Innovative Management of HPV-Associated Malignancies in Gynaecological Oncology

PhD thesis

Balázs Lajos Vida MD

Semmelweis University Doctoral School
Division of Cardiovascular Medicine and Research

Supervisor: Márton Keszthelyi MD, Ph.D.

Official reviewers:

Head of the Complex Examination Committee:
Szabolcs Várbíró MD, D.Sc.

Members of the Complex Examination Committee:
Attila Jósvai MD, Ph.D.
Lotti Lúcia Keszthelyi MD. Ph.D.

Budapest
2025

1. Introduction

Human papilloma virus (HPV) poses a significant challenge to healthcare systems worldwide. The high-risk types are associated with cervical, vulvar and oropharyngeal cancers, among others. Although, screening is recommended by multiple health organizations and guidelines, cervical cancer numbers remain high.

Vulvar cancer, a relatively rare malignancy is often associated with HPV. While the prognosis of the disease is influenced by various factors, such as histological type, grade, size of the tumor, age and comorbidities, one of the most crucial factor is the lymph node status of the patient. Therefore, complete lymphadenectomy was considered gold standard in particular cases, but due to its high rates of complications, it has been replaced by the sentinel lymph node SLN dissection technique. For this technique, the use of tracers are necessary. The mainly used tracers are: Tc99m isotope, indocyanine green (ICG), blue dyes, and superparamagnetic iron oxide (SPIO) particles.

High-risk HPV types are responsible for more than 90% of all cervical cancer cases. This is also the underlying cause in cervical cancer during pregnancy. But, with the increasing trend of delaying childbearing, the rates of malignancies, especially cervical cancer is rising. Cervical cancer during pregnancy is rare, therefore large scale studies with robust and diverse populations are also rare, thus guidelines often recommend case-by-case assessment for the management of the disease. The set of diagnostic tools and therapeutic measures in pregnancy are

often limited, which are further complicate the management of these patients.

2. Objectives

SLN biopsy represents a less invasive and increasingly preferred alternative to complete lymphadenectomy in early-stage disease. However, the optimal detection method remains debated, with multiple tracers: Tc99m, blue dye, ICG, and SPIO in clinical use. Each offers distinct strengths and limitations in detection efficacy, visual guidance, safety, and logistical feasibility.

1. This thesis first establishes a scientifically robust protocol for a meta-analysis comparing SLN detection methods in vulvar cancer.
2. The primary aim is to perform a systematic review and meta-analysis comparing detection rates (DRs) of individual and combined SLN mapping techniques.
3. Secondary objectives include assessing the influence of patient-specific factors (e.g., age, BMI), tracer dose variability, and procedural nuances on detection success.

In addition, the thesis explores another critical area where evidence is sparse: the management of cervical cancer during pregnancy. A rare, yet rising clinical scenario due to delayed childbearing.

4. Through an in-depth case study of a pregnant patient with cervical cancer treated with neoadjuvant chemotherapy, preterm delivery, and radical surgery, the

thesis examines real-world decision-making, oncological safety, and obstetric outcomes.

5. The analysis further evaluates the timing, feasibility, and safety of major interventions, including SLN biopsy and robot-assisted lymphadenectomy within the pregnant population.
6. Finally, this work advocates for individualized treatment strategies based on tumor biology, gestational age, and patient preference, emphasizing the role of multidisciplinary collaboration in optimizing outcomes for both mother and child.

3. Methods

3.1 Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: Protocol for a Systematic Review and Meta-Analysis

3.1.1 Registration

To ensure methodological transparency and reproducibility, a systematic review and meta-analysis protocol was prospectively developed and registered under PROSPERO (ID: CRD42024590774). The protocol adheres to international methodological standards, including the PRISMA 2020 statement and the Cochrane Handbook for Systematic Reviews of Interventions, thereby providing a solid framework for the investigation of SLN detection techniques in vulvar cancer.

The protocol formally predefines the study's objectives, methodological approach, inclusion criteria, and outcome measures. Its public registration ensures scientific integrity and prevents selective reporting.

3.1.2 Comparison of Interventions

The central objective of the protocol is to evaluate and compare the diagnostic performance of the most commonly used SLN detection tracers, namely Tc99m, ICG, blue dye, and SPIO. The analysis emphasizes per-patient and per-groin detection rates, with the goal of determining which methods offer the highest diagnostic accuracy, while minimizing surgical and infrastructural challenges.

3.1.3 Study Selection Criteria

Eligible studies include randomized controlled trials, as well as prospective and retrospective observational studies. Inclusion criteria are restricted to female patients, with histologically verified vulvar squamous cell carcinoma, specifically FIGO stages T1a to T2, with tumour size ≤4 cm, unifocal presentation, and no clinical lymph node involvement. No limits are placed on publication language or geographical setting, ensuring a comprehensive and globally relevant dataset.

3.1.4 Ethical Considerations

As this protocol does not involve the collection of new patient data, formal ethical approval is not required. Nevertheless, ethical principles remain central to the review.

All included studies will be accurately cited and responsibly interpreted, maintaining the highest standards of scholarly conduct.

3.2 Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: a Systematic Review and Meta-Analysis

3.2.1 Eligibility Criteria

Studies were selected based on a PICO-structured inclusion model. Eligible publications involved adult women with histologically confirmed vulvar cancer, undergoing SLN biopsy. Interventions included Tc99m, ICG, SPIO, blue dye, and their combinations (e.g., Tc99m + BD, Tc99m + ICG). Detection rate (DR) was defined, as the proportion of successfully identified SLNs either per patient or per groin. This was the primary outcome. Both randomized controlled trials and observational studies were included, contingent upon availability of extractable DR data.

3.2.2 Search Strategy and Sources

An extensive literature search was conducted across five major databases (PubMed, Embase, Web of Science, Cochrane CENTRAL, Scopus) up to August 15, 2024. The search syntax “vulv* AND sentinel” was applied broadly, with no language or publication status restrictions, ensuring sensitivity and inclusivity.

3.2.3 Study Selection and Data Extraction

After deduplication, a two-step screening process was executed independently by two reviewers. Disagreements were resolved through discussion or adjudication by a third expert. Rayyan.ai and EndNote X9 were used to manage references. Inter-reviewer reliability was assessed using Cohen's kappa statistic, confirming substantial agreement. A structured extraction sheet was used to collect variables including study design, sample size, tracer type, and DRs (both per-patient and per-groin). For midline tumours, bilateral groin data were emphasized.

3.2.4 Risk of Bias and Quality Appraisal

Risk of bias was evaluated using ROBINS-I (non-randomized studies) and RoB 2 (RCTs). Quality of evidence was assessed per GRADE methodology via GRADEpro GDT software, considering study design, imprecision, inconsistency, indirectness, and publication bias. Each outcome was classified as having high, moderate, low, or very low certainty.

3.2.5 Statistical Analysis and Data Synthesis

To accommodate heterogeneity, random-effects models were employed. Detection rates and 95% confidence intervals (CIs) served as primary effect measures. A three-level multivariate meta-analysis accounted for dependencies in studies reporting multiple tracer arms. Complementary two-level models were applied to detect publication bias and outlier effects, using funnel plots and Peters' test. Data

synthesis and visualization were conducted in R, employing the “meta”, “dmetar”, “metafor”, and “ggplot2” packages.

3.3 Cervical Cancer During Pregnancy: A Multidisciplinary Approach to a Complex Oncological Case

3.3.1 Patient Identification and Diagnosis

A 32-year-old woman was initially screened in December 2023 with an ASC-H cytology result. Follow-up testing confirmed HPV-18 positivity with signs of malignant transformation. Immunocytochemistry was negative, prompting conservative monitoring. In May 2024, during early pregnancy, repeat cytology revealed glandular neoplasia, leading to a LEEP conization. Histology confirmed grade 3 squamous cell carcinoma (HPV-associated), with no lymphovascular invasion.

3.3.2 Ethical Oversight

The case was conducted ethically under the Institutional Review Board of Semmelweis University approval, with informed consent obtained for both treatment and publication.

4. Results

4.1 Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: Protocol for a Systematic Review and Meta-Analysis

4.1.1 Expected Results

Although this section outlines a predefined protocol rather than empirical data, it offers informed projections based on existing literature and methodological design. The forthcoming systematic review and meta-analysis is expected to reveal key comparative insights regarding the efficacy of SLN detection techniques used in vulvar cancer surgery.

It is anticipated that dual-tracer approaches, particularly Tc99m combined with BD or ICG will demonstrate superior per-patient and per-groin detection rates compared to single-agent techniques. Tc99m, as a widely accepted radiotracer, is expected to show detection rates exceeding 90%, particularly when used with a visual adjunct.

ICG is projected to achieve high detection efficacy, with prior studies suggest ICG's performance may parallel that of dual-agent techniques. This analysis will critically assess such claims through pooled detection rate estimates.

SPIO, though not yet mainstream, is expected to yield promising results. Preliminary evidence indicates high detection rates without the logistical demands of radioactive tracers.

Conversely, BD alone is expected to show inferior sensitivity, reaffirming its limited utility as a standalone tracer. It is likely to be most effective only when used in combination with Tc99m or ICG.

4.2 Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: a Systematic Review and Meta-Analysis

4.2.1 Study selection

A systematic search across multiple databases yielded 1,418 articles. After removing duplicates, 967 unique records remained. Title and abstract screening by two independent reviewers excluded 748 studies based on pre-defined criteria. Of the 219 full texts reviewed, 94 met the inclusion criteria, with 6 later excluded during data extraction due to methodological or reporting issues. Ultimately, 61 studies (59 observational, 2 RCTs) were included. An additional 27 eligible observational studies were identified through citation tracking, resulting in a total of 88 studies involving 4,637 patients, constituting one of the most comprehensive analyses of SLN detection in vulvar cancer to date.

4.2.2 Characteristics of the included studies

Among the 88 studies included in this review, a wide range of SLN detection methods were assessed, often with multiple techniques evaluated per study. Blue dye, as a standalone tracer was used in 34 studies, including formulations like isosulfan and methylene blue. Tc99m, the gold standard in SLN mapping, appeared in 55 studies, alone

or with adjuncts, supporting its central role per international guidelines. ICG was assessed in 16 studies, either alone or in combination, while SPIO, a newer non-radioactive tracer, was examined in 3 studies.

Dual-tracer methods were common: 32 studies assessed Tc99m + BD, and 12 examined Tc99m + ICG. Many studies allowed for intra-study comparison of multiple techniques, reflecting real-world practice and contributing to the heterogeneity and breadth of the analysis. Full methodological and demographic details, including tracer combinations, study design, patient age, BMI, and tumour staging were also collected.

4.2.3 Detection rates

In this meta-analysis of 88 studies involving 4,637 patients, the overall pooled per-patient detection rate (DR) was 92% [95% CI: 89-94%], and the per-groin DR was 84% [95% CI: 80-87%]. For individual tracers, blue dye showed a per-patient DR of 82% [72-89%] and 78% [69-85%], with per-groin DRs of 69% [62-76%] and 66% [58-73%]. Tc99m achieved per-patient DRs of 98% [94-99%] and 93% [90-95%], and per-groin DRs of 93% [88-96%] and 87% [82-90%]. ICG demonstrated per-patient DRs of 86% [76-92%] and 88% [76-95%], with per-groin DRs of 90% [83-94%] and 87% [81-92%]. SPIO yielded per-patient DRs of 100% [0-100%] and 95% [81-99%], but per-groin DRs could not be pooled due to limited data. For dual-tracer techniques, Tc99m + blue dye showed per-patient DRs of 94% [91-96%] and per-groin DRs of 88% [83-92%] and 87% [83-91%],

while Tc99m + ICG reached per-patient DRs of 98% [91-99%] and 96% [90-99%], and per-groin DRs of 95% [87-98%] and 93% [88-96%], confirming the superior performance of dual-tracer approaches in SLN mapping for vulvar cancer.

4.2.4 Risk of bias assessment

The risk of bias was assessed using ROBINS-I for 86 observational studies and RoB 2 for 2 RCTs. Most observational studies had moderate risk due to non-randomized design, but several showed low risk due to strong methodology. RCTs were rated as low risk or with some concerns. Overall, study quality varied, but many met high standards of inclusion criteria, outcome reporting, and data consistency. Publication bias was explored through funnel plot analysis, indicating potential asymmetry and highlighting the importance of cautious interpretation of pooled results.

4.3 Cervical Cancer During Pregnancy: A Multidisciplinary Approach to a Complex Oncological Case

4.3.1 Imaging and Surgical Staging

Pelvic MRI showed a cervical lesion 30mm in diameter, confined to the cervix. Despite negative imaging for lymph node involvement, SLN biopsy and bilateral pelvic lymphadenectomy were performed via robot-assisted laparoscopy at 16 weeks of gestation. All 28 lymph nodes were negative for metastasis, demonstrating the feasibility

and safety of SLN staging during early pregnancy with minimally invasive techniques.

4.3.2 Neoadjuvant Chemotherapy and Disease Progression

The patient received three cycles of neoadjuvant chemotherapy (paclitaxel + carboplatin). However, follow-up MRI revealed tumour progression to 48 mm, prompting a change in management strategy.

4.3.3 Delivery and Radical Surgery

At 29 weeks of gestation, the patient underwent caesarean section, following corticosteroid administration for foetal lung maturation. A type C1 radical hysterectomy was performed concurrently. The neonate (1280 g) developed respiratory distress syndrome but responded well to NICU management, including non-invasive ventilation and surfactant therapy.

4.3.4 Final Pathology

Histopathological analysis confirmed a high-grade HPV-associated squamous carcinoma measuring 65 × 42 mm, with stromal invasion, but without vaginal wall and parametrial invasion. Surgical margins were negative, and all lymph nodes remained uninvolved. LVSI was identified in three microscopic foci.

4.3.5 Postoperative Course and Adjuvant Treatment

The patient was classified as intermediate risk due to tumour grade, stromal depth, and LVSI. Adjuvant treatment

included external beam radiotherapy (50.4 Gy in 25 fractions) with concurrent cisplatin-based chemotherapy. No postoperative complications occurred.

4.3.6 Clinical Implications

This case illustrates the feasibility of SLN mapping and robotic lymphadenectomy during pregnancy, enabling early staging without delaying oncologic treatment. It highlights the challenges of tumour progression under chemotherapy and underscores the importance of close monitoring and dynamic treatment planning. The case also exemplifies the importance of multidisciplinary coordination across oncology, obstetrics, neonatology, and surgery to optimize outcomes for both mother and child. Furthermore, it emphasizes the underutilized but potentially valuable role of SLN biopsy in guiding individualized treatment even during gestation, an area with limited prospective data.

5. Conclusions

This thesis discusses the diagnostic and managemental innovations in HPV-associated gynaecological cancers, while also emphasizing individualized care. Five main conclusions emerge:

1. ICG vs. Dual Tracers: ICG alone performs comparably to guideline-recommended combinations (Tc99m+BD, Tc99m+ICG), showing no statistical inferiority. This supports ICG's use as a standalone option when radiotracer access is restricted.

2. SLN Mapping in Vulvar Cancer: Our meta-analysis confirms that blue dye alone yields lower detection rates, while Tc99m remains effective but resource-dependent. ICG demonstrates comparable accuracy with fewer logistical barriers, suggesting its broader adoption in clinical practice.
3. SPIO as an Emerging Tracer: SPIO shows promise with detection rates similar to standard methods. Its non-radioactive nature and operational flexibility are advantageous, though current evidence is limited. Further multicentre studies are needed to establish its role.
4. Cervical Cancer in Pregnancy: A multidisciplinary, centralized approach is essential for managing cervical cancer during gestation. The case study demonstrates that early staging via robot-assisted SLN biopsy and lymphadenectomy is both feasible and safe, enabling timely neoadjuvant therapy without compromising foetal or maternal outcomes.
5. SLN Detection in Pregnancy: SLN mapping is viable during pregnancy, with surgical timing dependent on gestational age. ICG emerges as the most suitable tracer, offering high detection rates, real-time visualization, and a strong safety profile, supporting its use in pregnancy-associated malignancies.

Overall, this work contributes to refining SLN protocols, advocating for patient-specific, evidence-based approaches in gynaecologic oncology.

6. Bibliography of the candidate's publications

Publications related to the thesis:

Vida B, Lintner B, Veres DS, Várbíró S, Merkely P, Lőczi L, Ács N, Tóth R, Keszthelyi M. Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: a Systematic Review and Meta-Analysis. American Journal of Obstetrics and Gynecology. 2025 Apr 29:S0002-9378(25)00274-1.doi: 10.1016/j.ajog.2025.04.052 | PMID: 40311826. **IF: 8.7**

Vida B, Lintner B, Várbíró S, Merkely P, Lőczi LL, Ács N, Tóth R, Keszthelyi M. Assessing the Comparative Efficacy of Sentinel Lymph Node Detection Techniques in Vulvar Cancer: Protocol for a Systematic Review and Meta-Analysis. Life (Basel). 2024 Nov 23;14(12):1538. doi: 10.3390/life14121538 | PMID: 39768247 | PMCID: PMC11677005. **IF: 3.4**

Vida, B., Tóth, R., Merkely, P., Ács, N., Novák, Z., Balázs, B., Madaras, L., Bánhidy, F., Tabányi, Á., Keszthelyi, M., & Lintner, B. (2025). Cervical Cancer During Pregnancy: A Multidisciplinary Approach to a Complex Oncological Case. Reproductive Medicine, 6(3), 18. https://doi.org/10.3390/reprodmed6030018 **IF: 1.3**

Publications not related to the thesis:

Vida, B., Keszthelyi, E., Tóth, Z., Lőczi, L., Sebők, B., Merkely, P., Lintner, B., Bánhidy, F., Keszthelyi, A., Várbíró, S., Tóth, R., & Keszthelyi, M. (2025). The Neutrophil-to-Lymphocyte Ratio (NLR) as A Potential Predictor in Conization Outcomes

for Cervical Cancer. Cancers, 17(11), 1856. https://doi.org/10.3390/cancers17111856 **IF: 4.5**

Keszthelyi, M., Sebok, P., Vida, B., Szabó, V., Kalas, N., Várbíró, S., Lőczi, L., Ács, N., Merkely, P., Tóth, R., & Lintner, B. (2025). Efficacy of Fertility-Sparing Treatments for Early-Stage Endometrial Cancer—Oncologic and Reproductive Outcomes: Protocol of a Systematic Review and Meta-Analysis. Life, 15(7), 1133. https://doi.org/10.3390/life15071133 **IF: 3.4**

Kalas N, Szabó V, Vida B, Tóth Z, Lőczi L, Sebők B, Merkely P, Lintner B, Ács N, Keszthelyi A, Várbíró S, Tóth R, Keszthelyi M. The Platelet-to-Lymphocyte Ratio (PLR) as a Non-Invasive Biomarker for Cervical Malignancy in Conization Patients. Life (Basel). 2025 Jun 18;15(6):971. doi: 10.3390/life15060971. PMID: 40566623; PMCID: PMC12194268. **IF:3.4**

∑IF: 24,7