateral, limited to the ovary, without capsule invasion/rupture (equivalent to pT1a).
[d]LVSI: extensive/substantial, ≥5 vessels involved.
[e]Grade and histological type: Serous adenocarcinomas, clear cell adenocarcinomas, mesonephric-like carcinomas, gastrointestinal-type mucinous endometrial carcinoma, undifferentiated carcinomas, and carcinosarcomas are considered high-grade by definition. Non-aggressive histological types are composed of low-grade (grade 1 and 2) EECs. Aggressive histological types are composed of high-grade EECs (grade 3), serous, clear cell, undifferentiated, mixed, mesonephric-like, gastrointestinal mucinous type carcinomas, and carcinosarcomas.
[f]Micrometastases are considered to be metastatic involvement (pN1 (mi)). The prognostic significance of isolated tumour cells (ITCs) is unclear. The presence of ITCs should be documented and is regarded as pN0(i+). Macrometastases are > 2 mm in size, micrometastases are 0.2–2 mm and/ or > 200 cells, and isolated tumour cells are ≥0.2 mm and ≤200 cells.

# 11.3 Surgical protocol

**Sentinel node in endometrial cancer** CRF number ( by study coordinator)

Date

Surgeon……Assisting surgeon……..

OR nurse……..Circulating nurse…….

Included as/ planned surgery

☐ Low risk endometrial cancer. SLN only

☐ High risk endometrial cancer, SLN only

☐ High risk histologyendometrial cancer, SLN+ paraaortic LND due to preop MI >50%

Reason for an intraoperative deviation from a planned surgery

_____

| | | |
|---|---|---|
| **Previous surgery** | 1 | ☐ No |
| | 2 | ☐ App |
| | 3 | ☐ Pfannenstiel |
| | 4 | ☐ Midline upper |
| | 5 | ☐ Midline lower |
| | 6 | ☐ Laparoscopy/ robot |
| | 7 | ☐ other, specify |

**Weight (kg)**

**Height (cm)**

**Surgery** **(specify all)**

| | |
|---|---|
| 1 | ☐ Radical hysterectomy+adnex , QM type |
| 2 | ☐ Enkel hysterektomi+ adnex |
| 3 | ☐ Sentinel node UPP +LPP |
| 4 | ☐ Sentinel node UPP only |
| 5 | ☐ Full pelvic LND |
| 6 | ☐ Paraaortic LND to LRV |
| 7 | ☐ Paraaortic LND to IMA |
| 8 | ☐ Omentectomy |
| 9 | ☐ Other specify |

| | |
|---|---|
| **Pat in OR** | time. |
| **First Dr's procedure** | time |
| **Time for SLN Onset dissection incl reinj** | Minutes: |
| **Last stich** | time |
| **Pat out OR** | time |

**Uterus weight (g)**

**Insufflation technique**

☐ Palmer point direct entry

☐ Hasson periumbilical

☐ Veres needle

**Adhesiolysis before onset of surgery**

☐no

☐Yes

Total minutes ___ for adhesiolysis ( before and after docking)

**Nr robot instuments**

☐2

☐3

☐4

☐5

☐6

**Assistant trocar**

| | |
|---|---|
| ☐1 | ☐ 12mm ☐ disp ☐ non-disp |
| ☐2 | ☐ 15 mm ☐ disp☐ non-disp |
| ☐3 | ☐ 18 mm ☐ disp ☐ non-disp |
| ☐4 | ☐ 5 mm ☐ disp ☐ non-disp |

**Additonal instruments**

☐ Endobag,nr_____

☐ Tachyseal,/ floseal, nr_____

☐ Other specify_____

**Bleeding**

ml_____

**Transfusion**

☐ No

☐ Yes nr units:_____

**Conversion**

☐no

☐Yes to laparotomy

| | |
|---|---|
| **Cause of conversion** | ☐ Robot, technical problem,; specify:_____<br>☐ Surgery, specify:_____<br>☐ Anesthesiologically cause; specify:_____<br>☐ Oncological cause; specify:_____ |
| **Complications during insufflation or adhesiolysis** | ☐ None<br>☐ **Yes. Specify:**_____ |
| **Complications during SLN removal** | ☐ No<br>☐ **Yes. Specify**:_____ |
| **Complications during remaining pelvic LND** | ☐ No ☐ Not performed<br>☐ **Yes. Specify**:_____ |
| **Complications during remaining paraaortic LND** | ☐ No ☐ Not performed<br>☐ **Yes. Specify**:_____ |
| **Complications during hysterectomy** | ☐ No ☐ Not performed<br>☐ **Yes. Specify**:_____ |
| **Technique for removal of uterus** | ☐ vaginally without bag ☐ vaginally with Endobag<br>☐ Though abdominal wall in bag, specify why and where. |

_____

| | |
|---|---|
| **Technique for removal of nodes** | ☐ Trough assistant port in bag/container<br>☐ vaginally in bag |
| **Nodal tissue divided in abdomen to an enable removal through port?** | ☐ No ☐ yes specify |
| **Closure of fascia** | ☐ Assistant port<br>☐ Optics port ( SI robot)<br>☐ Other ports, specify;_____ |

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