FUTURE PERSPECTIVES OF SENTINEL NODE MAPPING IN GYNECOLOGICAL ONCOLOGY

EDITED BY: Angela Santoro, Fabio Martinelli, Andrea Papadia and Gian Franco Zannoni

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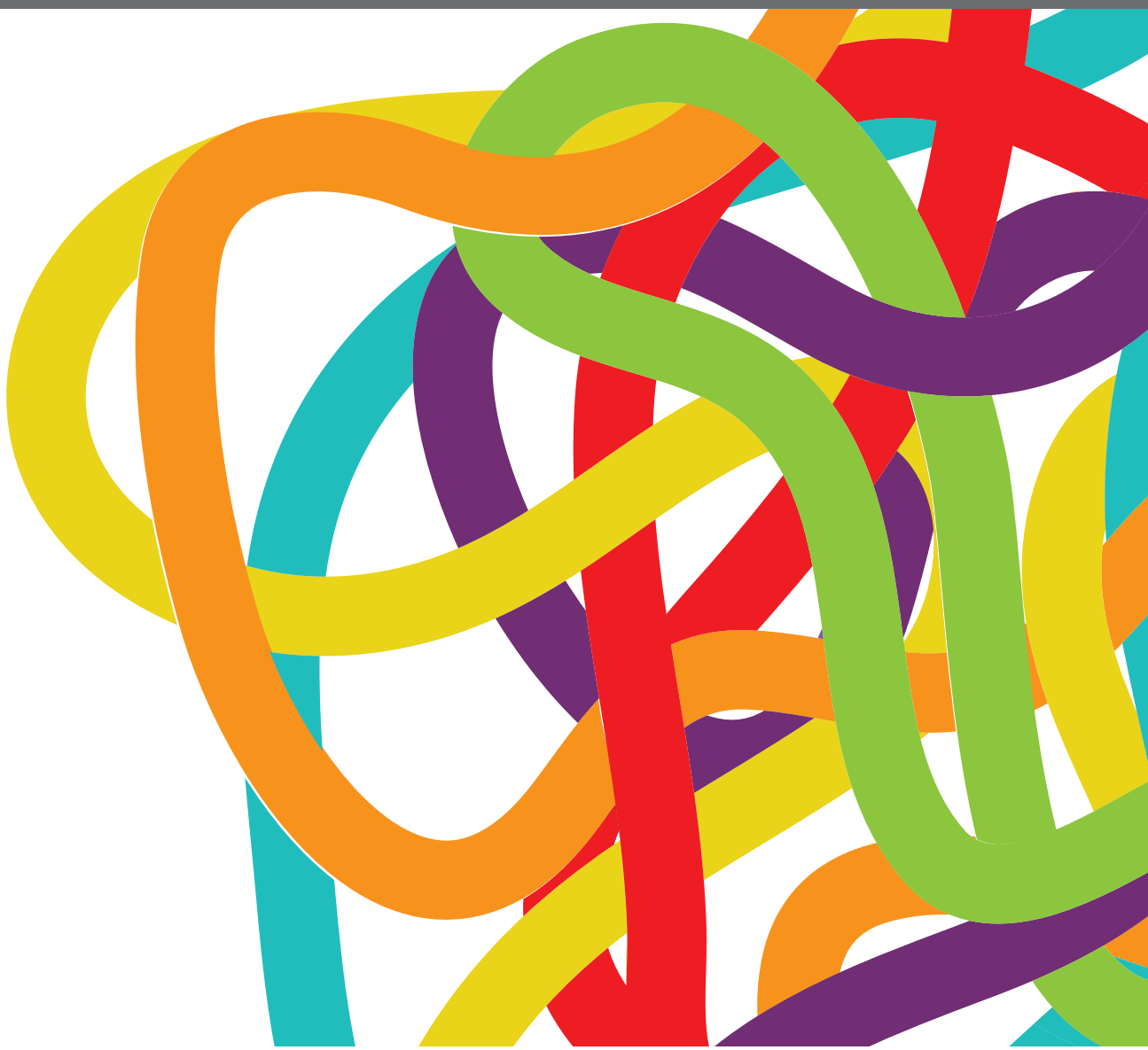

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FUTURE PERSPECTIVES OF SENTINEL NODE MAPPING IN GYNECOLOGICAL ONCOLOGY

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EDITORIAL
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Editorial: Future Perspectives of Sentinel Node Mapping in Gynecological Oncology

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Editorial on the Research Topic

Future Perspectives of Sentinel Node Mapping in Gynecological Oncology

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Lymph node (LN) metastasis in gynecological malignancies represents the most important negative prognostic predictor (1). Considering the high treatment-related morbidity of regional lymphadenectomy, sentinel LN (SLN) mapping has been increasingly used in the last years for staging purposes in gynecological tumors (1, 2). Clinical and pathological aspects specific to each gynecological site are discussed below.

In breast cancer the presence of metastatic SLNs still necessitates the recommended procedure for axillary staging of early neoplasms (3). Currently, circulating microRNAs (miRNAs) are recognized as promising non-invasive biomarkers and innovative prognostic factors, being correlated to LN status, occurrence of distant metastases, and recurrence. In their work, Escuin et al. showed the different expression profile of several circulating miRNAs in relation to the SLN status, suggesting the potential role of peripheral blood circulating nucleic acids as surrogate markers of LN metastases in early breast cancer patients.

SLN mapping in early-stage vulvar cancer represents the gold standard for patients with unifocal vulvar tumor, >1 mm in thickness and negative groin lymph nodes by clinical and imaging examination (Zhou et al., Siegenthaler et al.). Since the SLN utility and applications in vulvar cancer are still debated, Zhou et al. compared the safety of SLN biopsy (SLNB) with regional LN dissection (RLND) in patients with vulvar squamous cell cancer (Zhou et al.). Their findings indicate that SLNB is related to prolonged survival outcomes in patients with no metastatic or advanced-stage disease compared to RLND and no LN removed, irrespective of tumor size, surgery type, or invasion depth (Zhou et al.). A recent study has also established the clinical utility of ultrasound-guided fine-needle aspiration cytology (FNAC), stating that a positive result is sufficient to avoid an unnecessary SLN sampling, enabling the surgeon to perform a bilateral inguinofemoral lymphadenectomy (4).

The standard SLN detection technique involves a peritumoral injection of technetium-99m (99mTc) nanocolloid combined with an intraoperative injection of a blue dye. A novel, potentially interesting, SLN mapping technique has been proposed by Siegenthaler et al., demonstrating an

improvement of the SLN detection rate by using a combination of ^{99m}Tc-nanocolloid with indocyanine green.

Regarding SLN mapping in cervical cancer, the New ESGO/ESTRO/ESP guidelines incorporated SLN biopsy as an acceptable method of LN staging in early-stage cervical cancers, particularly in cases of small volume tumors (5). In the light of preliminary results of ongoing trials, and considering the long-term morbidity related to full pelvic LN dissection, a minimally invasive approach represents the standard of care in early-stage cervical cancer patients (5, 6). In this regard, Favre et al. in their randomized study, comparing early-stage patients undergoing SLN biopsy alone versus pelvic LN dissection, demonstrated no significant differences between the two groups in terms of overall survival and disease recurrence.

The gold-standard technique to process SLN in cervical cancer is the ultrastaging protocol (1, 7).

This technique, requiring LNs serial sectioning and immunohistochemistry, is utilized in pathology laboratories to confirm the negative status of a LN and also detect small-volume metastases, ranging between 0.2 and 2 mm in size. Recently, OSNA protocol, based on a quantitative measurement of target mRNA in a metastatic LN, has been proposed as an efficient alternative method for the intra-operative assessment of SLN in cervical cancer patients (8, 9). To date, the biological significance of small volume metastases in early cervical cancer is still highly debated. In the most detailed studies available, the presence of SLN micrometastases is related with a worse prognosis, representing an indication for adjuvant radiotherapy (1, 7). However, recently, SENTICOL1 trial results showed that SLNs micrometastases did not impact on progression-free survival in cervical cancer patients (10). Similarly, the biological significance and clinical management of SLN isolated tumor cells (ITCs) is still highly debated (1, 7).

As illustrated in the review article by Zhai et al., in endometrial cancer SLN mapping has emerged as a reliable alternative to pelvic LN dissection. Several studies have demonstrated a high sensitivity and negative predictive value to detect nodal metastases leading to similar oncological outcomes between patients undergoing SLN and pelvic lymphadenectomy (1, 2, Zhai et al.). Therefore, in 2020 National Comprehensive Cancer Network (NCCN) guidelines, SLN mapping has been recommended for staging purposes in EC patients (1). In this regard, according to the recent meta-analysis conducted by Gu et al., SLN mapping seems the more appropriate approach for both low- and high-risk EC patients given its lower surgical risk and patient morbidity in comparison to pelvic LN dissection. However, the article by Pineda et al., highlighted that high-risk EC patients could still benefit from pelvic LN dissection since sensitivity and negative predictive value observed in their cohort were 85.7 and 96.6% respectively (Gu et al.). On the other hand, SLN biopsy demonstrated high sensitivity and negative predictive values in intermediate-risk EC patients (Gu et al.).

Therefore, according to available literature data, full lymphadenectomy could be avoided by performing SLNB in patients of low and intermediate risk while additional data on larger cohorts need to be collected in order to demonstrate the staging and prognostic benefits of SLN biopsy in high-risk EC.

LN status is also a relevant prognostic factor in early ovarian carcinoma; however, para-aortic and pelvic lymphadenectomies carry a significant risk of intra- and post-operative morbidity (11, 12). SLN mapping could represent a safer staging alternative to lymphadenectomy, however studies on this topic are still limited to small cohorts. Preliminary results of a currently opened prospective multicenter study (SELLY: Sentinel-node biopsy in early-stage ovarian cancer: preliminary results) suggest that SLN can be difficult to identify even for experienced surgeons (12). Moreover, the authors pointed out that larger cohort studies are needed in order to determine the real sensitivity and negative predictive value of this technique (12).

In conclusion, SLN biopsy is an intraoperative procedure with potential for adequate staging with less treatment-related morbidity. It should be performed by a skilled multidisciplinary team, in oncology centers, preferably within the protection of clinical trials. Different methods of histopathological and molecular SLN assessment according to the different gynecological cancers have been proposed. This Research Topic, collecting several papers on this topic, discusses the need for standardization of pathological protocols, the molecular aspects of SLN evaluation in gynecological cancer, and the clinical benefits of this treatment option in routine practice.

AUTHOR CONTRIBUTIONS

All authors contributed to the Research Topic editorial and performed the literature search. AS and GA drafted the manuscript. GZ critically revised the work. All authors read and approved the final manuscript.

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SYSTEMATIC REVIEW
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Operative and Oncological Outcomes Comparing Sentinel Node Mapping and Systematic Lymphadenectomy in Endometrial Cancer Staging: Meta-Analysis With Trial Sequential Analysis

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Objective: To evaluate the utility of sentinel lymph node mapping (SLN) in endometrial cancer (EC) patients in comparison with lymphadenectomy (LND).

Methods: Comprehensive search was performed in MEDLINE, EMBASE, CENTRAL, OVID, Web of science databases, and three clinical trials registration websites, from the database inception to September 2020. The primary outcomes covered operative outcomes, nodal assessment, and oncological outcomes. Software Revman 5.3 was used. Trial sequential analysis (TSA) and Grading of Recommendations Assessment, Development, and Evaluation (GRADE) were performed.

Results: Overall, 5,820 EC patients from 15 studies were pooled in the meta-analysis: SLN group (N = 2,152, 37.0%), LND group (N = 3,668, 63.0%). In meta-analysis of blood loss, SLN offered advantage over LND in reducing operation bleeding (I^2 = 74%, P<0.01). Z-curve of blood loss crossed trial sequential monitoring boundaries though did not reach TSA sample size. There was no difference between SLN and LND in intra-operative complications (I^2 = 7%, P = 0.12). SLN was superior to LND in detecting positive pelvic nodes (P-LN) (I^2 = 36%, P<0.001), even in high risk patients (I^2 = 36%, P = 0.001). While no difference was observed in detection of positive para-aortic nodes (PA-LN) (I^2 = 47%, P = 0.76), even in high risk patients (I^2 = 62%, P = 0.34). Analysis showed no difference between two groups in the number of resected pelvic nodes (I^2 = 99%, P = 0.26). SLN was not associated with a statistically significant overall survival (I^2 = 79%, P = 0.94). There was no difference in progression-free survival between SLN and LND (I^2 = 52%, P = 0.31). No difference was observed in recurrence. Based on the GRADE assessment, we considered the quality of current evidence to be moderate for P-LN biopsy, low for items like blood loss, PA-LN positive.

Conclusion: The present meta-analysis underlines that SLN is capable of reducing blood loss during operation in regardless of surgical approach with firm evidence from TSA. SLN mapping is more targeted for less node dissection and more detection of positive lymph nodes even in high risk patients with conclusive evidence from TSA. Utility of SLN yields no survival detriment in EC patients.

Keywords: endometrial cancer, sentinel node mapping, lymphadenectomy, operation, lymph node assessment, oncological outcome

HIGHLIGHTS

- SLN is capable of reducing blood loss during operation in regardless of surgical approach with firm evidence from TSA.
- SLN mapping is more targeted for less node dissection and more detection of positive lymph nodes even in high risk patients with conclusive evidence from TSA.
- Utility of SLN yields no survival detriment in EC patients.

INTRODUCTION

Endometrial cancer (EC) is the most common gynecological malignancy in developed countries, and an estimated 65,620 new cases in United States in 2020 (1). The disease incidence has been climbing by 1.5 times over the last 10 years, and the death cases have increased by 58.4% according to latest statistics (1, 2). Though 5-year overall survival (OS) has reached at 80%, it has not made any progress since 1985, estimated in the US Surveillance, Epidemiology, and End Results (SEER) database (3).

Surgical staging is the step of final diagnosis and first treatment in most EC patients, and the standard operation includes hysterectomy, bilateral salpingo-oophorectomy, and lymph node assessment, allowing prognostic stratification and potentially benefited patients identification (3).

Lymph node status is a definite prognostic factor, albeit clinical trials showed no survival benefit in patients with nodal examination *versus* those not (4, 5). Ongoing controversy remains the extent of nodal dissection to tailor post-operation therapy. Traditional lymph node assessment contains systematic pelvic ± para-aortic lymphadenectomy (LND), and given low lymph nodal involvement rate, LND is prone to cause overtreatment and thus more surgery-related complications like lymphedema (6).

Sentinel lymph node mapping (SLN) has emerged as a reliable alternative in EC nodal assessment. Accumulating studies have demonstrated SLN was equal to LND in low- and high-risk EC patients and oncological outcomes were similar in both SLN and LND groups (7, 8). It has been recommended in low- and high-risk EC patients for surgical staging procedures in 2020 National Comprehensive Cancer Network (NCCN) guidelines (9). The superiority of SLN lies in pathological ultra-staging to avoid overtreatment and undertreatment.

A previous meta-analysis indicated SLN was superior to LND in nodal assessment (10), but given its limited data, further discussion about operative and oncological outcomes is still needed. The aim of this meta-analysis was to systematically review current evidence in comparison of two nodal assessment technologies, SLN and LND, in EC patients. The main outcomes contain surgery-related outcomes, nodal assessment, and oncological outcomes.

MATERIALS AND METHODS

This analysis has been registered in the International Prospective Register of Systematic Reviews (PROSPERO, https://www.crd.york.ac.uk/prospero, ID: CRD42020175099). And this meta-analysis was completed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (11).

Search Strategy

Comprehensive search was performed in MEDLINE, EMBASE, CENTRAL, OVID, Web of science databases, from the database inception to September 2020. The key words included "endometrial cancer," "sentinel node," and "lymphadenectomy". And three clinical trials registration websites, the Clinical trials.gov (www.clinicaltrials.com), WHO trial website (https://apps.who.int/trialsearch), and the Controlled Trials meta Register (www.controlled-trials.com), were searched as well. Details of search strategy is shown in **Supplement Files S1**.

Inclusion and Exclusion Criteria

Two independent reviewers (YG and HC) conducted selection of studies based on a protocol defined priorly. Studies were included if they met the following criteria: 1) patients diagnosed with endometrial cancer; 2) clinical trials concerning the comparison of sentinel node mapping and lymphadenectomy; 3) reported operative outcomes like operative time, blood loss, operative complications; lymph nodes assessment like the number of positive pelvic lymph nodes; oncological outcomes like overall survival and recurrence, but not limited to these above. The exclusion criteria as: 1) <10 patients; 2) review, case report, comment, and other types without original data; 3) full text could not be obtained; 4) written other than in English. At first screening, titles and abstracts of articles were assessed according to inclusion and exclusion criteria. Then full texts were read to identify eligibility. Consensus was made by discussion when disagreement occurring.

Data Extraction

Data were extracted using a modified form based on the Cochrane reviews handbook. The following information was collected: author, year of publication, study design, patients' characteristics, surgical approach, SLN technique, operative outcomes, nodal assessment and oncological outcomes, and so forth. Two reviewers (YG and LZ) conducted date extraction independently, and inconformity was resolved by discussion.

Quality Assessment

The Newcastle-Ottawa scale for cohort study was used for article quality assessment. This scale is comprised of three parts (selection, comparability, and outcome). With a maximum of nine stars, articles reaching six stars were included finally. Two reviewers (YG and YK) assessed articles independently, and consensus was reached by discussion in the event of disparity (**Supplement Files S2**).

Statistical Analysis

Software Revman 5.3 was used to pool data and generate forest plots. Mantel-Haenszel method was used in dichotomous data and the odds ratio (OR) was calculated. And for continuous data inverse variance and mean difference (MD) were applied. Random-effect model was used in analysis. Heterogeneity of included studies was assessed by I^2 and I^2>50% was defined as high heterogeneity. Subgroup analysis by SLN procedure or patients risk stratification was introduced when meeting high heterogeneity. When necessary, data, like operative time or blood loss, in form of (median, range) were transformed into (mean, standard difference) according to recommended methods (12). Hazard ratio (HR) and 95% confidence interval (CI) of overall survival (OS) and progression-free survival (PFS) were extracted from Kaplan-Meier curve using Engauge Digitizer software (10.7) and recommended methods (13). And for data failing to conducting meta-analysis, a narrative systemic review was performed. Trial sequential analysis (TSA) was performed by TSA software (version 0.9β) and we calculated sample size adjusted for this meta-analysis to testify whether the evidence is confirmed and conclusive. Pooled analysis was graded by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach, and the certainty of evidence was assessed as high, moderate, low, or very low, using GRADE pro website (https://gdt.gradepro.org).

RESULTS

A total of 2,048 articles were screened through the search strategy, and 21 articles were included after full text reading. Four were excluded for low quality assessment score (<6) (14–17), two were excluded for patients overlapping (18, 19), thus leaving 15 articles eligible for final analysis (7, 20–33) (**Figure 1**). Characteristics of the 15 studies are summarized in **Supplement Table S1**. Overall, 5,820 EC patients were pooled in the meta-analysis: SLN group (N = 2,152, 37.0%), LND group (N = 3,668, 63.0%), respectively.

Operative Outcomes

Data regarding operation related outcomes were available in seven studies (**Supplement Table S2**). In meta-analysis of blood loss, SLN offered advantage over LND in reducing operation bleeding; the MD was −54.40, 95% CI −85.36~−23.45 (I^2 = 74%, P<0.001; **Figure 2**). Z-curve of blood loss crossed trial sequential monitoring boundaries (TSMB) though did not reach TSA sample size, and indicating the result was true-positive (**Figure 3**). When intra-operative complications were measured, there was no difference between SLN and LND (I^2 = 7%, P=0.11, **Figure 4**).

When operative time was pooled, subgroup analysis failed to identify high heterogeneity (I^2 = 99%, P<0.01, **Supplement Figure 1A**), but a tendency of shorter operative time in SLN group was shown in **Supplement Table S2**. And TSA of operative time showed Z-curve crossed TSMB and highly surpassed TSA sample size (**Supplement Figure 1B**). Post-operative complications were assessed by Accordion Severity Grading System, Clavien-Dindo scale and MSKCC's Surgical Secondary Events Grading System. It seemed that SLN group had lower post-operative complications but more data are needed to conduct further analysis. When considering conversion rate, re-admission, re-operation, length of stay and frozen utility, potential advantage of SLN could be seen in shortening length of stay and frozen utility (**Supplement Figures 1C, D**). And TSA of length of stay showed inconclusive result for insufficient sample size (**Supplement Figure 1E**). Additionally, TSA of post-operative complications, conversate rate, re-admission, re-operation, and frozen utility were available for low sample size.

Lymph Node Assessment

The meta-analysis of nodal assessment was based on 10 trials (**Supplement Table S3**). SLN was superior to LND in detecting positive pelvic lymph nodes (I^2 = 36%, P<0.001, **Figure 5**). The Z-curve crossed TSMB and did not reach TSA sample size, and indicating the result was conclusive (**Figure 6**). While no difference was observed in detection of positive para-aortic nodes between two groups (I^2 = 47%, P = 0.76, **Figure 7**); and Z-curve did not cross TSMB and did not reach TSA sample size, and indicating the result was under discussion (**Figure 8**). In high risk patients, SLN had a higher pelvic nodes detection rate (OR 2.00, 95% CI 1.21~3.32, I^2 = 36%, P = 0.007, **Supplement Figure 2A**) and showed no difference in para-aortic nodes detection (OR 0.62, 95% CI 0.24~1.64, I^2 = 62%, P = 0.34, **Supplement Figure 2B**).

In pooling data of resected pelvic nodes, analysis showed no difference between two groups (I^2 = 99%, P = 0.26). Considering two SLN algorithm (subgroup1 SLN ± P-LND ± PA-LND; subgroup2 SLN+P-LND ± PA-LND) existed, subgroup analysis by SLN procedure was conducted and indicated that SLN procedure (SLN ± P-LND ± PA-LND) removed less pelvic nodes than LND(I^2 = 83%, P<0.01, **Figure 9**), and Z-curve did

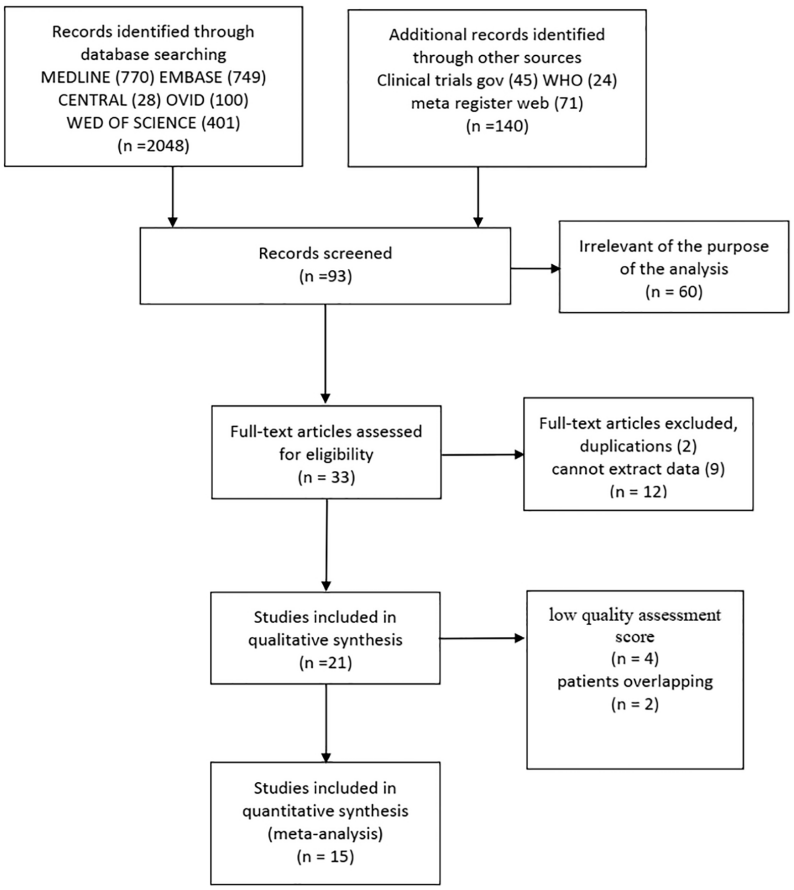

FIGURE 1 | Selection of studies for inclusion in the systematic review.

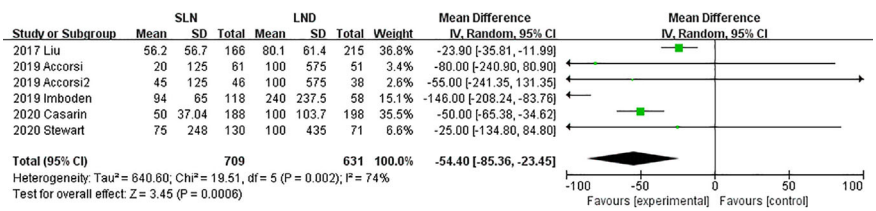

FIGURE 2 | Meta-analysis of blood loss.

not cross TSMB and did not reach TSA sample size, and indicating more studies were needed (**Figure 10**). The same subgroup analysis was undergone in pooling data of resected para-aortic nodes as well, and similar result was observed that SLN procedure removed less para-aortic nodes (I^2 = 0%, P<0.001, **Figure 11**), and Z-curve did not cross TSMB and did not reach TSA sample size, and indicating more studies were needed (**Figure 12**).

Oncological Outcomes

Supplement Table S4 reports data concerning disease characteristics. SLN was not associated with a statistically significant OS (I^2 = 79%, P = 0.81, **Supplement Figure 3A**). There was no difference in PFS between SLN and LND groups (I^2 = 52%, P = 0.31, **Supplement Figure 3B**). No difference was observed in overall recurrence (all sites, I^2 = 75%, P = 0.41, **Supplement Figure 3C**), and Z-curve did not cross TSMB and

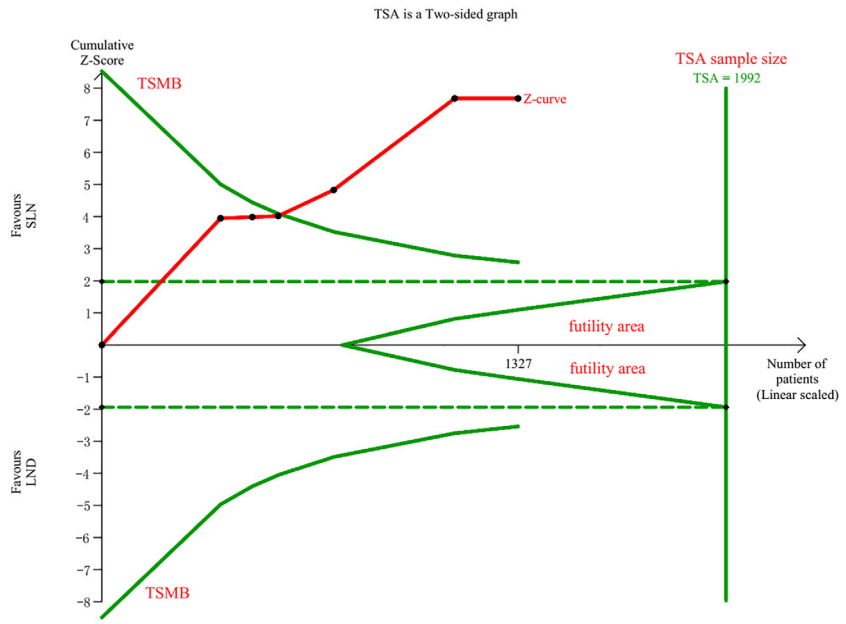

FIGURE 3 | TSA of blood loss, $\alpha = 0.05$, $\beta = 0.8$, two-sided test.

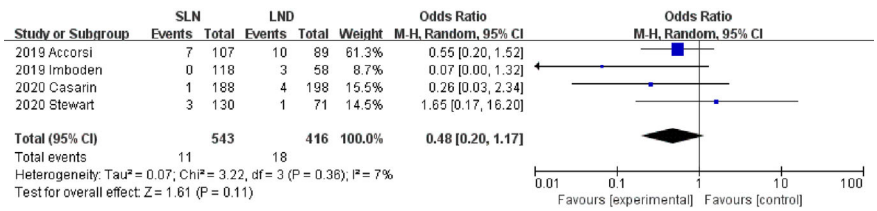

FIGURE 4 | Meta-analysis of intra-operative complications.

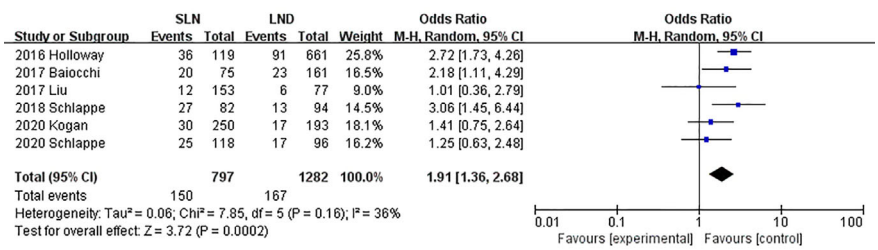

FIGURE 5 | Meta-analysis of pelvic lymph nodes positive.

did not reach TSA sample size, and indicating more studies were needed (**Supplement Figure 3D**).

In terms of recurrence pattern, analysis of nodal recurrence, locoregional recurrence, and multifocal recurrence showed no differences between SLN and LND (P>0.05); TSA showed further studies were needed (**Supplement Figures 4A–E**). Data on death of disease was available for two trials, and meta-analysis showed no difference between two groups (P>0.05, **Supplement Figure 5**).

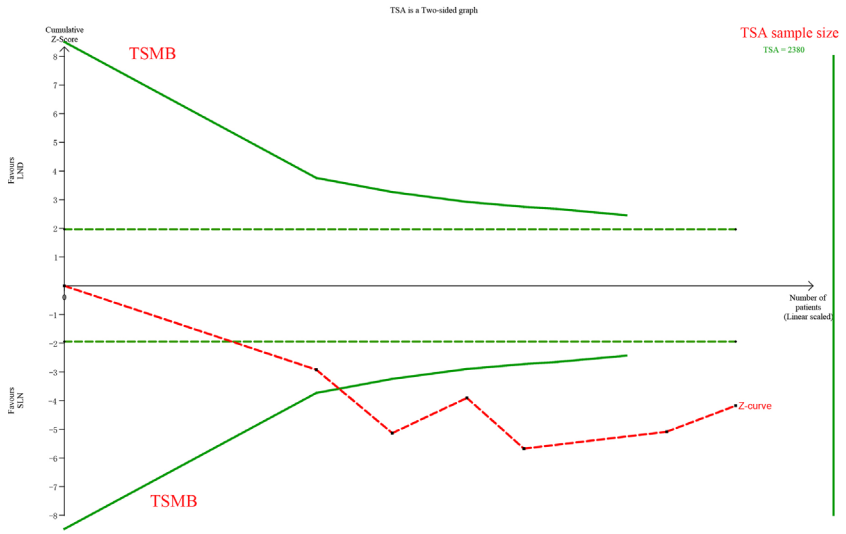

FIGURE 6 | TSA of pelvic lymph nodes, $\alpha = 0.05$, $\beta = 0.8$, relative risk reduction = −73.8%, incidence in control group = 16.4%, two-sided test.

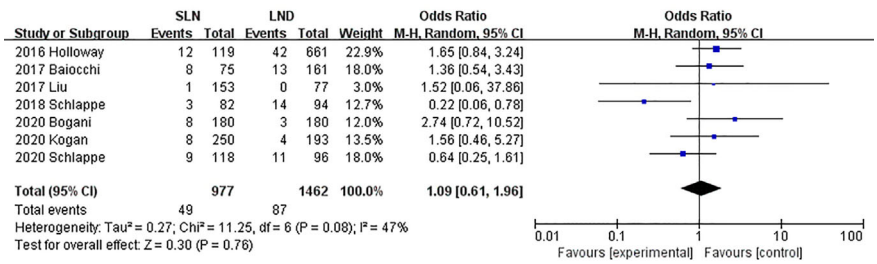

FIGURE 7 | Meta-analysis of para-aortic lymph nodes positive.

GRADE Assessment

Based on the GRADE assessment, we considered the quality of current evidence to be moderate for P-LN biopsy, low for items like blood loss, PA-LN positive. We postulated that because of basis on cohort studies, the grading hardly reached higher (**Table 1**).

DISCUSSION

The analysis reviewed present evidence in comparison of SLN and LND in EC patients, and the main findings covered operation-related outcomes, nodal assessment, and oncological outcomes. To our knowledge, this is the first meta-analysis discussing surgery-related outcomes, and found SLN was capable to reduce blood loss with firm evidence from TSA. The pooled date validates that SLN allows an accurate detection of positive lymph nodes in circumstance of less node removal, especially with conclusive evidence from TSA of P-LN positive patients. Additionally, no difference is observed in OS, PFS, and recurrence between two procedures, and TSA of recurrence showed further investigation are needed.

SLN has been gaining popularity in gynecological cancer staging over the past decades. Initial exploration of SLN by gynecological oncologist started at vulvar cancer (34) and subsequent studies validated its feasibility (35). It has experienced two stages of SLN employment in EC staging, and in the first stage multiple researches were focusing on the feasibility and reliability of SLN. Abu-Rustum et al. (36) identified a 100% sensitivity and low false-negative rate in grade 1 EC patients, with the methods of SLN procedure followed by systematic pelvic and para-aortic LND. After accumulating studies indicating the accuracy of SLN in EC staging, SLN has been evolved as a more targeted alternative for nodal assessment (37, 38). In 2014, SLN was firstly recommended by NCCN guidelines to stage I patients (39). A meta-analysis reported a>80% overall detection rate of SLN (40). A consensus from the Society of Gynecological Oncology (SGO) in 2017, approved the execution of SLN in low-risk patients (41). Till 2018, NCCN guidelines began to support SLN application in

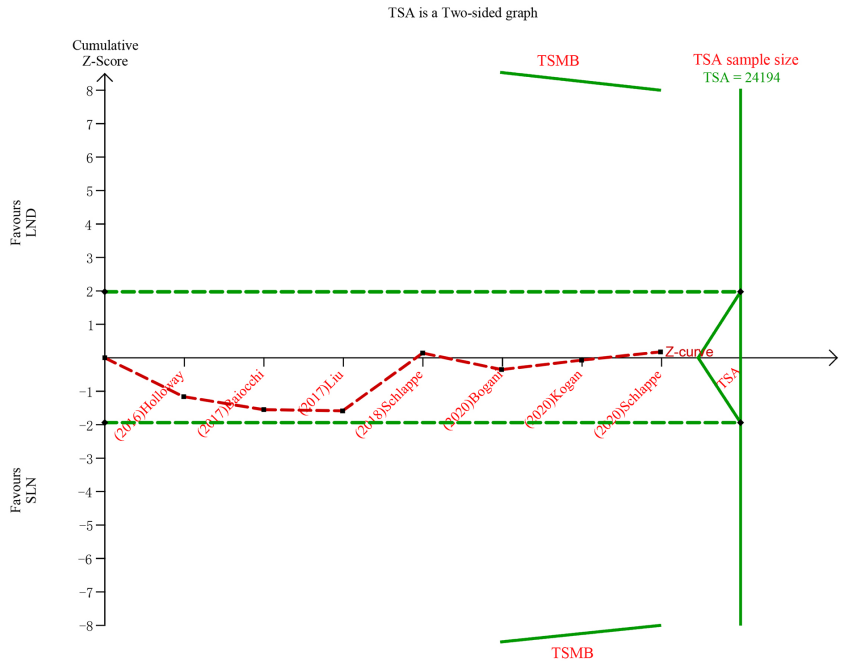

FIGURE 8 | TSA of para-aortic lymph nodes, $\alpha = 0.05$, $\beta = 0.8$, relative risk reduction = 15.4%, incidence in control group = 9.2%, two-sided test.

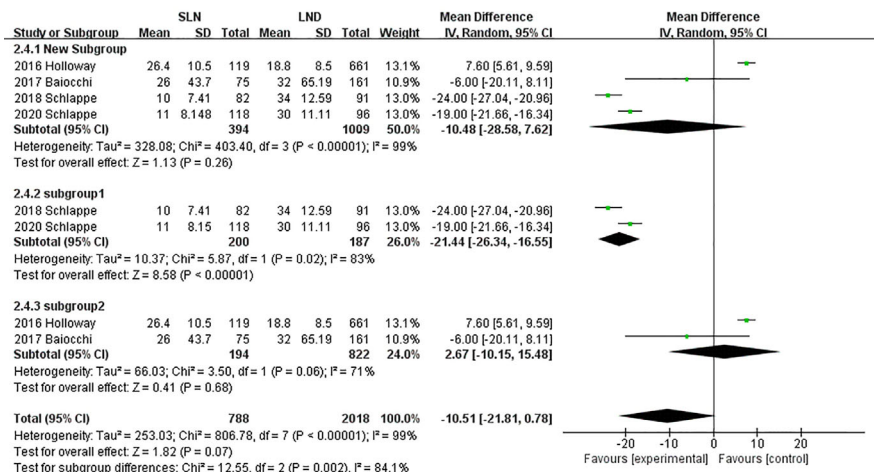

FIGURE 9 | Meta-analysis of pelvic lymph nodes removed.

all EC patients including those with high risk (42). At present the discussion about SLN *vs.* LND is still going on (43). A recent meta-analysis highlighted the safety and effectiveness of SLN (10), but given its limitation we conducted this analysis with latest evidence, bigger sample size and border outcomes measures.

SLN is introduced into EC staging with the aim of reducing LND-related morbidity and gaining prognostic factors of lymph node status (41). SLN techniques has been evolving during gynecologic oncology application. Three different tracers, patent blue, technetium 99, and indocyanine green (ICG), are the mainstay of SLN mapping (41). Considering the unreliability and radiation, the SGO recommended ICG dye with infrared imaging to EC patients for its high success and technical ease (41). The optimal site for tracer injection has been investigated in precious studies (44–46), out of common sites like myometrium, cervix injection is regarded as the most effective way to trace SLN (41).

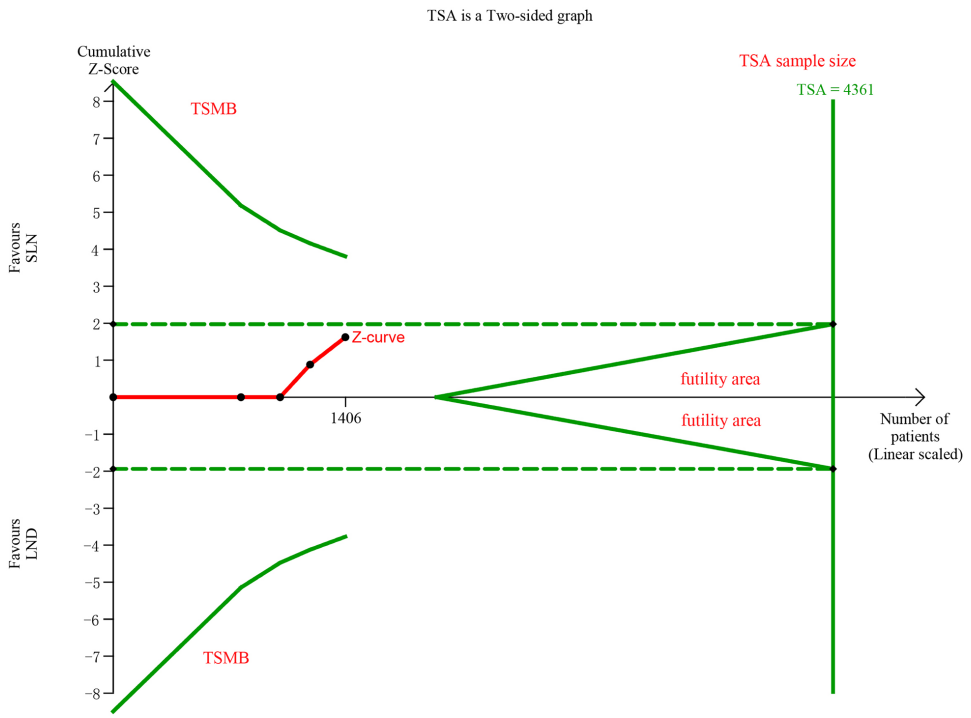

FIGURE 10 | TSA of pelvic lymph nodes removed, $\alpha = 0.05$, $\beta = 0.8$, two-sided test.

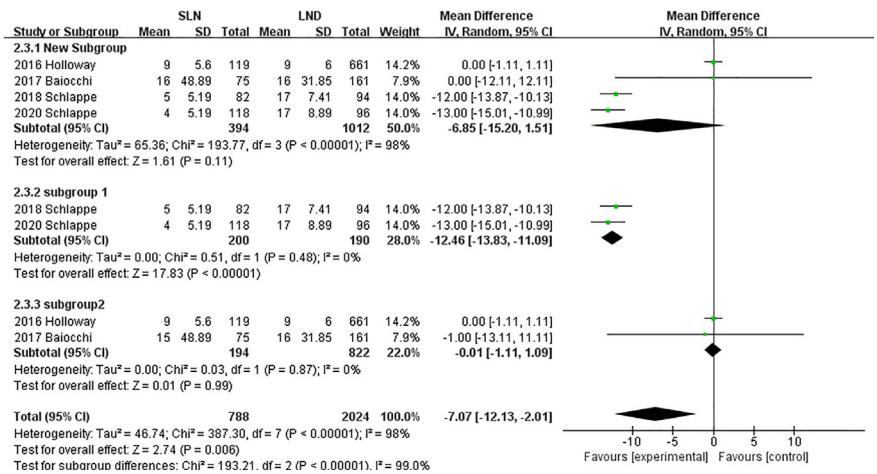

FIGURE 11 | Meta-analysis of para-aortic lymph nodes removed.

Recent studies focusing on operation-related outcomes indicated that SLN procedure could reduce operative time of minimal invasive surgery and laparotomy (23, 25, 26, 28, 30, 33). Stewart et al. (33) observed significant decrease in operative time (210 *vs* 170 min, P = 0.007) taking account of the surgery approach. And Valerio G. et al. (47) discussed robotic surgery in elderly gynecological cancer patients and demonstrated that minimally invasive could considered for older patients (even over 75 years old); and this illustrated SLN procedure during minimal invasive surgery could benefit patients more, especially these over 75 years old. The meta-analysis indicated that blood loss was significantly lower in SLN group. In terms of

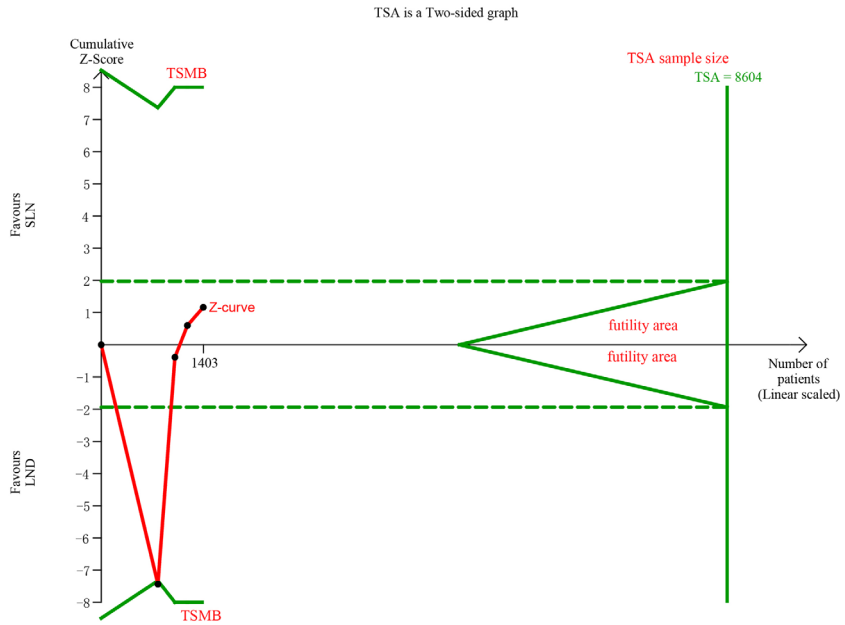

FIGURE 12 | TSA of para-aortic lymph nodes removed, $\alpha = 0.05$, $\beta = 0.8$, two-sided test.

complications, more evidence is needed for supporting intra- and post-operation complications declining. Accordion Severity Grading System and Clavien-Dindo scale are used to assess post-operation complications; two studies by Accordion Severity Grading System (30, 33) and two by Clavien-Dindo scale (26, 28) indicated SLN group occurred less post-operation complications. A retrospective study reported lower-limb lymphedema could only been seen in LND group (25). Leitao et al. (48) concluded that SLN was independently related to lower self-reported lower-extremity lymphedema rate than LND.

The meta-analysis indicates that SLN is more targeted for less node dissection and more detection of positive lymph nodes. The FIRES trial, enrolling stage I patients, yielded a high sensitivity of 97% and a negative predictive value of 99.6% by SLN mapping, and prevented more people from the morbidity of LND (38). Accumulating data indicated SLN was significantly associated with accurate detection of pelvic lymph nodes and was non-inferior to LND in para-aortic nodes assessment in high risk EC patients (7, 18, 21–23, 32, 41). The pathologic ultra-staging technique adopted by SLN mapping, defines positive lymph node as macro-metastasis (≥2 mm), micro-metastasis (≥0.2 mm), and isolated tumor cells (≤0.2 mm) (41). Ultra-staging could upgrade 10–40% patients in previous studies for identification of low volume metastasis in lymph nodes (49, 50).

This meta-analysis showed no survival and recurrence detriment in SLN mapping compared with LND, in accordance with the previous meta-analysis (10). Experience from a study with 1,135 low risk patients indicated that 3-year OS and PFS were similar in two groups (P>0.07) (15). A multicenter study in high risk patients showed HR for association of staging approach (SLN and LND) with progression and death was 3.12 (95% CI 1.02–9.57) and 0.69 (95% CI 0.24–1.95) respectively (32). Similarly, Multinu et al (18). reported the risk of progression and death were not significantly different between SLN *vs.* LND (HR 1.27, 95% CI 0.6–2.67; HR 2.10, 95% CI 0.79–5.58, respectively). Additionally, no difference in recurrence pattern was observed between two groups; this meta-analysis showed there was no difference in overall recurrence (all sites), nodal recurrence, locoregional recurrence, and multifocal recurrence. Multiple studies reported distant/multifocal recurrence was predominant; Schiavone et al. (24) found 74% patients occurred multifocal recurrence and 16% endured nodal recurrence. A 56% (19/34) of multifocal recurrence in all patients with recurrence was reported in retrospective cohort study (18).

The imitations of the meta-analysis are as follows. First, most of pooled studies are retrospective cohort studies, futured prospective studies comparing SLN and LND are warranted. Second, some included studies did not provide the needed data directly, therefore some statistical methods were utilized to obtain proper data, which may decrease inaccuracy. Third, this meta-analysis is based on observational studies, and fails to reach high GRADE assessment.

In conclusion, the present meta-analysis reviewed current evidence on SLN mapping in comparison of LND. SLN is capable of reducing blood loss during operation in regardless of surgical approach with firm evidence from TSA. Future studies on operation time and complications are needed for further analysis. SLN mapping is more targeted for less node dissection and more detection of positive lymph nodes even in high risk patients with conclusive evidence from TSA. Utility of SLN yields no survival detriment in EC patients.

TABLE 1 | GRADE evidence profile: Quality assessment.

Certainty assessment							Number of patients		Effect	Certainty	Importance
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	SLN(n)	LND(n)	Relative (95% CI)	Absolute(95% CI)	
Operation time											
8	Observational studies	Not serious	Serious[a]	Not serious	Not serious	None	959	2,243	–	MD 75.37 lower (106.36 lower to 44.38 lower)	⨁◯◯◯ VERY LOW	CRITICAL
Blood loss											
6	Observational studies	Not serious	Not serious	Not serious	Not serious	None	709	631	–	MD 54.4 lower (85.36 lower to 23.45 lower)	⨁⨁◯◯ LOW	CRITICAL
P-LN positive											
6	Observational studies	Not serious	Not serious	Not serious	Not serious	strong association[b]	150/797 (18.8%)[c]	167/1,282 (13.0%)	OR 1.91 (1.36 to 2.68)	92 more per 1,000 (from 39 more to 156 more)	⨁⨁⨁◯ MODERATE	CRITICAL
PA-LN positive											
7	Observational studies	Not serious	Not serious	Not serious	Not serious	None	49/977 (5.0%)	87/1,462 (6.0%)	OR 1.09 (0.61to 1.96)	5 fewer per 1,000 (from 21 fewer to 51 more)	⨁⨁◯◯ LOW	CRITICAL

GRADE, Grading of recommendation assessment, development, and evaluation; n, number of patients; CI, Confidence interval; MD, Mean difference; RR, Risk ratio; OR, Odds ratio; HR, Hazard Ratio; [a]Heterogeneity = 99%; [b]RR>2; [c][n/total n (%)].

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization: YG. Methodology: All authors. Project administration: YX. Supervision: YX. Writing—original draft: YG. Writing—review and editing: all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.580128/full#supplementary-material

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Sentinel Lymph-Node Biopsy in Early-Stage Cervical Cancer: The 4-Year Follow-Up Results of the Senticol 2 Trial

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Introduction: Senticol 2 is a randomized multicenter trial in the treatment of early-stage cervical cancer patients. The aim of the Senticol 2 study was to compare the effect of sentinel-lymph-node biopsy (SLNB) to that of SLNB + pelvic lymphadenectomy (PLND), and to determine the postoperative lymphatic morbidity in the two groups. Here, we report a secondary objective of this study: the follow up.

Material and Methods: In the Senticol 2 trial, patients underwent a laparoscopy with a sentinel-node-detection procedure and were randomized into two groups, namely: Group A, in which participants received SLNB, and Group B, in which participants received SLNB + PLND. Patients with an intra-operative macroscopically suspicious lymph node, were given a frozen-section evaluation and were randomized only if the results were negative. All of the patients received follow up with a clinical examination at 1, 3, and 6 months after surgery, and then every 3–4 months after that. The median follow up was 51 months (4 years and 3 months).

Results: Disease-free survival after 4 years for the SLNB group and the SLNB + PLND group were 89.51% and 93.1% (p = 0.53), respectively. The only statistical factor associated with recurrence in the univariate analysis was the adjuvant radiotherapy. No other factors, including the age of the patients, histological type, tumor size, lymph vascular space invasion (LVSI), and positive nodal status, were significant in the univariate or multivariate analyses. The overall survival rates after 4 years in the SLNB and SLNB + PLND groups were 95.2% and 96% (p = 0.97), with five and four deaths, respectively. The univariate and multivariate analyses did not find any prognostic factors.

Conclusions: This randomized study confirmed the results of the Senticol 1 study and supports the sentinel lymph node (SLN) technique as a safe technique for use in patients with early-stage cervical cancer treated with SLNB only. Disease-free survival after 4 years

was similar in patients treated with SLN biopsy and patients who underwent a lymphadenectomy.

Keywords: cervical cancer, sentinel lymph node, lymphadenectomy, survival, lymph node biopsy

INTRODUCTION

Cervical cancer results in over 300,000 deaths worldwide every year (1).

Advances in cervical-cancer screening have resulted in a lower incidence of cervical cancer but a higher incidence of early-stage disease diagnosis in developed countries (2, 3). One of the most important prognosis factors in the early stages is the pelvic lymph-node status.

It has been demonstrated that the lymph-node status directly impacts the 5-year survival rate of patients with International Federation of Gynecology and Obstetrics (FIGO 2009) Stage IA1 to IIB cancers (4).

According to the international guidelines for the treatment of early-stage cervical cancer, the gold-standard treatment includes pelvic-lymph-node dissection (PLND) in order to adapt the treatment to a potential lymphatic metastasis. In the Senticol 1 trial (5), we demonstrated the feasibility and safety of the sentinel lymph node (SLN) technique when used with bilateral detection. A review of the literature by Tax et al. showed 99% sensitivity and a 97–100% negative predictive value (NPV) for the bilaterally detected sentinel lymph node (SLN) technique (3).

A lymph-node metastasis is present in 27% of early cervical cancers, leading to a high rate of overtreatment with unnecessary PLND in three out of four patients (2, 3). Moreover, this lymphatic surgery is known to induce significant morbidity and to lead to a decreased quality of life (6).

A SLNB procedure can accurately detect metastases for several other diseases such as breast, penile, skin, and vulvar cancer. In 2015, the National Comprehensive Cancer Network (NCCN) Guidelines (7) stated that SLN mapping should be considered an option for PLND in cervical cancer (category 2B).

Furthermore, we showed previously that an SLN biopsy can decrease both early and long-term morbidity and can improve quality of life compared with a complete pelvic lymphadenectomy (6).

The aim of this study was to assess the disease-free survival and overall survival of early-stage cervical cancer patients included in the randomized controlled multicenter Senticol 2 study.

PATIENTS AND METHODS

Patients

The Senticol 2 protocol (clinical trial #NCT01639820) was approved by an ethics committee (Comité de Protection des Personnes Sud-Est IV, decision A08-223) and all of the patients provided written informed consent before inclusion.

We performed a randomized controlled trial from December 2008 to November 2011. A total of 25 centers were included, all surgical team were experimented (>20 cases). The number of cases per center and the name of the surgeon is described in **Supplementary Table 2** in the **Appendix**.

Patients were followed for a minimum of 3 years after their inclusion in the study.

The inclusion criteria were as follows: Women aged 18 or older diagnosed with cervical carcinoma of FIGO 2009 stage IA1 with LVSI, to IIA1, including any histological subtype (except neuroendocrine carcinoma). The patients were eligible for laparoscopy and written informed consent was obtained from all of the patients.

The exclusion criteria were as follows: Pregnant women, stage IB (by downstaging), evolving or recurrent cervical cancer, other cancer diagnosed during treatment, history of pelvic node surgery, or severe allergy or contraindication to radioactive tracer or Patent Blue.

Methods

All patients received injections of the radioactive tracer colloidal rhenium sulfide labeled with technetium (99mTc; Nanocis®, CIS Bio International) on the day of (60 MBq; short protocol) or the day before the surgery (120 MBq; long protocol), after which 2 mL of vital dye (Patent Blue®, Laboratoire Guerbet) diluted with 2 mL of water was injected into the cervix at the 3, 6, 9, and 12 o'clock positions.

In addition, a pre-operative lymphoscintigraphy was performed in order to detect SLNs during surgery, especially in unexpected locations.

The patients underwent a laparoscopy using a sentinel-node-detection procedure and were randomized into the following two groups: Group A, which received sentinel lymph node biopsy (SLNB), and Group B, which received SLNB + PLND.

The patients with intra-operative macroscopically suspicious sentinel or eventually non sentinel lymph nodes (NSLNs) received a frozen-section evaluation and were randomized only if the results were negative.

All of the SLNs were analyzed using the histological ultrastaging method (200 µm sections) and were stained with hematoxylin eosin saffron (HES) or hematoxylin phloxine saffron (HPS). An additional immunohistochemistry (IHC) analysis with a pan-cytokeratine antibody was performed in case of a negative SLN.

For the definite node-negative patients, we proceeded with an additional surgery—either a radical hysterectomy or radical trachelectomy (an extrafascial hysterectomy or simple trachelectomy were performed for tumors <2cm without lymph vascular space invasion (LVSI)). Adjuvant treatments were defined following the final histology.

For the definite node-positive patients, we proceeded with an additional treatment using chemo-radiotherapy after first considering a laparoscopic para-aortic lymphadenectomy.

All of the patients received clinical examination follow ups 1, 3, and 6 months after surgery, and then every 3–4 months afterwards. The median follow-up duration was 51 months.

Statistical Methods

Disease-free survival rates were estimated using the Kaplan Meier method. The survival curves were compared with a Log-rank test (unilateral test with a 5% significant threshold). Recurrence-free survival was defined as the time from surgery to disease recurrence or death due to any cause. A second analysis regarding patients with no evidence of recurrence or death was done at the date of the last follow-up. A multivariate analysis was performed including factors with $p < 0.15$. The multivariate analysis of the recurrence-free survival was performed using a Cox's proportional hazard model. All of the analyses were performed on an intention-to-treat basis.

RESULTS

Characteristics of Patients

Between December 2008 and November 2011, 267 patients participated in Senticol 2. Of these, 61 patients were excluded —2 (3.3%) had not had a lymphoscintigraphy before surgery, 12 (19.7%) had an incomplete SLNB procedure, 11 (18%) had an absence of SLN detection, 21 (34.4%) had a unilateral detection of SLN, and 15 (24.6%) had positive SLN on the frozen sections.

A total of 206 patients were randomized, with 105 patients assigned to Group A (SLNB) and 101 to Group B (SLNB + PLND).

The baseline characteristics of the patients at inclusion are summarized in **Table 1**. The median ages were 42.2 and 41.7 years, respectively. Most patients (88.4%) had FIGO stage IB disease. Histological subtypes represented were mainly epidermoid carcinoma (64.8% and 72.3%, respectively). There was a nonsignificant difference between the two groups in terms of the size of the tumors (median sizes were 19 and 15 mm, respectively).

All patients received injections of the 96 technetium and 2 mL of vital dye (Patent Blue®) diluted with 2 mL of water injected into the cervix at the 3, 6, 9, and 12 o'clock positions. We had no cases with discrepancy between the lymph-nodes marked with patent blue and lymph-nodes marked with technecium. During the SLN procedure, there was no difference in sentinel mapping between the two groups (**Supplementary Table 1** in the **Appendix**). The main location for the sentinel node was ilio-obturator and external iliac area (85.8%). The second main location was the common iliac area (9.6%) of SLN.

The surgical approaches were similar in the two groups (p = n.s.), with a radical hysterectomy rate of 78.7% and 80.5%, a radical trachelectomy rate of 16.9% and 13.4%, and a simple hysterectomy and trachelectomy rate of 4.5% and 6.1%, respectively.

The final histological analysis (including ultrastaging) of the SLNs indicated 12 (11.4%) patients with a positive node in the SLNB group (three with macro-metastasis, three with micro-metastasis, and six with isolated tumor cells) and 9 (8.9%) patients with a positive node in the SLNB + PLND group (three with macro-metastasis, four with micro-metastasis, and two with isolated tumor cells) (p = n.s.). In this group there were 9 positive SLN and 1 positive NSLN in a patient with a positive SLN also. The correspondence between SLN and NSLN was 100%.

The rate of postoperative adjuvant therapy was similar in the two groups (**Table 2**), including brachytherapy (32 in the SLNB group and 27 in the SLNB + PLND group), radiotherapy (13 and 16, respectively), and chemotherapy (9 and 11, respectively). Nine patients from the SLNB group underwent a secondary lymph-node dissection in raison of positive SLN on the final pathology. This secondary dissection was performed according to the protocols of the different centers. One patient had only a pelvic lymphadenectomy, 6 patients had pelvic and para-aortic dissection, and 2 patients had only para-aortic dissection. We observed one

TABLE 1 | Patient characteristics at baseline.

	SLNB n = 105	PLND n = 101	p value
Age (median)	42.2	41.7	0.81
BMI (median)	22.7	22.6	0.92
History of abdominal surgery (n (%))	69 (65.7)	70 (69.3)	0.66
PS score	88 (95.7)	88 (96.7)	0.19
3	4 (4.3)	2 (2.2)	
2	0	1 (1.1)	
1			
History of pregnancy (n (%))	85 (81)	87 (86.1)	0.43
Menopausal status (n (%))	29 (28.2)	30 (30)	
FIGO 2009 stage at inclusion	7 (6.7)	2 (2.2)	0.29
I A1 with LVSI	5 (4.8)	6 (6.0)	
I A2	90 (85.7)	91 (91.0)	
I B1	3 (2.9)	1 (1.0)	
II A1			
Histology (n (%))	68 (64.8)	73 (72.3)	0.68
Epidermoid carcinoma	33 (31.4)	24 (23.8)	
Adenocarcinoma	2 (1.9)	2 (2.0)	
Adenosquamous carcinoma	2 (1.9)	2 (2.0)	
Other			
Preoperative conization (n (%))	68 (64.8)	63 (62.4)	0.52
Presence of LVSI in the biopsy (n (%))	19 (27.9)	16 (25.4)	0.84
Surgical approach of radical hysterectomy:	6	4	0.87
Laparotomy	42	40	
Laparoscopy	41	38	
Vaginal-assisted laparoscopy			

SLNB, sentinel lymph node biopsy; PLND, pelvic lymph node dissection; BMI, body mass index; PS, performance status; LVSI, lymph vascular space invasion.

TABLE 2 | Post-operative adjuvant therapy.

Additional treatment	SLNB	SLNB + PLND	p value
Brachytherapy	32 (30.8%)	27 (26.7%)	0.54
Radiotherapy	13 (12.5%)	16 (15.8%)	0.55
Chemotherapy	9 (8.7%)	11 (10.9%)	0.64

SLNB, sentinel-lymph-node biopsy; PLND, pelvic lymph-node dissection.

case of positive para-aortic node in a patient with micrometastasis in the SLN, and one patient with 2 pelvic positive nodes on the secondary pelvic lymphadenectomy after metastatic SLN.

Survival Outcomes

The mean follow-up duration was 50 months (3–89 months), with a median of 51 months (4 years and 3 months).

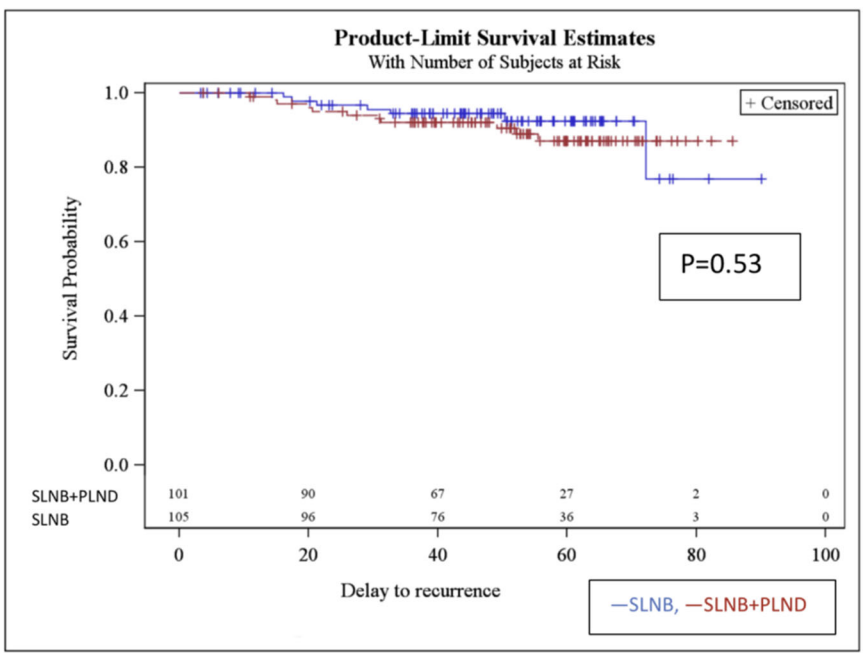
Disease-free survival after 4 years in the SLNB and SLNB + PLND groups was 89.5% and 93.1% (p 161 = 0.53), respectively. There was no significant difference between the two groups. There were 11 recurrences in the SLNB group and 7 in the SLNB + PLND group (**Figure 1**). The most statistically significant factor identified in the univariate analysis was radiotherapy. In fact, we observed 11 recurrences in the patients treated with adjuvant radiotherapy (15.5%). Other factors that influenced disease-free survival after 4 years in the univariate and multivariate analyses included the age of the patients, histological type, tumor size, presence of LVSI, positive nodal status, surgical approach, and adjuvant treatment. However, a trend was observed, depending on the case, for LVSI. In the multivariate analysis, both of these covariates (LVSI and adjuvant radiotherapy) were significant (**Table 3**). The type of recurrences is described in **Table 4**; there were 2 lymphatic recurrences: one pelvic in the SLNB group and one para-aortic (associated with liver metastasis) in the SLNB + PLND group.

During the follow-up period, nine deaths were observed (four in the SLNB + PLND group and five in the SLNB group). The overall survival (OS) for the patients who received SLNB was 95.2, and 96% for those who received SLNB + PLND (p = 0.97). In the univariate analysis, none of the risk-factor covariates that were analyzed were statistically significant for any of the clinical events at a p = 0.05 level. However, a trend could be observed, depending on the case, for the covariates—namely adjuvant radiotherapy and LVSI. When we retained covariates with a p-value of <0.15 in the univariate analysis in a multivariate model, none were statistically significant (**Table 5**).

DISCUSSION

Full pelvic lymph-node dissection is associated with early and long-term morbidity. Limited dissection of SLNB is associated with lower morbidity and a better quality of life (6).

Moreover, the SLN procedure has been intensely assessed, and has shown safe and relevant results as follows: considering bilateral SLN detection with histological ultrastaging, a 99–100% sensitivity with a 97–100% NPV was demonstrated (3). While this technique has been used in tumors up to 4 cm in size, the best detection rates and mapping results are in tumors less than 2 cm (7). The ultrastaging techniques, combining serial sectioning and IHC, improved the rate of nodal metastasis detection and revealed that 8.1% of apparently node-negative patients were classified as node-positive. Low-volume metastasis (micrometastasis and isolated tumor cells) is usually only



SLNB = sentinel lymph-node biopsy
PLND = pelvic lymphadenectomy

FIGURE 1 | Disease-free survival rate at 4 years.

TABLE 3 | Univariate and multivariate analyses of the association between disease-free survival and risk factors.

Risk factors	Total number	Recurrences	*P* value (univariate analysis)	*P* value (multivariate analysis)
Histological type			0.97	
Squamous	135	12 (8.9%)		
Adenocarcinoma	56	5 (8.9%)		
Adenosquamous	6	1 (16.7%)		
Other	9	0		
Tumor size			0.92	
<2 cm	28	2 (7.2%)		
>2 cm	176	16 (9%)		
Lymph node involvement			0.87	
Yes	21	2 (9.5%)		
No	185	16 (8.6%)		
Type of surgery			0.97	
Simple hysterectomy	5	0		
Simple trachelectomy	4	0		
Radical hysterectomy	171	16 (9.3%)		
Radical trachelectomy	26	2 (7.7%)		
Type of radical hysterectomy			0.13	
Type B	158	10 (6.3%)		
Type C	13	3 (23%)		
Lymph-vascular space invasion			0.09	0.16
Pos	48	7 (14.9%)		
Neg	159	11 (6.9%)		
Adjuvant Radiotherapy			**0.02**	0.25
Yes	71	11 (15.5%)		
No	135	7 (5.2%)		

TABLE 4 | Type of recurrences.

Group	Recurrence	Time of recurrence	Death
SLNB	Lungs	18 months	Yes
SLNB	Lungs	14 months	No
SLNB	Parametrium	15 months	Yes
SLNB	Lungs	49 months	No
SLNB	Pelvic	19 months	Yes
SLNB	Inguinal	49 months	No
SLNB	Lymphatic (right iliac)	52 months	No
SLNB	Vaginal	9 months	Yes
SLNB	Liver	26 months	Yes
SLNB	Peritoneum	30 months	No
SLNB	Vaginal	30 months	No
SLNB + PLND	Carcinosis and lungs	50 months	Yes
SLNB + PLND	Parametrial	72 months	No
SLNB + PLND	Lungs	17 months	Yes
SLNB + PLND	Lungs and liver	15 months	Yes
SLNB + PLND	Lymphatic (para-aortic) and liver	20 months	Yes
SLNB + PLND	Vaginal	28 months	No
SLNB + PLND	Peritoneum	31 months	No

SLNB, sentinel lymph node biopsy; SLNB+PLND, sentinel lymph node biopsy + pelvic lymphadenectomy.

detected *via* ultrastaging. Its clinical significance is currently being debated. The FIGO 2018 (8) classification considers only macro- and micro-metastases to be significant lymph-node metastases; the presence of isolated tumor cells does not change the stage. Our recent study, presented at the ASCO 2020 Congress (9) and recently published (10), demonstrated that the sentinel-node technique is reliable even from the point of view of low-volume metastasis in NSLN. The limitation of the ultrastaging technique is that it cannot be performed intraoperatively as it is too cumbersome and time-consuming. Considering the convenience of a single-step approach in early stage cervical cancer, the one-step nucleic acid amplification (OSNA) assay has recently been investigated in several tumor types, including cervical cancer patients (11). More important results are expected on the study of this technique in cervical cancer in order to be able to draw conclusions about its reliability.

In our study, the results showed that for women with early-stage cervical cancer from FIGO (2009) IA1 to IIA1, the SLN procedure alone did not result in a significant disease recurrence compared to complete pelvic lymph-node dissection. In addition, no significant difference was found in the overall survival between the two groups.

Considering that a great majority of recurrences normally appear within 3 years after treatment, SLNB seems to be a safe and efficient alternative to lymphadenectomy, and should be proposed to every patient as a routine protocol for early-stage cervical cancer in cancer centers where surgeons are familiar with this technique and follow the correct SLN protocol. It should still be considered that the study was not designed to compare the survival and the risk of recurrence between the two groups (SLNB and SLNB + PLND). This subgroup of patients with early-stage cervical cancer indeed had a good survival with a low recurrence rate. In conclusion, too few events were observed during long-term follow up (4 years) to allow for a tangible analysis of the risk factors for survival or disease-free survival.

However, this was the first randomized controlled prospective study evaluating the follow up of SLNB alone in comparison with SLNB + PLND, and it confirmed the Senticol 1 results.

Considering the other factors associated with risk of recurrence, only the adjuvant radiotherapy was statistically significant ($p = 0.02$). This result can be explained by the fact that the patients who presented major risk criteria (according to the Seidlis criteria) (12) were treated with radiotherapy. In the multivariate analysis, no factors were significant for either the risk of recurrence or for survival.

Recently, the LACC study by Pedro Ramirez (13) demonstrated the superiority of open surgery for better survival and disease-free survival in the treatment of early-stage cervical cancer. Our study was designed before Ramirez's study, and the vast majority of our patients were operated on *via* laparoscopy. Open surgery was performed only in the case of complications during laparoscopic surgery. It is interesting that in our study, we found a survival and

TABLE 5 | Univariate and multivariate analyses of the association between risk factors and overall survival.

Risk factors	Total number	Death	P stat (univariate analysis)	P stat (multivariate analysis)
Histological type			0.93	
Squamous	135	7 (5.2%)		
Adenocarcinoma	56	2 (3.6%)		
Adenosquamous	6	0		
Other	9	0		
Tumor size			0.25	
<2 cm	28	2 (7.2%)		
>2 cm	176	7 (3.9%)		
Lymph-node involvement			0.88	
Yes	21	1 (4.8%)		
No	185	8 (4.3%)		
Type of surgery			1	
Simple hysterectomy	5	0		
Simple trachelectomy	4	0		
Radical hysterectomy	171	9 (5.3%)		
Radical trachelectomy	26	0		
Type of radical hysterectomy				
Type B	158	9 (5.7%)		
Type C	13	0		
Lymph-vascular space invasion			0.12	0.22
Pos	48	4 (8.5%)		
Neg	159	5 (3.1%)		
Adjuvant radiotherapy			0.07	0.11
Yes	71	6 (8.5%)		
No	135	3 (2.2%)		

disease-free survival similar to that of the open-surgery group in Ramirez's study. This comparison is limited, as our study was designed for laparoscopic treatment and there were a large number of cases where the colpotomy was performed vaginally with protective measures. A vaginal colpotomy may protect from the risk of tumor dissemination and recurrence (14).

CONCLUSIONS

SLN biopsy was found to be a safe technique allowing for accurate nodal staging in early cervical cancer.

Furthermore, this surgery led to less morbidity and to a clearly improved quality of life for patients. Given that disease recurrence after 4 years was similar in patients who underwent an SLN procedure, this study strongly supports the extension of this surgical approach to all clinically node-negative patients (cN0) affected by early cervical cancer.

A strong and influential study called Senticol 3 (15) and SentiX (16) is under way in order to confirm the equivalent survival in SLNB and SLNB + PLND patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes Sud-Est IV, decision A08-223. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, GF, VB, BG, FL, and PM. Methodology, LM. Software, LM. Validation, VB, FL, BG, and PM. Investigation, FL and PM. Resources, BG, PM, and FL. Data curation, GF, BG, PM, and FL. Writing—original draft preparation, GF and BG. Writing—review and editing, GF, VB, FL, BG, and PM. Visualization, GF, VB, FL, BG, and PM. Supervision, FL and PM. Funding acquisition, PM. All of the authors have read and agreed to the published version of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.621518/full#supplementary-material

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Avoiding Full Lymphadenectomies in Intermediate- and High-Risk Endometrial Cancer by Sentinel Lymph Node Biopsy Implementation

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Objective: To evaluate the role of sentinel lymph node biopsy (SLNB) to avoid staging lymphadenectomies by detecting nodal metastasis in intermediate- and high-risk endometrial cancer (EC).

Methods: A single institutional retrospective study was performed including all patients with intermediate- and high-risk EC who underwent surgical nodal staging between January 2012 and December 2019. Patients with disseminated disease detected on imaging techniques or at the time of surgery were excluded. Patients were evaluable if they underwent nodal staging with SLNB and pelvic (PLD) and paraaortic (PALD) lymph node dissection. We analyzed the accuracy of the sentinel lymph node technique. Only patients with at least one sentinel lymph node (SLN) detected were included in the sensitivity and negative predictive value (NPV) analyses. The tracers used were technetium 99m, blue dye, and indocyanine green.

Results: Eighty-eight patients presented intermediate- and high-risk EC (51 patients and 37 patients respectively) and underwent SLNB with consecutive PLD and PALD. The median (range) number of sentinel nodes retrieved was 2.9 (0–11). The global detection rate of SLN was 96.6% with a bilateral detection of 80.7% when considering all tracers used. However, when combination of indocyanine green and technetium was used the bilateral detection rate was 90.3%. Nodal metastases were detected in 17 (19.3%) cases, 8 (47%) of them corresponded to low volume metastasis (LVM), 7 (87.5%) of them diagnosed at ultrastaging pathologic exam. Finally, we obtained a sensitivity of 90%, a NPV of 97.5%, and a false negative rate (FNR) of 10% in the intermediate-risk EC compared to sensitivity of 85.7%, NPV of 96.6%, and FNR of 14.3% in the high-risk EC group. The only patient with isolated paraaortic nodal metastasis was found at the high-risk group, 1.1%.

Conclusions: According to our results, full lymphadenectomy could be avoided by performing SLNB in patients with intermediate-risk EC because the only false negative case detected was at the beginning of ICG learning curve. For high-risk EC patients we did not find enough evidence to support the systematic avoidance of staging full lymph node

dissection. Nevertheless, SLNB should be performed in all cases of EC as it improves LVM diagnosis substantially.

Keywords: high-risk endometrial cancer, intermediate-risk endometrial cancer, early stage endometrial cancer, sentinel lymph node biopsy, systematic lymphadenectomy, isolated metastatic aortic lymph nodes

INTRODUCTION

Endometrial cancer (EC) is the most common gynecological malignancy in developed countries, with an estimated incidence of 65,620 new cases in 2020, causing 12,590 deaths annually in the USA. Globally, 382,069 new cases of EC were diagnosed in 2018, with 89,909 deaths worldwide (1, 2).

Classic surgical staging of early-stage EC included pelvic and para-aortic lymphadenectomy in order to collect prognostic information and to guide the adjuvant treatment. However, the inclusion of systematic lymphadenectomy in the surgical treatment of EC has not showed any additional improvement in overall survival and disease-free survival of the patients while it increases perioperative morbidity (3, 4). Currently, complete lymphadenectomy is a standard surgical procedure in high-risk EC patients since 19% of these patients could present lymph node metastases (14% endometrioid histology and 32% non-endometrioid histology) (5).

Over the last decade, several clinical trials as SENTI-ENDO or FIRES (6, 7), showed that SLN biopsy seems to be as accurate as systematic lymphadenectomy to evaluate the nodal status of early-stage EC reporting a high sensitivity and negative predictive value to detect nodal involvement (84 and 97% *vs* 97.2 and 99.6%, respectively (6–8). These studies included mostly no high-risk disease for lymph node involvement which could influence the false negative rate.

Some studies reported a false negative rate in SLNB in EC ranging from 5 to 20% among high-risk patients (9, 10). The main drawback of SLNB technique in high-risk tumors is the lack of para-aortic assessment so isolated lymph node metastases would not be detected. In addition, the cervical injection of the tracer (the most extended method) would prevent from para-aortic drainage through the infundibulopelvic ligament.

Soliman et al. evaluated the accuracy of SLNB in high-risk EC reporting a sensitivity of 95% and a FNR of 4.3%, which developed an update in several clinical guidelines including the SLNB in the standard management of endometrial cancer (10).

The last National Comprehensive Cancer Network (NCCN) guideline and the recent update of the European guideline of Gynecological Oncology include the use of SLNB in high-risk EC as a reasonable option (11, 12).

The aim of our study was to evaluate the role of sentinel lymph node biopsy (SLNB) to avoid staging lymphadenectomies by detecting nodal metastasis in intermediate- and high-risk endometrial cancer.

MATERIAL AND METHODS

We carried out a retrospective single-institutional study that included all consecutive patients initially diagnosed of intermediate- and high-risk EC and treated at our institution between January 2012 and December 2019. Data were collected from the medical records after Institutional Review Board approval (#PI-3846). All women with presumed intermediate- or high-risk EC by European risk classification (12) and Federation of Obstetrics and Gynecology (FIGO) stage I-II were assessed for eligibility. Intermediate-risk cases were defined as those endometrioid histotypes presenting ≥50% myometrial invasion and histological grade 1–2; or <50% of myometrial invasion and histological grade 3. High-risk cases were defined as those endometrioid histotypes presenting cervical stromal involvement; or ≥50% of myometrial invasion and histological grade 3; or high-risk histology including serous, clear cell, and carcinosarcoma tumors based on preoperative biopsy.

All patients underwent a preoperative imaging work-up with vaginal ultrasound or MRI to evaluate myometrial and cervical invasion. CT-scan or PET/CT was performed in high-risk cases in order to exclude nodal involvement or metastatic disease. Patients with suspected disseminated or locally advanced disease were excluded.

All patients included underwent total hysterectomy, bilateral salpingo-oophorectomy, SLNB, PLD, and PALD up to the left renal vein level in most of cases. Patients where SLN mapping or complete PLD and PALD were not performed were excluded from the study. In addition, patients with peritoneal disease or nodal macroscopic involvement identified intraoperatively were also excluded from the study.

Our SLNB protocol included the next steps: Firstly, the day before surgery two cervical injections at 3 and 9 o'clock (5 mm superficial and 15 mm deep) of 2 ml of technetium sulfur colloid (Tc99) were administered with a 25-gauge spinal needle (13). Lymphoscintigraphy images were obtained 2 h after the injections with the integration of single-photon emission computed tomography (SPECT/CT); Second, intraoperatively, 4 ml of methylene blue or indocyanine green (ICG) dilution 2.5 mg/ml were injected in the same location that technetium (2 ml per side, 5 mm superficial and 15 mm deep); Third, during surgery all the pelvic areas were carefully inspected for lymph ducts, following the main lymphatic drainage pathways. Lymph nodes marked by technetium (hot lymph nodes) and/or those marked by ICG/blue were identified and removed.

The tracers used during the study period were: from January 2012 until October 2014 Tc99 + methylene blue; from October 2014 until December 2018 Tc99 + ICG; and finally, from 2019 ICG alone has been used as single tracer.

All sentinel lymph nodes were ultrastaged postoperatively by multiple sectioning at 200 µm intervals. Each section was also divided at 50-µm intervals and stained with hematoxylin and eosin. An additional slide of each interval was used for an immunohistochemistry exam (IHC) with an anticytokeratin antibody dilution (cytokeratins AE1–AE3). Non-sentinel lymph nodes were evaluated by routine sectioning and H&E staining. Lymph node status was defined using the criteria of American Joint Committee on Cancer for breast cancer (2002): Isolated tumor cells (ITCs) were defined as a focus of metastatic disease measuring ≤0.2 mm; micrometastasis (MIC) was defined as a focus of metastatic disease between >0.2 and 2 mm; and macrometastasis (MAC) was defined as a focus of metastatic disease >2 mm (14). Those lymph nodes without tumor present on pathologic evaluation were reported as negative and lymph nodes with MAC, MIC, or ITCs were considered to be positive on final pathology. LVM was defined as ITCs and MIC together.

An analysis of diagnostic test was performed including the sensitivity, false negative rate (FNR), and negative predictive value (NPV) of SLNB, considering the gold standard the complete lymphadenectomy. Only patients with at least one sentinel lymph node detected were included in the sensitivity and negative predictive value analyses comparing to final pathology. We also estimated overall and bilateral detection rates among intermediate- and high-risk patients, considering the bilateral detection rate according to the tracer used. The sensitivity of SLNB was described as the proportion of patients with node-positive disease with successful SLN mapping who had metastatic disease correctly identified in the sentinel lymph node.

The overall detection rate was defined as the proportion of patients in which at least one SLN was identified. False negative rate was defined as the proportion of cases with negative bilateral SLNB and positive non-SLN at final pathology. Qualitative variables were reported with absolute numbers and percentages. Quantitative variables were reported as median and range. Categorical variables were compared using the chi-square test for univariate analysis. All statistical analysis were performed using the software SPSS Statistics v.24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Flow diagram of patient inclusion is showed in **Figure 1**. A total of 101 cases of intermediate- and high-risk were enrolled, among them, 88 cases of endometrial cancer were evaluable and included in the study, 51 cases of intermediate-risk and 37 cases of high-risk endometrial cancer.

Demographic and final clinicopathological features are summarized in **Table 1**. The majority of patients, 86 cases (97.7%), were operated by laparoscopic approach and extraperitoneal paraaortic approach was performed in 65 cases (73.9%). The upper border of PALD was the left renal vein in 81 patients (92%), in the remaining 7 cases (8%) the dissection was up to inferior mesenteric artery (IMA) due to intraoperative complications. On final pathology 69 patients (78.4%%) presented early stage and advanced stage was presented in 19 patients (21.6%%) being the most frequent histology endometrioid, 61 patients (69.3%%), and serous, 15 cases (17%), adenocarcinoma (**Table 1**).

At least one SLN was retrieved in 85 cases being overall SLN detection rate 96.6%. Bilateral pelvic detection rate was achieved in 71 cases (83.5%). When we analyzed the data in each risk group, the bilateral detection rate in the intermediate-risk group was 81.6% and in the high-risk group was 86.1%. Regarding the use of different tracers, combination of Tc99 with blue dye has been used in 40 patients (47.1%), combination of Tc99 and ICG in 39 cases (45.9%), and ICG in 6 patients (7.1%) Bilateral detection rates based on tracer used are shown in **Table 2**.

Among 85 cases in which SLN was detected, a total of 251 SLN were removed. The median number of SLN retrieved was 2.9 (range 0–11) per patient and the median numbers of pelvic and paraaortic nodes were 14.7 (range 4–36) and 16.7

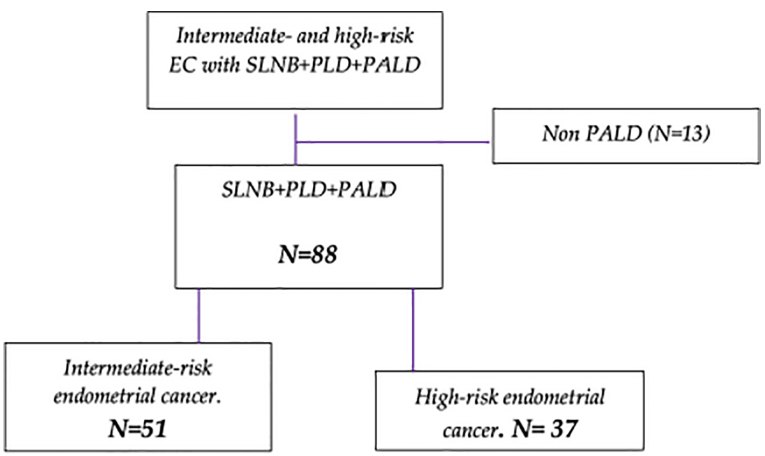

FIGURE 1 | Enrollment of patient diagram. (SLNB = Sentinel lymph node biopsy. PLD = Pelvic lymph node dissection. PALD = Paraaortic lymph node dissection).

TABLE 1 | Demographic characteristics of the study population.

Variables	N
Age (years) Median (range)	66 (45–85)
BMI Median (range)	28 (17–40)
Histology	
Endometrioid	61 (69.3%)
Serous	15 (17%)
Clear cell	6 (7%)
Carcinosarcoma	6 (7%)
Grade	
G1	24 (27.3%)
G2	28 (31.8%)
G3	36 (40.9%)
Surgical approach	
Laparoscopy	86 (97.7%)
Laparotomy	2 (2.3%)
Extraperitoneal PALD	23 (26.1%)
Transperitoneal PALD	65 (73.9%)
Upper border of PALD	
Left renal vein	81 (92%)
Inferior mesenteric artery	7 (8%)
SLN mapping	88(100%)
FIGO stage	
IA	35 (39.7%)
IB	31 (35.2%)
II	3 (3.4%)
IIIA	2 (2.3%)
IIIC1	11 (12.5%)
IIIC2	6 (6.8%)

BMI, body mass index; PLD, pelvic lymph node dissection; PALD, paraaortic lymph node dissection.

TABLE 2 | Sentinel lymph node unilateral and bilateral detection rates with different tracers.

Tracer used	Unilateral detection rate N (%)	Bilateral detection rate N (%)	N (%)	
ICG+Tc99	5 (12.8)	34 (87.2)	39 (45.9)	
Tc99+Blue dye	9 (22.5)	31 (77.5)	40 (47.1)	P = 0.27
ICG	0 (0)	6 (100)	6 (7.1)	
Overall detection	14(16.5)	71 (83.5)	85(100)	

(range 2–39) respectively. The anatomical distribution of SLN was as follows: 54.9% in obturator area, 38.5% in external iliac vessels area, 5.5% in iliac common vessels area, and finally 1.1% in paraaortic area (**Figure 2**). There were 15 (17.6%) cases with positive SLN with the following metastasis distribution: 5 (37.5%) corresponded to isolated tumor cells, 3 (12.5%) to micrometastasis, and 7 (50%) to macrometastasis.

On final pathology there were 17 patients (19.3%) with nodal metastatic disease, among them, the only positive node was the SLN in 10 patients (58.8%). Concerning LVM disease, 4 (23.5%) patients presented MIC and 4 (23.5%) patients presented ITCs, 7 (87.5%) of them diagnosed at ultrastaging pathologic exam. Finally, 2 patients (11.8%) presented nodal disease in non-SLN with negative SLN at final pathology, therefore, considered as two false negative cases. Details of all nodal involved cases are included in **Table 3**.

When we analyzed the accuracy of SLNB by groups we obtained a sensitivity of 90%, NPV of 97.5%, and FNR of 10% in the intermediate-risk EC group. When we studied the accuracy of SLNB in this group in the last 4 years, the sensitivity and NPV increased up to 100% with 0% of false negative rate. Whereas in the high-risk EC group, we observed a sensitivity of 85.7%, NPV of 96.6%, and FNR of 14.3%. The only case of isolated para-aortic lymph node metastasis found was the unique false negative case in the high-risk group.

On the other hand, 71 patients (80.7%) presented negative nodes, corresponding to practically half of each group of patients. In the intermediate risk group, 41 (80.4%) patients presented negative nodes being also negative in 30 (81.1%) patients in the high-risk group.

The three patients (two cases of intermediate-risk and one of high-risk) for whom no SLNs were identified went on to full lymphadenectomy, none of the additional nodes evaluated were malignant.

DISCUSSION

The inclusion of SLNB would avoid the performance of complete lymphadenectomies which have not shown any impact on the survival of patients with early stage of EC according to two randomized clinical trials [ASTEC trial (2009), Benedetti et al. trial, (2008)] (15, 16).

In the last decade, SLN biopsy has obtained enough scientific evidence to relegate complete lymphadenectomy to the past, demonstrating the same oncological accuracy in nodal staging in early stages of endometrial cancer. The most important clinical trial to validate the accuracy of SLNB, FIRES trial, included only 28% of high-grade histology in the study population (6). The main criticism of these studies was the low percentage of high-grade histology included which has the highest risk of metastasis and isolated paraaortic disease. Therefore, despite recent SGO recommendations on the inclusion of SLN biopsy in early stages of EC (17), the role of the technique in high-risk disease remains controversial.

In this retrospective study of SLNB, we analyzed the accuracy of the technique including that cases in which at least one SLN was identified. The global detection rate of SLN was 96.6% with a bilateral detection rate of 83.5% in accordance with the literature (18). While our study was not able to demonstrate significant differences in detection rates by mapping technique, ICG is associated with higher detection rate and bilateral rate according with previous literature (8).

In our validation analyses of SLNB in patients with intermediate- and high-risk EC, this technique detected 90 and 85.7% of patients with positive nodes respectively. Our study demonstrated in the intermediate-risk group, high sensitivity and NPV with only one false negative case identified at pelvic level. This case occurred during the early learning curve of ICG mapping and probably it had an impact on our SLN mapping accuracy. On the other hand, no cases of isolated paraaortic metastasis were identified in this group. With the improvement of our learning curve in SLN mapping, no more false negative

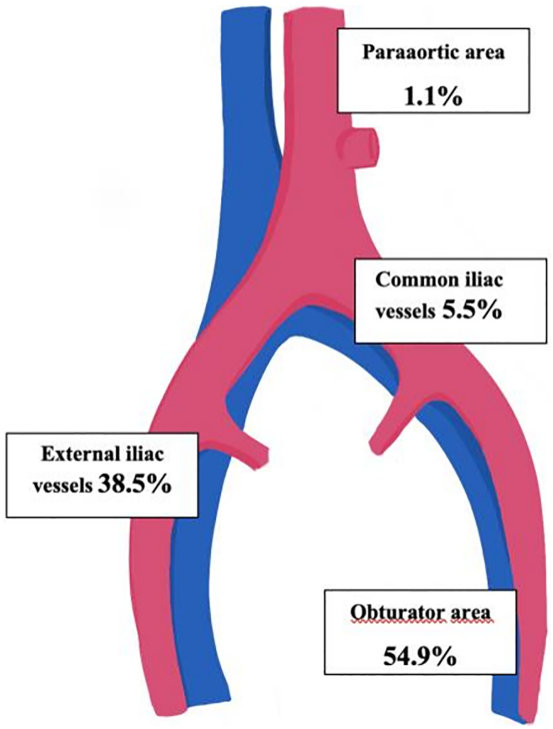

FIGURE 2 | Anatomic distribution of sentinel lymph nodes.

TABLE 3 | Nodal metastatic disease distribution.

Presurgical FIGO Stage	Grade	Histology	Status SLN	Status pelvic nodes	Status paraaortic nodes
II	1	Endometrioid	MAC	–	–
IB	2	Endometrioid	MIC	–	–
IB	3	Endometrioid	MIC	–	–
IB	2	Endometrioid	MIC	–	–
IB	1	Endometrioid	MAC	–	–
IB	1	Endometrioid	MIC	–	–
II	2	Endometrioid	ITCs	–	–
IB	3	Serous	MAC	MAC	MAC
IA	2	Endometrioid	–	MIC	–
IB	1	Endometrioid	MAC	–	MAC
II	3	Serous	MAC	MAC	MAC
IB	2	Endometrioid	MAC	–	–
IB	3	Serous	ITCs	–	MAC
IB	1	Endometrioid	MIC	–	–
IB	1	Endometrioid	ITCs	–	–
IB	2	Clear cells	MAC	MAC	MAC
IB	3	Clear cells	–	–	MAC

MAC, macrometastasis; MIC, micrometastasis; ITCs, isolated tumor cells; - = negative nodes.

cases were recorded during the last 4 years. Therefore, the inclusion of SLN biopsy in this group could be considered following the NCCN surgical SLN algorithm as it has demonstrated good accuracy and a false negative rate <5% in the detection of nodal metastases in recent prospective studies (10, 19).

However, in the high-risk group the sensitivity and NPV dropped slightly to 85.7 and 96.6% respectively. Concerning false negative rate, it increases up to 14.3%, less compared to other retrospective series described in the literature with up to 20% false negative rate (9).

Nevertheless, more recent prospective and retrospective studies have demonstrated more promising results on this subject. The retrospective study by Touhami et al. (2017) described a sensitivity and NPV of 95.8 and 98.2% respectively (19) and Holloway et al. (2017) reported on a prospective study a sensitivity of 97.5%, a NPV of 99.3%, and a FNR of 2.5% applying SLN mapping with different tracers in intermediate- and high-risk EC (18).

The retrospective study of Papadia et al. (20) aimed to validate the laparoscopic ICG SLN mapping in patients with grade 3 or high-risk histology. This group reported 23.8% of Lymph node metastasis with only one false negative case which corresponded to a metastatic non-SLN isolated para-aortic metastasis, according with our results in high-risk group. This study showed a sensitivity, FNR, and NPV of 90, 10, and 97.1% respectively which is consistent with our results (20).

In the last year, two large prospective studies on this topic were published. SHREC trial by Persson et al. (21) included 275 patients with intermediate- and high-risk EC who underwent SLN biopsy followed by robot-assisted pelvic and para-aortic lymphadenectomy (in 81% of cases). The analyses reported a

sensitivity of 98% and a NPV of 99.5% applying surgical SLN algorithm with ICG. Two cases of false negative SLN were detected in the analyses. The authors concluded that when SLN algorithm was performed by experienced surgeons, it has the potential to safely replace lymphadenectomy in these cases of EC without the need for para-aortic LND (21).

Recent publication of SENTOR study by Cusimano et al. (22), described a SLNB sensitivity of 96% and a NPV of 99% with only one false negative case. Comparing this study with ours, we identified one more false negative case, one of them an isolated paraaortic metastasis. We should consider that 100% of our population underwent PALD while in SENTOR and in SHREC studies PALD was performed in 80 and 81% of their population respectively (22).

The main strength of our study is that all patients included presented a comprehensive surgical staging based on SLNB, PLD, and PALD, which allowed us to accurately define the sensitivity, NPV, and FNR of SLNB in intermediate- and high-risk EC.

Another important consideration of SLN biopsy in high-risk EC is the proportion of patients who present additional non-SLN metastasis in the presence of metastatic SLN because these patients could benefit from complete lymphadenectomy. The recent study of Taskin et al. (23) evaluated the feasibility of SLN mapping in uterine confined endometrial cancer, it reported 60% of patients with macrometastatic SLN who also presented non-SLN involvement. All of these patients received chemotherapy but there is not consistent evidence suggesting that leaving these nodes *in situ* has a detrimental effect on survival (23). Retrospective studies of Buda et al. (24) evaluated the impact of SLN mapping compared to SLN plus complete lymphadenectomy on the prognosis in patients with intermediate and high-risk EC and concluded that the 5-year recurrence free survival was similar in both groups (79.2 *vs* 81.6 respectively, p = 0.831) (24, 25). Therefore, the most important concept in high-risk EC is achieve an adequate nodal staging in order to target adjuvant therapy properly.

Moreover, high-risk EC has the highest risk for metastasis and isolated paraaortic metastasis, the only false negative case in our high-risk population was an isolated paraaortic metastasis. In order to improve this lack, the study of Ruíz et al. (26) included dual cervical and fundus injection of ICG in SLNB with aortic SLN detected in 59.5% of cases (26).

A further important concept of SLN biopsy is that ultrastaging of pelvic SLN nodes decreased the true prevalence of isolated paraaortic dissemination with LVM detection. The study of Multinu et al. (27) showed that ultrastaging of pelvic nodes reduced by 30% true isolated paraaortic metastasis prevalence identifying occult LVM (27). In our study, 47% of LNM corresponded to LVM, among them, 87.5% were only detected at SLN ultrastaging.

In conclusion, our study gives another argument for the inclusion of SLNB in surgical staging of intermediate- and high-risk endometrial cancer. SLN biopsy seems to be an accurate alternative to systematic lymphadenectomy in patients with intermediate-risk endometrial cancer after improvement of our learning curve with the new tracer. On the contrary, in high-risk endometrial cancer we would need to improve the false negative rate of the technique to be able to avoid systematic lymphadenectomy. A proper accuracy of SLNB was not achieved in this group, probably by the low number of cases included.

Nevertheless, SLN mapping should be included as part of nodal staging in both intermediate- and high-risk disease since it increases the overall detection of nodal metastasis when compared to routine systematic lymphadenectomy. Although SLNB ultrastaging has shown an increase in the low volume metastasis detection, its oncological impact remains controversial.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: patient data are protected and cannot be disclosed. Requests to access these datasets should be directed to VP, virginiagarciapineda@gmail.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board approval (#PI-3846). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VP contributed to designing the study, analyzing the data, and writing the manuscript. IZ and AH contributed to the writing of the manuscript. The rest of the authors contributed in data collection. All authors agree to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.

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Exploratory Study of the Clinical Value of Near-Infrared Sentinel Lymph Node Mapping With Indocyanine Green in Vulvar Cancer Patients

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Background: This study aimed to evaluate the clinical value of indocyanine green sentinel lymph node (SLN) mapping in patients with vulvar cancer. The conventional procedure of SLN mapping in vulvar cancer includes peritumoral injection of technetium-99m nanocolloid before surgery and intraoperative injection of a blue dye. However, these techniques harbor some limitations. Near-infrared fluorescence imaging with indocyanine green has gained popularity in SLN mapping in different types of cancer.

Methods: We analyzed retrospectively vulvar cancer patients at our institution between 2013 and 2020 undergoing indocyanine green SLN mapping by applying video telescope operating microscope system technology.

Results: 64 groins of 34 patients were analyzed. In 53 groins we used technetium-99m nanocolloid, in four patent blue, and in five both techniques, additionally to indocyanine green for SLN detection. In total, 120 SLNs were identified and removed. The SLN detection rate of indocyanine green was comparable to technetium-99m nanocolloid (p=.143) and higher than patent blue (p=.003). The best results were achieved using a combination of ICG and technetium-99m nanocolloid (detection rate of 96.9%). SLN detection rates of indocyanine green were significantly higher in patients with positive lymph nodes (p=.035) and lymphatic space invasion (p=.004) compared to technetium-99m nanocolloid.

Conclusion: Indocyanine green SLN mapping in vulvar cancer is feasible and safe, with reasonable detection rates. Due to its easy application and few side effects, it offers a sound alternative to the conventional SLN mapping techniques in vulvar cancer. In patients with lymph node metastasis, indocyanine green even outperformed technetium-99m nanocolloid in terms of detection rate.

Keywords: vulvar cancer, sentinel lymph node, indocyanine green, near-infrared imaging, Technetium-99m

INTRODUCTION

Inguinal lymph node status represents the most significant prognostic factor for survival in vulvar cancer patients (1). Lymphadenectomy therefore plays a crucial role in both surgical treatment and staging of vulvar cancer. Complete inguinofemoral lymph node dissection leads to a short- and long-term morbidity, consisting of wound infections or dehiscence and lymph edema in up to 50% of all patients (2). However, only one third of patients with stage I or II disease have lymph node metastasis and consequently up to two thirds undergo unnecessary lymphadenectomy (3), associated with high morbidity and prolonged hospitalization (4).

The introduction of sentinel lymph node (SLN) biopsy in vulvar cancer, first described by Levenback in 1994 (5), provides a less invasive technique for staging of vulvar cancer than complete inguinofemoral lymph node dissection, with significant reduction in lymphedema, wound infection, and dehiscence without compromising groin recurrence rates or survival rates (6, 7). SLN biopsy has been shown to be oncologically safe in unifocal squamous-cell vulvar cancer up to a tumor size of 4 cm with clinically negative lymph nodes (6, 8) and with a low false negative rate of approximately 3% (9).

The conventional technique of SLN mapping in vulvar cancer involves a peritumoral injection of technetium-99m (^{99m}Tc) nanocolloid before surgery combined with an intraoperative injection of a blue dye. Preoperative 3D single photon emission tomography imaging helps detecting the SLN more precisely regarding number and anatomical localization (10). However, these techniques harbor some limitations: (a) the preoperative injection of radiotracers involves a painful procedure for the patient; (b) the transport and storage of radioactivity requires complex logistics; (c) blue dyes may lead to staining of the injection site and to allergic reactions; and (d) visualization of the blue dye is limited when the lymphatic tissue is covered by skin or fat – resulting in a lower detection rate.

In cervical and endometrial cancer, SLN mapping with near-infrared fluorescence imaging using indocyanine green (ICG) has shown better overall and bilateral detection rates as compared to a combination of blue dye and ^{99m}Tc-nanocolloid, a better safety profile than blue dyes, and an easier application than ^{99m}Tc-nanocolloid (11–14). Furthermore, ICG and near-infrared fluorescence imaging outperformed blue dye for SLN detection in skin cancer (15) and in breast cancer (16).

Until now, several studies demonstrated the technical feasibility and safety of ICG in SLN mapping in vulvar cancers, though most of them are case series characterized with methodological variations and lack of standardization. The aim of this study was to evaluate the SLN detection rate of ICG in vulvar cancer compared to the conventional technique using ^{99m}Tc-nanocolloid and blue dye in a large cohort of patients and to analyze its applicability in different risk groups.

MATERIALS AND METHODS

We retrospectively investigated patients with histologically proven vulvar cancer who were operated at the certified cancer center of the Bern University Hospital, Switzerland between April 2013 and April 2020. The experimental protocols was approved by the Ethics Commission of the Canton of Bern, Switzerland (reference number: 261/2015) and meets the guidelines of the responsible governmental agency. All patients signed informed consent. Demographic, clinical, and intraoperative data were retrieved from an electronic database.

Surgical Procedure

All patients underwent inguinal SLN mapping using near-infrared fluorescence imaging with ICG, applying video telescope operating microscope system technology (VITOM ICG® by Karl Storz GmbH, Germany) (**Figure 1**). In every case, at least one additional tracer (^{99m}Tc-nanocolloid and/or patent blue) was used. After skin incision, a gentle dissection of the fatty tissue was performed. Under near-infrared imaging, the groin was inspected for fluorescence. The groin was tested systematically for radioactivity using a handheld gamma probe and, if applicable, for blue staining by visual inspection. In accordance with international guidelines, a bilateral SLN biopsy was performed if the tumor site was located 1 cm or less from the midline. All ICG, ^{99m}Tc-nanocolloid, or patent

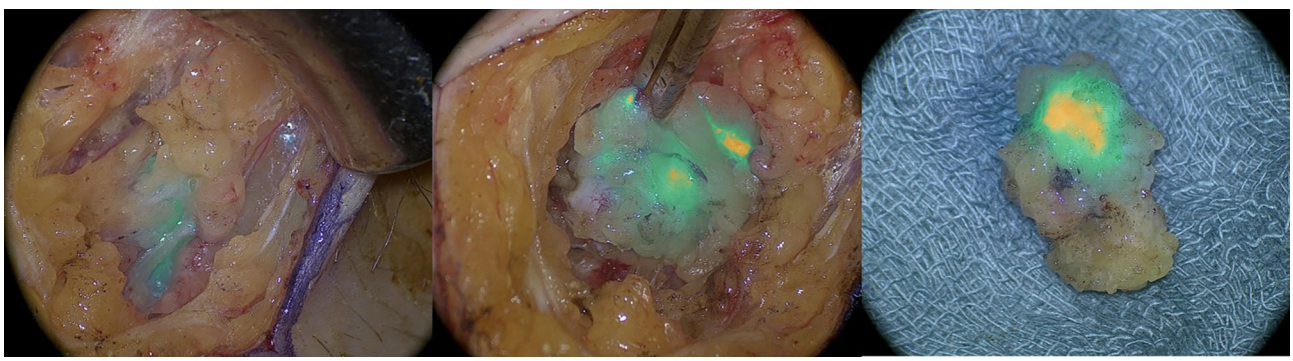

FIGURE 1 | Intraoperative imaging of indocyanine green positive lymphatic channels and inguinal sentinel lymph node with near infrared imaging.

blue-positive lymph nodes were excised and sent for frozen section. If frozen section analysis revealed lymph node metastases, a complete inguinofemoral lymphadenectomy was performed. Following the SLN extirpation procedure, tumor excision was performed, consisting of a radical local excision or a vulvectomy, in function of the size and location of the tumor. Surgeries were undertaken by a team of three experienced gynecologic oncologists. For final pathology, ultrastaging of all SLN was performed.

Sentinel Lymph Node Marking Techniques

Injection of ^{99m}Tc-nanocolloid: A SLN scintigraphy was performed one day before surgery. Four aliquots of 15 MBq ^{99m}Tc-labeled nanocolloids (Nano-HSA®, produced by Rotop Pharmaka GmbH, Dresden, Germany, particle size ≤80 nm) were injected intradermally adjacent to the tumor. After this procedure, a single-photon emission computed tomography combined with conventional computed tomography (SPECT/CT) was carried out.

Injection of ICG: One vial of 25mg ICG powder (Verdye®, produced by Diagnostic Green GmbH, Germany) was suspended in 10 ml of sterile water and injected intradermally directly before surgery at four injection sites around the tumor (**Figure 2**).

Injection of patent blue: A peritumoral intradermal injection of a total amount of 4ml patent blue (Patentblau V Guerbet® 25mg/ml, produced by Guerbet AG, Zurich, Switzerland) was performed immediately before surgery.

Statistical Analysis

A false negative SLN was defined as a SLN with negative tumor involvement detected with one SLN mapping technique in combination with a metastatic SLN detected with another SLN mapping technique or a metastatic non-SLN. The SLN detection

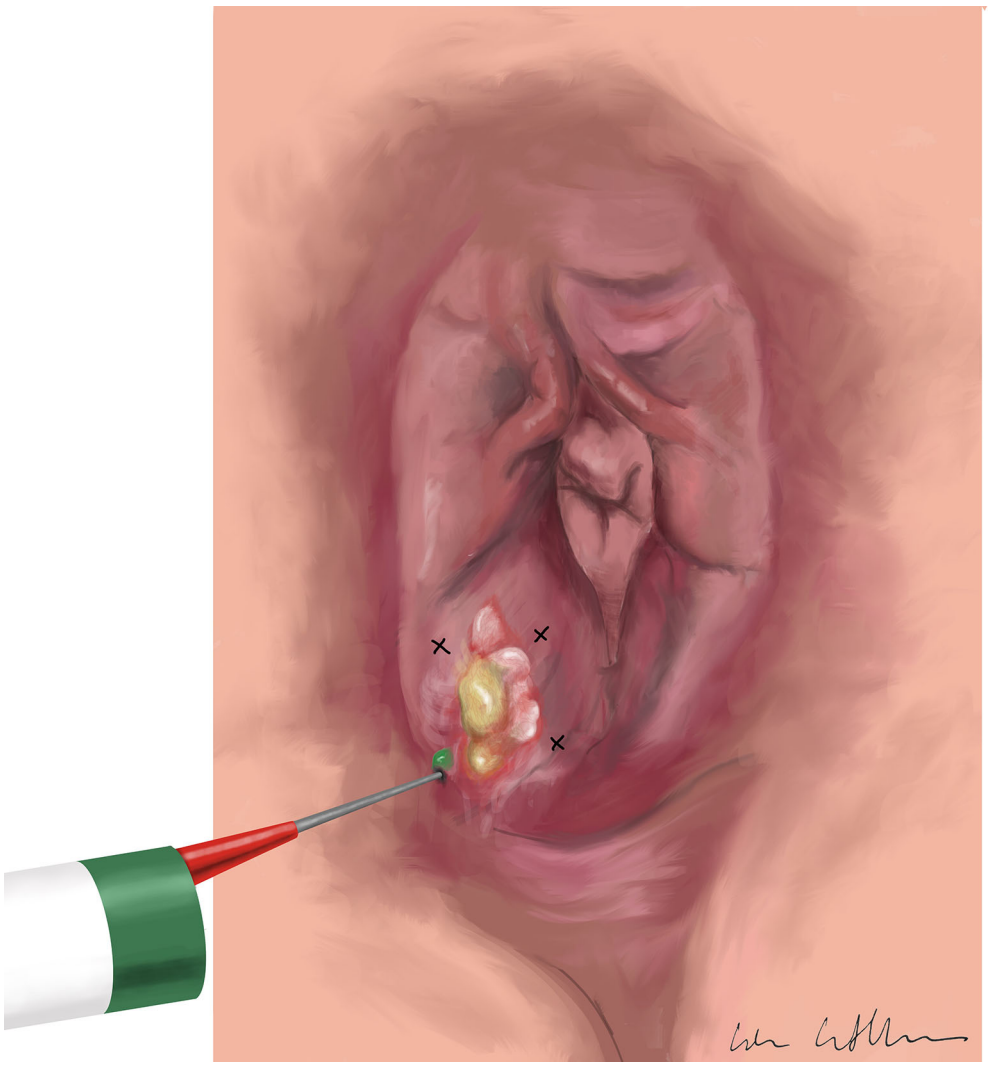

FIGURE 2 | Injection of indocyanine green intradermally at four injection sites around the tumor.

rate was calculated for each SLN mapping technique, defined as the number of procedures in which at least one SLN was identified divided by the total number of procedures performed. Detection rates among the different subgroups were compared using the chi-square test. Statistical calculations were performed using the Statistical Package for Social Sciences (IBM SPSS Statistic Version 25.0).

In accordance with the journal's guidelines, we will provide our data for the reproducibility of this study in other centers if this is requested.

RESULTS

Between April 2013 and April 2020, 34 patients were analyzed retrospectively for this study. Patient demographics and operative data are presented in **Table 1**. The majority of the patients had FIGO stage IB disease with a median age of 71.0 years and a median body mass index (BMI) of 27.85 kg/m^2. The median tumor size in final pathology was 2.50 cm with a median depth of infiltration of 5.50 mm. Final histology was a squamous epithelial carcinoma in all of the cases. Adjuvant treatment was performed in eight patients. Groin recurrence rate was 2.9% with a mean follow up time of 29.9 months.

Of the 34 patients, SLN mapping was performed in 64 groins with 30 patients having bilateral SLN biopsy and four patients having only unilateral. The mean amount of ICG injected was 8.4 ml (range 5 to 10 ml). No intra- or postoperative complications occurred due to the administration of ICG. In 51 groins (79.7%), a SLN biopsy alone was performed while in 13 groins (20.3%) an additional complete inguinofemoral lymphadenectomy was performed. The mean number of SLNs per groin removed was 1.88. In addition to using ICG for SLN mapping, in 53 groins we used ^{99m}Tc-nanocolloid, in four groins patent blue, and in five groins both methods. In total, 120 SLNs were identified and removed, of which 103 (85.8%) were positive for ICG. In 10 groins (15.6%), we found lymph node metastases; in eight of these a SLN was detected. In seven groins no further positive lymph nodes were identified at final pathology in addition to the SLN, while in one groin two positive non-SLNs were detected in the final pathology of the complete lymph node dissection. No additional SLN was found to have metastatic disease using ultrastaging in the final pathology. In one patient (both groins affected), SLN mapping was unsuccessful using each of the three techniques. This patient therefore underwent bilateral complete inguinofemoral lymph node dissection. No false negative sentinel lymph nodes were recorded with ICG in the 13 patients who underwent complete lymphadenectomy.

The SLN detection rate of ICG (87.5%) was comparable to ^{99m}Tc-nanocolloid (89.7%, p=.143) and significantly higher than patent blue (77.8%, p=0.003) (**Table 2**). The best detection rates were achieved using a combination of ICG and ^{99m}Tc-nanocolloid (96.9%).

Risk Group Analysis

In 19 groins the tumors showed lymph vascular space invasion and in 10 groins showed positive lymph nodes. In these cases, the SLN detection rate of ICG was significantly higher than that of ^{99m}Tc-nanocolloid (p values of .004 and .035 respectively) (**Table 3**). Furthermore, we observed a higher detection rate of ICG compared to ^{99m}Tc-nanocolloid in obese patients (BMI > 30 kg/m^2), although statistically not significant (p=.707).

DISCUSSION

Accurate SLN mapping is a crucial part of vulvar cancer staging, and enables avoiding unnecessary inguinofemoral lymphadenectomies. The current standard SLN procedure consists of a combination of a radioactive tracer and a blue dye. The SLN detection rates reported in the literature are 63-82%, 88-96%, and 91-98% for blue dye, ^{99m}Tc-nanocolloid, and the

TABLE 1 | Patients' characteristics and operative data.

Median age, years (range)	70.00 (44)
Median body mass index, kg/m^2 (range)	27.85 (23.8)
FIGO stage, n (%)	
• IA	1 (2.9)
• IB	22 (64.7)
• IIIA	5 (14.7)
• IIIB	1 (2.9)
• IIIC	4 (11.8)
• IVA	1 (2.9)
Tumor grading, n (%)	
• 1	6 (17.6)
• 2	20 (58.8)
• 3	7 (20.6)
Median operating time, min (range)	159.0 (274)
Median blood loss, ml (range)	100.0 (350)
Intraoperative complications, n (%)	0 (0)
Major postoperative complications, n (%)	3 (8.9)
Postsurgical treatment, n (%)	
• Chemoradiation	6 (17.6)
• Inguinal radiation only	2 (5.8)

n, number of patients.

TABLE 2 | Sentinel lymph node detection rates among different sentinel lymph node mapping techniques.

	SLN detection rate (%)
ICG (n=64)	87.5
^{99m}Tc (n=58)	89.7
Patent blue (n=9)	77.8
ICG + ^{99m}Tc (n=58)	96.9

SLN, sentinel lymph node; ICG, indocyanine green; ^{99m}Tc, Technetium-99m; n, number of groins.

TABLE 3 | Sentinel lymph node detection rates among different risk groups.

	SLN detection rate ICG (%)	SLN detection rate ^{99m}Tc (%)	p-value
Positive lymph nodes	80.0	62.5	0.035
Lymphatic space invasion	89.5	78.9	0.004
Obesity	94.7	88.2	0.707

SLN, sentinel lymph node; ICG, indocyanine green; ^{99m}Tc, Technetium-99m.

combination of both, respectively (17–19). Although these techniques show reasonable results in terms of detection and false negative rates, they have some shortcomings, including painful injections, complex logistics, and allergic reactions. SLN mapping with near-infrared fluorescence imaging has recently gained popularity in gynecological cancers (11, 13). Advantages include easier application, absence of radioactivity, and fewer side effects.

In our study, SLN mapping with ICG and near-infrared fluorescence imaging in vulvar cancer was feasible and safe. SLN detection with near-infrared fluorescence imaging performed equally well as ^{99m}Tc-nanocolloid (87.5% vs 89.7%, p=0.143) and significantly better than patent blue alone (87.5% vs 77.8%, p= 0.003); the best results were achieved using a combination of ICG and ^{99m}Tc-nanocolloid (96.9%). In patients with lymph node metastases or lymph vascular space invasion, ICG alone outperformed ^{99m}Tc-nanocolloid, with a significantly higher detection rate. For the conventional SLN mapping techniques, a compromised detection rate in lymph node positive patients is described in the literature, as a result of the complete replacement of true SNL by tumor cells and a redirection of the lymphatic vessels to other nodes (17, 20). However, particularly in these patients, a reliable SLN mapping is of utmost importance. 2019 Frumovitz et al. described a superior detection rate of ICG compared to blue dye in case of metastatic sentinel lymph nodes in endometrial and cervical cancer patients (21). Furthermore, several studies describe a restricted application for ICG in obese patients due to its limited tissue penetration, with an increased BMI identified as a potential risk factor for failure in SLN mapping (22–25). Results obtained from our cohort do not support this assumption, as the detection rates of ICG and ^{99m}Tc-nanocolloid did not differ significantly in obese patients, even with a slight tendency towards a higher detection rate with ICG in obese patients (94.7 vs 88.2%, p=0.707). Over all groin recurrence rate was 2.9%, which is consistent with the literature (6). The only patient with recurrence was successfully mapped with ICG and ^{99m}Tc-nanocolloid revealing two negative SLNs.

Up to now, several studies reported reasonable results in ICG SLN mapping for vulvar cancer patients (**Table 4**). The largest cohort was described by Broach et al. with ICG SLN mapping in 85 patients with different histological subtypes of vulvar cancer, including melanomas and less frequent tumors (26). The further studies are mainly case series of fewer than 20 patients (22, 24, 27–30), with methodological variations. For instance, ICG was administered in different formats: either absorbed in human serum albumin (23, 27) or as the hybrid tracer ICG-^{99m}Tc-nanocolloid (28, 30). Different near-infrared fluorescence imaging devices were applied, some of which were custom made (22, 29) and others commercially available [VITOM® II ICG exoscope (24, 31), the Mini-FLARE™ imaging system (23, 27, 28), Photodynamic Eye (30)]. In one exploratory study, the imaging device was changed during the course of the study from SPY® to PinPoint® (32). In addition, few case reports have been published on feasibility and safety of robot-assisted SLN mapping with ICG in vulvar cancer patients (33, 34). This

TABLE 4 | Studies using indocyanine green for sentinel lymph node mapping in vulvar cancer patients.

Author, year of publication	Type of study	No of patients	Histologic subtype	SLN marking tracers	Imaging system	ICG SLN detection rate
Broach et al. (26)	Retrospective cohort study	85	SCC Melanoma Others	ICG ^{99m}Tc Blue dye	NR	96.3%
Buda et al. (24)	Retrospective cohort study	6	NR	ICG ^{99m}Tc	VITOM-ICG®	100%
Hutteman et al. (27)	NR	9	SCC	ICG-HSA ^{99m}Tc Blue dye	Mini-Flare™	NR
Verbeek et al. (28)	Prospective trial	12	NR	ICG-^{99m}Tc Blue dye	Mini-Flare™	100%
Crane et al. (22)	Feasibility pilot study	10	SCC	ICG ^{99m}Tc Blue dye	Custom-made	NR
Laios et al. (29)	Prospective pilot study	11	NR	ICG Blue dye	Custom-made	91%
Mathéron et al. (30)	NR	15	SCC Melanoma	ICG-^{99m}Tc Blue dye	Photodynamic Eye	NR
Schaafsma et al. (23)	Double-blind randomized trial	24	SCC	ICG-HSA ^{99m}Tc Blue dye ICG	Mini-Flare™	63%
Soergel et al. (31)	Prospective trial	27	NR	ICG ^{99m}Tc Blue dye	VITOM-ICG®	NR
Prader et al. (32)	Exploratory study	33	SCC	ICG ^{99m}Tc	SPY PinPoint	87.5%

No, Number; SLN, sentinel lymph node; ICG, indocyanine green; SCC, squamous cell carcinoma; ^{99m}Tc, Technetium-99m; ICG-HSA, ICG adsorbed to human serum albumin; ICG-^{99m}Tc, combined tracer of indocyanine green and Technetium-99m.

minimally invasive approach might be a valid option to further reduce short- and long-term morbidity in these patients. However, follow-up data on a larger cohort of patients are needed. Several studies focused on the sensitivity of ICG compared to ^{99m}Tc-nanocolloid (23, 27, 30, 31). After injection, ICG travels *via* lymphatic vessels to the SLN as well as to echelon and second-echelon lymph nodes, potentially leading to the removal of additional, non-SLNs. Therefore, the number of lymph nodes removed is less important: the crucial point is removing the right lymph nodes. In cervical and endometrial cancer, a retrospective analysis demonstrated that a higher SLN count did not seem to increase the accuracy of SLN mapping (35). In our opinion, the SLN detection rate is the more reliable variable to investigate. Another important test characteristic is the false negative rate. As the majority of our patients did not undergo complete lymphadenectomy, we are not able to establish a false negative rate with our data.

To our knowledge, this study contains one of the largest cohort of ICG SLN mapping in squamous cell vulvar cancer patients to date. Beside its relatively large sample size, its major strengths include the risk group analysis of patients. This research adds to a growing body of literature supporting the use of ICG in SLN mapping in vulvar cancer patients. One of its most interesting aspects is the improvement of the SLN detection rate using a combination of ^{99m}Tc-nanocolloid with ICG. Based on our findings, a combination of ICG and ^{99m}Tc-nanocolloid offers a reasonable alternative to the conventional SLN mapping techniques in vulvar cancer. However, the major limitation of our study is the inability to determine if ICG alone improves the SLN detection rate; specifically the value of the additional information of the SPECT/CT performed preoperatively cannot be defined in this setting.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Commission of the Canton of Bern, Switzerland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SI, AP and MM contributed to conception and design of the study. SM and LK organized the database. FS and SI performed the statistical analysis. FS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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High Incidence of Gestational Trophoblastic Disease in a Third-Level University-Hospital, Italy: A Retrospective Cohort Study

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Introduction: to assess incidence, prognosis and obstetric outcome of patients treated for gestational trophoblastic disease GTD in a twenty-year period. Incidence, prognosis and obstetric outcome of gestational throphoblastic disease

Methods: retrospective study.

Results: Fifty-four cases of GTD: 46 (85.18%) cases of Hydatidiform mole (HM); 8 cases of Persistent Gestational Trophoblastic Neoplasia (GTN) (14.81%): 6/8 cases (75%) GTN not metastatic; 2/8 cases (25%) GTN metastatic. In both cases, the metastases occurred in the lungs. In 3 out of 8 GTN cases (37.5%) a histological picture of choriocarcinoma emerged. The incidence of GTD cases treated from 2000 to 2020 was 1.8 cases per 1000 deliveries and 1.3 cases per 1000 pregnancies. Of the 54 patients, 30 (55.56%) presented showed normal serum hCG levels without the need for chemotherapy. On the other hand, 24 patients (44.44%) developed a persistent trophoblastic disease and underwent adjuvant therapy. The negative prognostic factors that affected the risk of persistence of GTD were: serum hCG levels at diagnosis > 100,000 mUI/ml; characteristic "*snow storm*" finding at the ultrasound diagnosis; a slow regression of serum hCG levels during follow-up; the persistence of high serum hCG levels (especially if > 1000 mUI/ml one month after suction curettage) that was the main risk factor for resistance to first-line chemotherapy. There were 10 pregnancies in total following treatment. Patients' survival in our study was 100%.

Discussion: Although GTD is a rare disease, its incidence was 1.3 cases per 1,000 pregnancies in Sardinia, Italy, higher if compared with mean national and worldwide incidence.

Keywords: gestational throphoblastic disease, serum human chorionic gonadotrophin, obstetric outcome, epidemiology, prognostic

INTRODUCTION

Gestational trophoblastic disease (GTD) is a heterogeneous group of epithelial tumors which originate from placental trophoblastic tissue after abnormal fertilization and relate to a pregnancy event (i.e., abortion, extra-uterine or term/preterm pregnancy). Trophoblast shows limited histolytic, angiotropic, and invasive power, not exceeding thin myometrial basal decidua. Chorionic neoplasms, which are histologically similar to the chorionic villus, have invasive morphological and proliferative attitudes (1).

From a clinical-pathological point of view, we can distinguish the hydatidiform mole (HM) (complete and partial), which represents the most common form (80% of cases) and is a premalignant disease and the malignant gestational trophoblastic neoplasia (GTN), that may be nonmetastatic or metastatic (1, 2). The latter can include: Invasive mole (15% of cases); choriocarcinoma (a rare form that makes up about 5% of cases); placental site trophoblastic tumor (PSTT) (3), extremely rare; epithelioid trophoblastic tumor (ETT), even rarer (4, 5).

GTD burden can vary: in North America and Europe the incidence ranges from 0.57 to 1.1 cases per 1,000 pregnancies, whereas in Asia ~2.0 cases per 1,000 pregnancies (1). The estimated worldwide incidence of the HM and chorioncarcinoma is ~1 and 0.02/0.07 per 1,000, respectively (2, 6).

In the pre-chemotherapy era, invasive mole mortality was ~15%, caused by hemorrhage, sepsis, embolism, or surgical complications, whereas choriocarcinoma mortality was ~100% and ~60% in case of metastatic and non-metastatic disease, respectively (7, 8).

Currently, the cure rate is 90%. The risk of further molar pregnancy as well as chemotherapy-related fertility problems are the main issues (7–13).

Aim of the present study was to assess the incidence, prognosis, and obstetric outcomes of GTD patients admitted at an Italian university hospital. Furthermore, it was assessed the relationship between serum hCG levels and early identification of patients at risk of disease persistence.

MATERIALS AND METHODS

GTD cases were retrospectively reviewed from 2000 to 2020. Patients were retrieved using the report of histological examinations performed on surgical specimens and that were analyzed in the Institute of Pathology of the University of Sassari.

Each patient was then evaluated through a critical collection of the anamnestic, clinical and epidemiological information reported in their medical records.

This analysis was not reviewed by the local Ethics Committee of University of Sassari, Italy, because it was a retrospective study.

Demographic, epidemiological, and clinical characteristics were collected, including its the persistence and resistance to chemotherapy.

Serum hCG levels were measured after 1, 2, 3, and 4 weeks after uterine vacuum aspiration by Karman's cannula (suction curettage) to predict the risk of GTD persistence.

All patients underwent a weekly follow-up based on the assessment of serum hCG levels, a gynecological examination, and ultrasound. The clinical evaluation was interrupted after two to three consecutive negative serum hCG levels.

We evaluated risk factors associated with the persistence of disease such as, serum hCG levels rise (or not reduction), maternal age >40 years and volume of endocavitary material.

An *ad hoc* electronic form was used to collect demographic, epidemiological, and clinical variables. Qualitative variables were described with absolute and relative (percentage) frequencies. Quantitative variables were summarized with means (standard deviations, SD) and medians (interquartile ranges, IQR) in case of normal and non-normal distribution, respectively. Individuals with and without persistent pathology were compared: chi-squared or Fisher exact test was used for the qualitative variables, Student t or Mann-Whitney test was used for normal and non-normal quantitative variables, respectively. ROC curve was used to assess the accuracy of serum hCG levels (1, 2, 3 weeks, and one month) in the prediction of the persistence of trophoblastic disease.

Sidak's adjustment was carried out for multiple comparisons.

RESULTS

Study Population and Diagnosis

A total of 54 patients were reported in the study period (2000–2020): forty-six (85.18%) were HM and 8 (14.81%) GTN (6, 75%, with a non-metastatic disease). Three HM were randomly diagnosed after histological examination on specimen recovered during suction curettage performed in 1 case for miscarriage and in 2 cases for voluntary termination of pregnancy. Two out of 8 cases (25%) GTN were metastatic. In both cases, the metastases occurred in the lungs, in particular in one case the radiological image found was the characteristic "*snow storm*" picture.

Three out of 8 (37.5%) GTN cases were choriocarcinoma. In one case the diagnosis was made on surgical specimen recovered by suction curettage carried out two months after spontaneous delivery; another case was found to be a primitive tubal choriocarcinoma after laparotomic salpingectomy performed urgently for acute hemoperitoneum; the third case was diagnosed with certainty only after the hysterectomy performed after chemotherapy with methotrexate (MTX) and folinate calcium (FC).

In most of the cases (53), the diagnosis was performed using the surgical specimen collected through uterine vacuum aspiration by Karman's cannula (suction curettage).

Twenty-four (44.44%) patients, including those with a histopathological diagnosis of GTN, showed steady or slightly increase of serum hCG levels. Then, (after chest XR and total body CT to check for distant metastases), they were exposed to a first-line chemotherapy with MTX and FC; unfortunately, 6 (25%) were resistant and were treated with second-line drugs EMA/CO (etoposide, methotrexate, actinomicina-D, ciclophosphamide, and vincristine).

Six out of 24 (25%) patients undergoing chemotherapy, received total hysterectomy in order to report serum hCG levels to normal in three consecutive draws at the end of chemotherapy treatment, because being women over 40 years and not desiring pregnancy.

Incidence of GTD

The incidence of GTD was 1.8 cases per 1,000 deliveries and 1.3 cases per 1,000 pregnancies (**Table 1**); in particular, the incidence of HM was 1.6 cases per 1,000 deliveries and 1.2 cases per 1,000 pregnancies. The incidence of GTN was 0.3 cases per 1,000 deliveries and 0.2 cases per 1,000 pregnancies.

Among GTN, incidence of choriocarcinoma was: 0,1 cases/1000 deliveries and 0,07 cases/1000 pregnancies.

The highest prevalence was found in patients aged 31 to 40 years (33.3%) and 41–50 (31.4%). 50% were multiparous, with 16 (29.6%) with ≥1 miscarriage. The diagnosis was performed between the 7th and 11th weeks of gestational age. One patient had a previous history of GTD (3 molar pregnancies in 1988, 1991 and 1993). A patient developed a vesicular mole during an ovarian stimulation treatment for medically assisted reproduction technologies.

Sign and Symptoms, Serum hCG Levels, Follow-Up, and Therapy

72% complained symptoms at diagnosis: 87% with vaginal bleeding and 51% with an increased uterine volume.

Almost half of the cases (25, 46.3%) showed the "*snow storm*" ultrasound finding.

Thirty (55.6%) patients had complete regression of serum hCG levels to normal without chemotherapy; 24 (44.4%) patients with a persistent trophoblastic disease were treated (**Table 2**). 18 (75%) patients had a complete remission after a mean of 3 cycles.

Seven (29.2%) patients underwent laparoscopic or laparotomic hysterectomy at the end of the chemotherapy cycles and after three negative serum hCG levels. The surgically treated patients had an average age of 43 years (with a range of 41–51), at least two children and did not want to preserve fertility. The surgical treatment was carried out at the end of chemotherapy therapy, after regression of serum hCG levels to normal in three consecutive draws.

Of the 24 patients undergoing chemotherapy: 7/24 (29,17%) underwent hysterectomy with a mean age of 43 years, multiparous and without further desire for pregnancies; 8/24 (33,33%) were between the ages of 40 and 50 and had at least one child.

Serum hCG Monitoring and Risk Factors for Disease Persistence

Serum hCG levels >100,000 mUI/ml at diagnosis increased the risk of disease persistence (OR: 10; p-value: 0.001).

Serum hCG levels >10,000 mUI/ml 1 week after suction curettage increased the risk of persistence (OR: 8.6; p-value: 0.007). Furthermore, a 1-month serum hCG levels between 100 and 1,000 mUI/ml, as well as at two-month between 10 and 100 mUI/ml, significantly increases the risk (OR 52.2; p-value: 0.001; OR: 9; p-value: 0.004, respectively). The risk can be increased by a positivity at three months (OR: 16; p-value <0.001). The ultrasound finding of the "*snow storm*" at diagnosis similarly increased the risk of disease persistence (OR: 3.3; p-value: 0.04).

ROC curve for serum hCG levels at 3 weeks after the suction curettage predicted the persistence of trophoblastic disease with a sensitivity of 70.8% and a specificity of 92.6% (**Figure 1**).

Serum hCG levels between 100 and 1,000 mUI/ml at 1 month after suction curettage increased the risk of resistance to chemotherapy (OR: 14.1; p-value: 0.03). Similarly, a 2-month serum hCG levels between 10 to 100 mUI/ml and >100 mUI/ml increased the risk by 8 and 17 times, respectively.

TABLE 1 | Incidence of gestational trophoblastic disease (GTD) x 100000 deliveries and x 100000 pregnancies.

Year	Incidence HM/ deliveries	Incidence HM/ pregnancies	Incidence GTN/ deliveries	Incidence GTN/ pregnancies	GTD/deliveries	GTD/pregnancies
2000	135.59	94.25	0.00	0.00	135.59	94.25
2001	260.76	182.98	65,19	45,74	325.95	228.73
2002	127.71	89.69	63.86	44.84	191.57	134.53
2003	180.94	131.93	0.00	0.00	180.94	131.93
2004	0.00	0.00	60.31	43.71	60.31	43.71
2005	316.46	224.92	0.00	0.00	316.46	224.92
2006						
2007	127.71	91.19	127.71	91.19	255.43	182.40
2008	64.39	45.21	0.00	0.00	64.39	45.21
2009	191.08	139.02	0.00	0.00	191.08	139.02
2010	125.39	92.38	0.00	0.00	125.39	92.38
2011						
2012	76.28	52.86	76.28	52.86	152.56	105.71
2013	236.78	180.07	0.00	0.00	236.78	180.07
2014	412.54	305.81	0.00	0.00	412.54	305.81
2015	78.37	58.17	0.00	0.00	78.37	58.17
2016	86.88	62.00	86.88	62.00	173.76	123.99
2017	265.49	187.27	0.00	0.00	265.49	187.27
2018	354.30	255.92	0.00	0.00	354.30	255.92
2019	69.93	50.35	0.00	0.00	69.93	50.35
2020	303.03	210.38	101.01	70.13	404.04	280.50
2000–2020	**155.37**	**111.63**	**27,02**	**19.41**	**182.39**	**131.05**

HM, hydatidiform mole; GTN, gestational trophoblastic neoplasia; GTD, gestational trophoblastic disease.
Bold values: overall incidence of GTD in the last 20 years.

TABLE 2 | Patients treated by first-line (MTX/CF) and second-line (EMA/CO) chemotherapy. CT).

Patient	Histology	CT (MTX/CF)	Response to therapy	EMA/CO	Hysterectomy (Hy) and bilateral anesectomy (Ba)
1	HM	3 cycles	Complete	–	–
2	HM	2 cycles	Complete	–	–
3	HM	2 cycles	Complete	–	–
4	Chorioncarcinoma	3 cycles	Resistance	8 cycles with CR	–
5	GTN	3 cycles	Resistance	6 cycles with CR	–
6	HM	3 cycles	Complete	–	–
7	HM	2 cycles	Complete	–	–
8	HM	2 cycles	Complete	–	–
9	GTN	3 cycles	Resistance	4 cycles con CR	–
10	HM	3 cycles	Complete	–	–
11	HM	3 cycles	Complete	–	–
12	GTN	8 cycles	Resistance	3 cycles with CR	–
13	GTN	3 cycles	Resistance	4 cycles with CR	–
14	HM	2 cycles	Complete	–	Hy+Ba after CT
15	HM	4 cycles	Complete	–	–
16	GTN	3 cycles	Resistance	2 cycles with CR	Hy+Ba after CT
17	HM	2 cycles	Complete	–	–
18	HM	4 cycles	Complete	–	Hy+Ba after CT
19	HM	4 cycles	Complete	–	Hy+Ba after CT
20	HM	3 cycles	Complete	–	Hy+Ba after CT
21	Chorioncarcinoma	7 cycles	Complete	–	Hy+ Ba during CT
22	HM	4 cycles	Complete	–	–
23	HM	3 cycles	Complete	–	Hy+Ba after CT for recurrent metrorragies
24	Primitive tubal chorioncarcinoma	5 cycles	Complete	–	–

HM, hydatidiform mole; CR, complete remission; GTN, gestational trophoblastic neoplasia.

Three month serum hCG levels positivity increased the risk of resistance to first-line chemotherapy by 12 times (OR 12.3; p-value: 0.005).

Serum hCG levels >100,000 mUI/ml were risk factors for the development of a trophoblastic disease. Maternal age >40 years and increased volume of endocavitary material increased the risk of disease persistence without any statistical significance.

GTD and Obstetric Outcome

Nine out of 24 patients who had chemotherapy, afterwards 6 (66.67%) had at least one pregnancy following chemotherapy treatment. There were 10 pregnancies in total, including: 6 term deliveries without obstetric complications and no newborns presented chromosomal pathologies; 1 miscarriage at the first trimester; 2 interruptions of pregnancy; 1 extrauterine pregnancy.

Thus, 6 (66. 7%) patients had ≥ 1 pregnancy following chemotherapy. The survival of the cohort was 100%.

DISCUSSION

The incidence of GTD in our university hospital was 1.8 cases per 1,000 deliveries and 1.3 cases per 1,000 pregnancies during the time period 2000 to 2020. It is higher if compared with the Italian incidence (0.7–0.8 cases per 1,000 deliveries and 0.5 cases per 1,000 pregnancies) (13–21). The increased epidemiological burden of GTD could be explained by unknown genetic factors, selected in Sardinia island such as autoimmune diseases (e.g., diabetes mellitus type 1, multiple sclerosis, and celiac disease) whose prevalence and incidence are highest. This hypothesis is supported by a study carried out in indigenous villages in Alaska, where the incidence of the hydatidiform mola was 3.9 cases per 1,000 deliveries (22). Mutations of the NLRP7 gene on chromosome 19q were found in families with recurrent vesicular mole (23). This gene involved also in mediating inflammatory pathways may represent a milestone in linking GTD with autoimmune diseases, further investigations will be attempted in our Institution to clarify the role of this genetic pattern in Sardinian women.

The incidence of choriocarcinoma was 0.072 cases per 1,000 pregnancies and was higher than that estimated in Europe and USA (0.02 cases per 1,000 pregnancies), whereas it was comparable to that of Japan (0.075 cases per 1,000 pregnancies) (23, 24).

It was found a reduction of GTD incidence when the current analysis was compared with a previous one performed for a cohort recruited between 1976 and 1989 (1.8 per 1,000 deliveries VS. 3.6 per 1000 deliveries, respectively) but a slight increase if compared with the incidence of the cohort enrolled between 1974 and 1983 (1.46 per 1,000 deliveries). On the other hand, the incidence of choriocarcinoma was almost comparable (0.04 per 1,000 deliveries of 1974–1983 VS. 0.06 per 1,000 deliveries of 1976–1989 VS. 0.07 per 1,000 deliveries of 2000–2020) (12, 13).

The results on the factors associated with the persistence of the disease are in agreement with previous findings; serum hCG levels >100,000 mUI/ml are a risk factor for the development of a trophoblastic disease (25–28). Other risk factors associated with the persistence of disease are maternal age >40 years and increased volume of endocavitary material; in our cohort both factors increased the risk of disease persistence without any statistical significance.

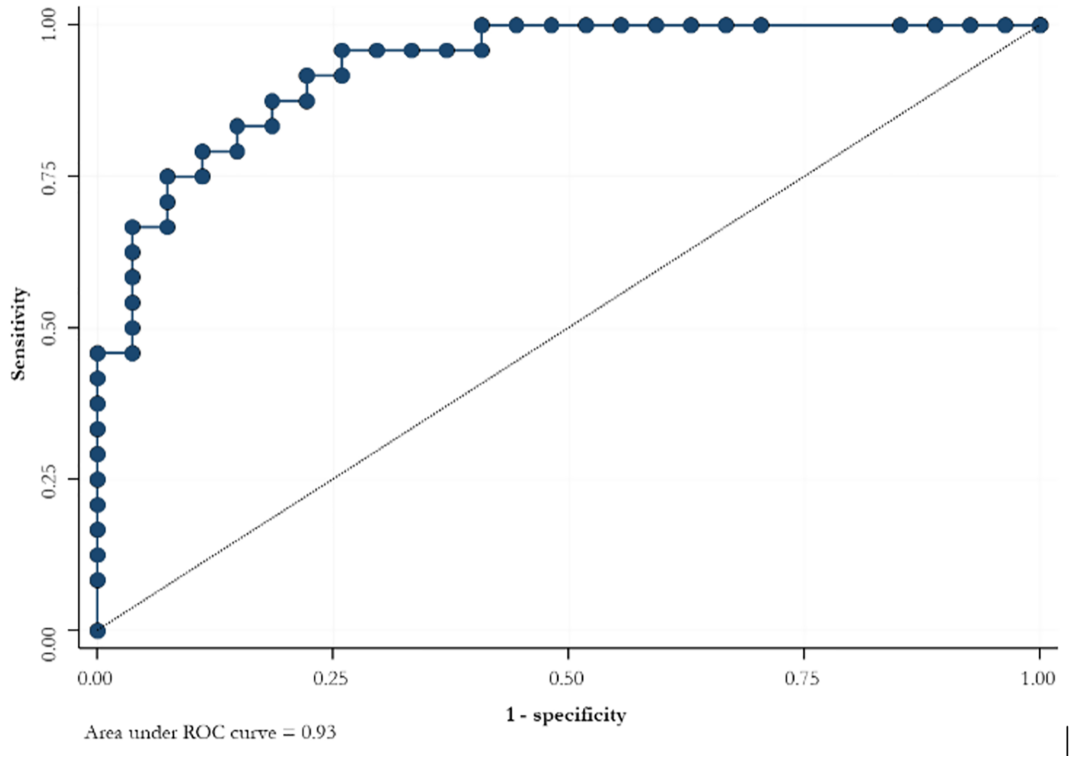

FIGURE 1 | ROC curve for the slope of serum hCG levels at 3 weeks after suction curettage to predict the persistence of trophoblastic disease.

Serum hCG levels monitoring is mandatory in the follow-up of patients with molar pathology to perform an early diagnosis of persistent trophoblastic disease and to diagnose patients with resistance to first-line chemotherapy (9, 29–31).

It would be helpful to identify a serum hCG levels threshold: the analysis carried out in our cohort found a good specificity and a limited sensitivity. A large sample size could increase the accuracy. The identification of an appropriate threshold of hCG serum levels in monitoring GTD is of great relevance, also considering the recent findings of a large meta-analysis which documented a very favorable obstetric outcome in women receiving conservative management of complete/partial molar pregnancy (32), thus highlighting the need to properly follow up women with GTD.

We acknowledge that the retrospective nature, and the long period of patients' enrollment represent major study limitations; however, the homogeneity of investigated population is certainly a relevant strength.

CONCLUSIONS

Although GTD is a rare disease, its incidence was 1.3 cases per 1,000 pregnancies in Sardinia, Italy, higher if compared with mean national and worldwide incidence. Genetic factors could concur to the increased burden.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Project development: GC and ET. Data collection and manuscript writing/editing: LS and FD. Data collection and manuscript editing: MP and MM. Project development, data management, and manuscript editing: GV, AO, and DS. Manuscript writing/editing: AC, SD, and PC. Data analysis: GS. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Sentinel Lymph Node Mapping in Endometrial Cancer: A Comprehensive Review

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Endometrial cancer (EC) is known as a common gynecological malignancy. The incidence rate is on the increase annually. Lymph node status plays a crucial role in evaluating the prognosis and selecting adjuvant therapy. Currently, the patients with high-risk (not comply with any of the following: (1) well-differentiated or moderately differentiated, pathological grade G1 or G2; (2) myometrial invasion< 1/2; (3) tumor diameter < 2 cm are commonly recommended for a systematic lymphadenectomy (LAD). However, conventional LAD shows high complication incidence and uncertain survival benefits. Sentinel lymph node (SLN) refers to the first lymph node that is passed by the lymphatic metastasis of the primary malignant tumor through the regional lymphatic drainage pathway and can indicate the involvement of lymph nodes across the drainage area. Mounting evidence has demonstrated a high detection rate (DR), sensitivity, and negative predictive value (NPV) in patients with early-stage lower risk EC using sentinel lymph node mapping (SLNM) with pathologic ultra-staging. Meanwhile, SLNM did not compromise the patient's progression-free survival (PFS) and overall survival (OS) with low operative complications. However, the application of SLNM in early-stage high-risk EC patients remains controversial. As revealed by the recent studies, SLNM may also be feasible, effective, and safe in high-risk patients. This review aims at making a systematic description of the progress made in the application of SLNM in the treatment of EC and the relevant controversies, including the application of SLNM in high-risk patients.

Keywords: sentinel lymph node, endometrial cancer, lymphadenectomy, low-volume metastases, high risk, sentinel lymph node biopsy, sentinel lymph node mapping

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INTRODUCTION

Endometrial cancer (EC) is known as a common female genital malignancy with rapidly increasing incidence these years. In 2021, there will be an estimated 66,570 new cases and 12,940 deaths, making uterine cancer the second most prevalent cancer in women in U.S. after breast cancer (1). Surgery is the mainstay for treatment include total hysterectomy + bilateral salpingooophorectomy + pelvic lymphadenectomy +/− para-aortic lymphadenectomy (TH+BSO+PLAD+/−PALAD). LAD represents a significant component of comprehensive staging for patients with EC. However, studies have revealed that LAD may not be conducive to the prognosis of EC patients (2, 3). Besides, the

selective lymphadenectomy (SLAD) based on "Mayo criteria" shows a high sensitivity with a low specificity (4), and 80% of the high-risk patients undergo excessive lymph node dissection (5). Additionally, lymph node resection brings a series of complications like vascular nerve injury, lymphedema, lymphatic cysts, and so on (6). Therefore, SLNM or sentinel lymph node biopsy (SLNB) can be effective in addressing this drawback. SLNM does not compromise patient outcome by providing enough information on lymph node directing adjuvant therapy (7), meanwhile, it improves the quality of life by shortening operation time and reducing complications (8). This review is to give a comprehensive view of the application of SLNM in EC, thus providing further choices regarding the lymph node dissection.

DISPUTES ABOUT LAD FOR EC

The I–IV staging system of EC was initiated in 1962 and transferred from clinical staging to surgical pathologic staging in 1988 (9). Furthermore, staging protocol was re-edited in 2009 for setting IIIC1 as positive pelvic lymph nodes, while IIIC2 refers to the positive para-aortic lymph nodes (10). LAD is an essential part of staging surgery for it provides the lymph node information thus indicating adjuvant therapy, evaluating prognosis, and acting as a therapeutic role. Patients with pelvic or para-aortic lymph node metastasis has dramatically decreased survival rate (10). Additionally, it is believed that LAD eliminates not only existing metastases but also occult or potential metastasis (11). Large retrospective studies showed that LAD is associated with prolonged survival outcome, especially in high-risk EC (12).

However, the therapeutic role and survival benefit of LAD have been in controversial in recent years with the publication of a series of high-quality research. Two large randomized controlled clinical trials (RCT) in 2008 and 2009 included 514 and 1408 patients with EC found no statistical significance in PFS and OS between LAD or not (13, 14). Though the two studies are blamed for varying design defects, such as LAD group, did not perform PALAD, the two groups of high-risk patients were not balanced, the proportion of low-risk patients was larger, and adjuvant therapy was not standardized, but it did arouse intensive debates (15). A more recent multicenter study performed by Bougherara et al. (3) demonstrated that LAD brings no survival benefits in intermediate-risk EC group and Zhang et al. (16) analyzed SEER databases and found that after balancing mixing factors, LAD has no survival difference for patients in clinical stage IA with any histologic grade. Besides, LAD increased the incidence of intraoperative complications (prolonged operation time, excessive bleeding, vascular nerve injury, etc.) and post-operative complications (lymphedema, lymphocyst, intestinal obstruction, deep venous thrombosis), thus affecting the quality of life for patients (17). Beesley et al. (18) followed up 643 EC patients and found that the incidence of lymphedema was related to the number of lymph nodes removed, the risk climbed to 50% when cutting more than 15 lymph nodes. Volpi et al. pointed out that LAD and PALAD are independent risk for lymphedema and lymphocele (6).

At present, the most commonly used strategy is "SLAD" according to "Mayo Criteria" proposed by Mariani et al. (19) in 2000. That is to say, LAD could be omitted in low-risk group (meet all of the following conditions: (1) endometroid type, grade G1 or G2; (2) myometrial invasion< 1/2; and (3) tumor diameter < 2 cm), while LAD should be applied in high-risk group (not in accordance with any of the above). However, evidence has confirmed the ability of the method to identify patients with low risk (1%–2.4%) or high risk [11.4%–19% (5, 20, 21)] with a high sensitivity of 90%, which remains the most sensitive method in determining which patients can be omitted from LAD (22), while the specificity is only 36% (4). Nearly 80% of the high-risk group without metastases undergo LAD. In addition, the criteria depend on intraoperative frozen section (FS) and the coincidence rate with postoperative pathology declines when the histology grade and myometrial invasion degree increases, which result in approximately 18% of EC patients up-staged when final pathologic reports come (23, 24).

Therefore, the emergence of SLNM provides an alternative for both systemic LAD and SLAD. Not only does it reduce complications and improve the quality of life of patients, it provides sufficient staging information for evaluating prognosis and guiding adjuvant therapy. Most importantly, it seems not to compromise the survival outcomes of EC patients.

THE CONCEPT AND ORIGIN OF SLN

SLN refers to one or several lymph nodes that first receive lymphatic fluid from an organ or regional tissue, or the first lymph node that is impacted by the lymphatic metastasis of the primary malignant tumor through the regional lymphatic drainage pathway, thus indicating the involvement of the whole drainage area (25). Theoretically, if SLN is negative, lymphatic metastasis of the drainage area does not occur yet, thus avoiding LAD with following surgical trauma (11). If SLN is found positive in FS, the LAD can be performed directly during the operation. If the H&E staining and/or ultra-staging of SLN is positive after surgery, patients can either choose adjuvant therapy or a second operation. It is noted that FS of SLN is not a routine in many institutions due to its cost and inaccuracy in finding low volume metastatic disease (LVMD) (10, 26), while some send corpus uterine for FS assessment when SLN map failure occurs (27), which is also mentioned in the latest NCCN guideline.

In 1960, Gould et al. first discovered and defined SLN in parotid carcinoma (28). In 1977, Cabanas first used SLN lymphangiography in penile cancer (29). SLNM gradually became a routine procedure for the treatment of breast cancer and skin melanoma. Burke was the first to perform SLNM on 15 patients with EC back in 1996 (30). In the most recent decade, SLN has developed rapidly in EC and has been applied to the treatment of gynecological tumors such as vulvar cancer, cervical

cancer, and EC. By resecting two to four high-quality lymph nodes, SLNM may have the same diagnostic advantages as LAD and minimize surgical injuries.

THE TECHNIQUE ADVANCES OF SLNM

Detection Method

At the present time, SLN detection methods include blue dye method, radionuclide tracing method, indocyanine green (ICG), carbon nanoparticle (CNP), and combination method.

Blue dye method, also known as bioactive dye tracing method, including methylene blue, isosulfur blue, and patent blue. The dye can reach lymphatic vessels and lymph nodes around the tumor, and SLN is the first lymph node to show color. The method features simplicity and cost-effectiveness. However, the blue dye can diffuse to parametrial area thus interfering with the discovery of regional SLN (31). Some methylene blue may leak into the capillaries, resulting in reduced dye volume in lymphatic pathway and decreased SLN DR (32). Also, the risk of allergy cannot be ignored (33).

Radioactive tracers like technetium(Tc)-99^{m} can remain highly concentrated in the SLN, and emit gamma-rays, which will be detected by gamma detector and single-photon emission computed tomography (SPECT-CT). Radioisotopes can transmit signals through deep tissues. However, the higher cost of detection and imaging equipment, inconvenience, and potential radioactive contamination limit its use (31). The cervical injection site can also stimulate gamma detectors, which makes it difficult to be distinguished from parametrial lymph nodes (34).

ICG fluorescence labeling relies on ICG, a near-infrared fluorescent dye, to drain through lymph nodes and stimulate fluorescence under near-infrared light (700-900 nm) (11) (**Figure 1A**). It is the most recommended tracer in researches and guidelines, especially for patients with minimally invasive surgery and obesity, due to its highest DR and bilateral detection rate (BDR) (35–37). A randomized non-inferiority trial of 180 patients with uterine and cervical cancer showed that, ICG detected 97% of the total lymph nodes dissected whereas blue dye identified only 47% (38). Recent research from Germany compared ICG with blue dye in EC and cervical cancer, as a result, ICG improved the DR (78% *vs.* 61%, p=0.006) and therefore decreased the LAD rate from 28% to 9% (p=0.001) when mapping failure occurs (39). However, the method relies on near-infrared device (40). Also, ICG enhanced the visualization of lymphatic channels, which leads to an increase in "empty node," which may be compromised by the combination of ICG and Tc-99m (41). Though the adverse reaction rate is extremely low (0.07% to 0.5%) (42), it should be avoided in patients with iodine allergy and liver failure, since it is completely metabolized through liver (10).

CNP suspension derives from carbon nanoparticles with a diameter of 150 μm (43). It enters the lymphatic system by macrophage and is excreted through the respiratory and gastrointestinal tract (44). It owns the advantages of unique lymphatic system tendency, small-size, fast diffusion, and long-lasting color rendering (43). Meanwhile, it can adsorb anti-cancer drugs and is difficult to leak out when lymphatic channels are cutoff intraoperatively (43). There are no adverse reactions reported yet, and the DR is quite high. Data from our hospital showed that the combination of CNP and ICG resulted a higher BDR of SLN in cervical and endometrial cancer comparing to CNP or ICG alone ($p<0.05$) (45, 46).

The combined method is usually a combination of TC-99 and blue dye or ICG. Despite its high DR and low false negative rate (FNR), it is inconvenient and costly.

Injection Route

Injection routes include cervix and uterine corpus (47).

Cervical injection is the most common and simplest way. It is stable because of the rarity of cervical deformation caused by uterine fibroids, tumor infiltration or conization history (48). Anatomical studies have confirmed that cervical injection can penetrate into uterine vessels, isthmus, parametrial, and uterine body (15), while deep injection can reach para-aortic lymph nodes through pelvic funnel ligament (**Figure 1B**). The DR of pelvic SLN is higher using cervical injection as confirmed by large-scale studies (more than 100 patients), with a rate over 80% normally (49). However, the possibility of missing occult para-aortic lymph nodes (PAL) remains disputable. This may be compensated by rare incidence, ranging from 0.5% to 3.8% (47, 50, 51), of isolated para-aortic lymph node (IPL) metastasis, which is negative pelvic lymph node with positive PAL. Also, patients with any site lymph node metastases will receive adjuvant therapy, which theoretically eliminates potential metastatic lesions in para-aortic region (24). In brief, the main protocol for cervical injection is superficial injection (1–3 mm) with deep injection (1–2 cm or 3–4 cm) at 3- and 9-o'clock, or 3-, 6-, 9-, and 12- o'clock 2-, 4-, 8- and 10-o'clock points (48, 52) (**Figure 1C**).

Uterine corpus injection includes hysteroscopic or transvaginal ultrasound-guided peritumoral (subendometrial) injection (53) preoperatively and subserosal or myometrial injection intraoperatively. Hysteroscopic way can visualize the tumor directly and reflect the real lymphatic metastatic pathway, thus it seems a better method for the evaluation of para-aortic area. The DR ranged from 73% to 100%, the DR of PAL ranged between 13% and 56%, and the DR of IPL ranged from 3.4% to 20% (54–56). A multicenter RCT showed that hysteroscopic injection has a higher rate in identifying PAL (29% *vs.* 19.5, p=0.18) and IPL (5.8% *vs.* 0%) than cervical injection; however, there is no statistical difference (57). A recent retrospective analysis of 221 patients undergone hysteroscopic injection resulted in a 94.1%, 62.5%, and 2.7% DR, BDR, and IPL DR, which contributed to an 88.5% sensitivity and 96.5% NPV (58). However, the technique is complicated and not suitable for tumors with large size. Besides, the potential risk of tumor spreading through fallopian tubes is under concern (59). However, the risk of tubal leakage can be avoided by lower intrauterine pressure (<40 mm Hg) when performing hysteroscope and even tubal leakage may not result in tumor dissemination (60). The hysteroscopic way is usually injected

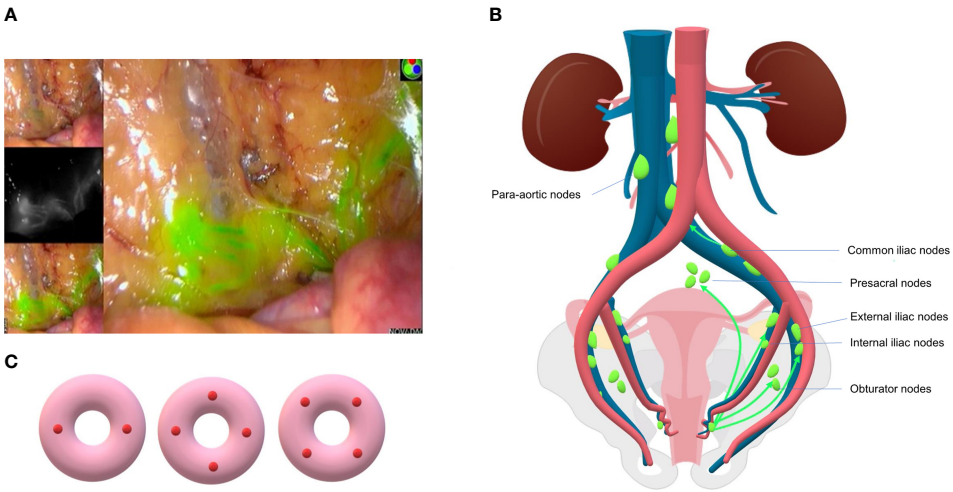

FIGURE 1 | **(A)** SLN and lymphatic vessel mapped in surgery using ICG dye (Liaoning Pharmaceutical Co., Ltd.) and intraoperative fluorescence imaging system (PC9000, Novadaq Technologies Inc.). **(B)** Common lymphatic drainage pathway of endometrial cancer. SLNs are mostly located in external iliac and obturator region and less commonly in presacral and common iliac area. **(C)** Three patterns of cervical injection sites of SLNM: two sides or four quadrants.

around the tumor with 111MBq Tc 99m or 8 ml blue dye (60). Though subserosal injection at fundus is relatively easy, it remains difficult to show the parametrial lymphatic drainage, and most early ECs do not invade or penetrate to the serosa layer. Moreover, patients with uterine fibroids may cause uterine deformation, which made it difficult to inject. The reporting DR of subserosal injection varies from 73% to 95% (49). The injection site is generally at the midpoint of the uterine fundus, anterior wall, and posterior wall. Cervical isthmus and peritumoral regions can also be injected.

Overall, Cormier et al. conducted a systematic review of cervical injection in 1,102 cases and corpus injection in 300 cases, which led to a conclusion that the overall DR of cervical injection ranged from 62% to 100%, corpus injection varied from 73% to 95% (49), and the DR of PAL was 39%, 17%, and 2%, respectively, in fundus, deep cervix, and superficial cervix injection (49). Cervical injection is simpler, faster, and more effective, which is accepted and recognized as mandatory by worldwide gyn-oncologist in latest consensus and surgical assessment tool of SLNM in EC. It is noted that, in the consensus, cervical injection is obligatory, whereas hysteroscopic or myometrial injection is not suggested. Also, it recommends the utilization of ICG, although blue dye and Tc-99m are available (61).

THE DIAGNOSTIC ACCURACY OF SLNM

Key Concepts

DR (49) refers to the percentage of patients with at least one SLN detected of all the patients tested. BDR refers to the proportion of patients with at least one SLN detected in each pelvic cavity to all the patients tested. False negative rate (FNR) refers to the proportion of patients with negative SLN but non-SLN positive to the total number of patients with SLN metastasis. Sensitivity is defined as the proportion of patients with positive SLN to the total number of patients with metastasis. NPV refers to the proportion of patients with SLN-negative and confirmed that no other lymph node metastasis to the total number of patients with SLN-negative. SLNM is supposed to show high sensitivity and low FNR.

SLNM Shows Good Feasibility and Accuracy

The diagnostic value of SLNM requires the institution to perform LAD after SLNM and do pathologic evaluation of the lymph nodes resected by SLNM and LAD, respectively, to determine the abovementioned indicators. Researches have demonstrated high DR, sensitivity, and NPV in patients with early-stage EC using SLNM with pathologic ultra-staging. SLNM + LAD was performed in 125 patients with FIGO stage I-II EC by SENTI-ENDO multicenter research (62). The DR was 88.8%. The sensitivity, FNR, and NPV was 84%, 2.4%, and 97%, respectively. To improve the sensitivity and NPV, MSKCC proposed that, unilateral or bilateral LAD should be added if SLN map failure occurs in one side or both sides, all suspicious enlarged lymph nodes and peritoneal lesions should be removed, and ultra-staging pathology should be performed after operation. It is called MSKCC algorithm and is recommended in NCCN guideline. In a retrospective study involving 498 patients performed by Barlin et al. (63), the DR was 81%. After the SLN algorithm was applied, the FNR declined sharply from 14.9% to 1.9%, the sensitivity increased from 85.1% to 98.1%, and the NPV increased from 98.1% to 99.8%. When the MSKCC algorithm was retrospectively applied to 14 studies including SENTI-ENDO, NPV increased from 95% to 99.2% (47). The FIRES study (64) included 385 patients with EC from 19 surgeons in 10 institutions. The DR and BDR was 86% and

52%. The sensitivity, NPV and FNR was 97.2%, 99.6%, and 2.8%. However, these studies are mostly retrospective or prospective in nature, and there are no RCT yet.

The diagnostic value of SLN is thoroughly evaluated in several meta-analysis and systematic reviews, with the DR of SLN ranging from 80% to 100%, FNR varied between 0% and15%, and sensitivity ranged from 86 to 100%. The meta-analysis of 26 studies performed by Kang et al. (65) indicated that the DR and sensitivity was 78% and 93%, respectively. When learning curve deviation was considered, the DR and sensitivity with less than 30 patients were 82% and 88%, and those with more than 30 patients were 78% and 93%, respectively. Cormier et al. (49) conducted a systematic review of 17 studies, with the studies fewer than 30 patients excluded. The DR varied from 60% to 100%, and the DR exceeded 80% in subgroup of over 100 patients. After retrospective application of SLN algorithm, the sensitivity, NPV, and FNR was 95%, 99%, and 5% respectively. These results prove that surgeon experience and standard surgical procedures are favorable in improving diagnostic accuracy of SLN. Bodurtha et al. (66) published a meta-analysis of 4,915 patients in 55 studies. The DR and BDR was 81% and 50%. DR of PAL was 17%. The sensitivity and NPV was 96% and 99.7%, respectively. ICG and cervical injection could increase the DR ($p < 0.05$). The similar results were reached by Lin et al. (67) that ICG, cervical injection, and robotic-assisted surgery may improve the DR and sensitivity. While in a recent meta-analysis published by How et al. (68), with 5,348 patients and 48 studies included, the DR, BDR, and PAL DR was 87%, 61%, and 6%, respectively. It is noted that the study showed that SLNM failed to impair the diagnostic value in high-risk histology types, and compared with LAD, SLNM failed to affect survival outcome or increase recurrence risk.

Factors Associated With Diagnostic Value

Surgeon Experience

Plenty of studies have demonstrated the learning curve effect. The accumulation of surgeon experience is associated with an increase in DR and sensitivity. Khoury et al. (69) compared the DR in early (2005–2007) and late (2008–2009) periods of MSKCC, which revealed an increase from 78% to 94%, suggested that the experience of more than 30 cases played an important role. Also, researchers from University of North Carolina finds 40 cases as a plateau of the learning curve for successful SLN mapping (70).

Tracer Type and Injection Site

ICG and cervical injection has gained worldwide acceptance for its ability in detecting SLN with a relatively high sensitivity. However, some researchers are working on combination dyes or special injection methods to improve the DR of both pelvic and para-aortic area and compromise the drawback of single method. Cabrera suggested adding Tc-99m to ICG for the increased BDR (69% *vs.* 41%, $p = 0.012$) and decreased empty node rate (0% *vs.* 4%, $p = 0.032$), which is known as a disadvantage to ICG alone (41). Our work shows an increased BDR in identifying SLNs when adding CNP to ICG comparing with CNP or ICG alone (45). Cervical reinjection when mapping failure occurs has been demonstrated as a feasible strategy to increase the DR and BDR of SLNM (71, 72). Eoh et al. (73) and Ruiz et al. (74) carried out "two-step method", which was a combination of fundus injection and cervical injection of ICG, showing a relatively high DR in both pelvic and para-aortic regions. The overall DR of pelvic SLN and para-aortic SLN was 92.79% to 100%, and 86%, respectively. The sensitivity, specificity, and NPV ranged from 94.44% to 100%. Torne et al. developed transvaginal ultrasound-guided myometrial injection of radiotracer (TUMIR), presenting an 82.1% DR, 92.3% sensitivity, and 97.7% NPV (53).

Patient's Condition

Age, obesity (BMI > 40), pelvic anatomical abnormality (vascular tortuosity), pelvic adhesions (history of operation and radiotherapy), and lymphatic vessel obstruction or destruction (tumor metastasis, deep myometrial infiltration, and endometrial inflammation), all could impact the DR of SLN (32, 37).

Pathology Examination

Some scholars believe that routine HE staining is possible to miss LVMD in SLN, which could be identified by immunohistochemistry staining (IHC) and serial section, also known as ultra-staging, which is discussed in later paragraphs.

Other Factors

At present, lymph vascular space invasion (LVSI), non-endometroid histology is seen as independent risk factors for failed mapping (75). The false negative SLN was more likely to appear in unilateral mapping failure patient. Higher SLN detection rate is also reported to be associated with tumor size and patient age, as well as tracer volume (76). However, the role of tumor size, depth of myometrial invasion, pathological type and grade, operation time, and scope, as well as LVSI are still lacking strong evidence. The other factor includes the surgical approach, like robotic or laparoscopic procedure. Cela et al reported 23 patients who underwent robotic-assisted surgery showing a 78.26% DR and 60.9% BDR (77). While, Chaowawanit et al summarized 76 patients with laparoscopic surgery and 33 patients with robotic approach. The result showed that laparoscopic procedure was superior than robotic in DR (97% *vs.* 83%, $p = 0.046$) and BDR (88% *vs.* 73%), whereas the two groups showed similar SLN detection and dissect time (78).

THE THERAPEUTIC SAFETY OF SLNM

Whether SLNM alone affects the long-term prognosis of patients with EC has been of great concern. Studies have been carried out to compare the oncologic outcome of SLNM-only *vs.* LAD without SLNM, or SLNM only *vs.* SLNM+LAD, or SLNM+LAD *vs*. LAD group, suggesting that SLNM failed to compromise survival outcome. Even though SLNM resects only a few lymph nodes, the overall DR of metastatic lesions in SLNM group is higher than regular LAD (79), which benefit accurate staging, thus guiding adjuvant therapy. In addition,

SLNM can improve the quality of life for patients by minimizing operation complications (7, 8).

SLNM Detected More Metastases Thus Facilitate Adjuvant Therapy

It is worth noting that even though SLNM may only remove two to four lymph nodes at a time, with a certain FNR and the risk of missing occult lymph nodes, the overall DR of metastatic lymph nodes is higher compared to conventional LAD (79). Leitao et al. (80) conducted a retrospective study on 507 EC patients. As indicated by the results, LAD rate decreased gradually and the number of removed lymph nodes was in decline accordingly (Y1 20; Y2 10; Y3 7; $p < 0.001$). However, there was no difference spotted in the detection of cases with lymph node metastasis found every year (Y1 7.0%, Y2 7.9%, Y3 7.5%, $p = 1.0$), so SLNM failed to reduce the diagnosis of stage IIIC. Despite this, it did reduce the need for LAD and the probability of surgical injury. In addition, Holloway et al. (79) found out that compared with LAD group (661 cases), SLN + LAD group (119 cases) showed a higher DR of metastases (30.3% *vs.* 14.7%, $p < 0.001$), more stage IIIC cases (30.2% *vs.* 14.5%, $p < 0.001$). SLN was the only metastasis in 50% of lymph node positive patients, and the FNR was 2.8%. SLN + LAD improved the DR of lymph node metastasis (OR3.29, $p < 0.001$). Raimond et al. (81) recruited 304 patients, and the incidence of lymph node metastasis in SLN was three times higher than in non-SLN (16.2% *vs.* 5.1%, $p = 0.03$). Among SLN positive, 8.1% were detected by ultra-staging. Furthermore, SLNM exerted no impact on recurrence-free survival (RFS). Buda et al. (82) found out that, in the early-stage patients, the DR of positive pelvic lymph nodes in SLN group (145 patients) was higher than in LAD group (657 patiens) (16.7% *vs.* 7.3%; $p = 0.002$), including 80 type II EC, and there was no difference observed in 3-year RFS and mortality between the two groups.

The improved detection rate of metastases probably attributes to the application of ultra-staging pathology, which help find previously neglected metastases, and the identification of lymph nodes located outside the routine lymph node dissection area. As revealed by the FIRES studies, 17% of lymph node-positive patients were found in non-traditional sites (presacral, parametrial areas, and deep iliac) (64). Therefore, the improvement to DR of metastatic lesions may mitigate the false negative consequences of SLN.

SLNM Did Not Impair Survival Outcome

Although long-term follow-up studies and RCTs for the comparison of survival outcome between SLNM and LAD are lacking, current results showed promising results that SLNM did not compromise the survival prognosis of EC patients (47).

In the SENTI-ENDO study conducted by Darai et al. (83), the outcomes of 125 stage I-II EC patients were assessed. There was no difference observed in recurrence rate (12.6% *vs.* 28.6%; $p = 0.23$) and RFS between successful SLN detection group and failed group. There was no difference in RFS between lymph node metastasis group and non-metastasis group ($p = 0.23$). However, the adjuvant therapy in the study was not standardized and it is difficult to validate the accurate survival effect of SLNM. Eriksson et al. (84) applied two lymph node dissection methods to patients with low-risk EC in MSKCC and Mayo Clinic, respectively. MSKCC applied SLN algorithm (642 cases), and Mayo Clinic applied SLAD (493 cases). The results indicated that the DR of metastasis was higher in SLN group. The pelvic lymph node metastasis rate (including LVMD) was 5.1% and 2.6% ($p=0.03$), respectively, while the PAL metastasis rate was 0.8% and 1.0% ($p=0.75$), respectively. There was no difference in 3-year disease-free survival (DFS) (94.9% *vs.* 96.8%), despite that the adjuvant treatment rate in SLNM group was higher than in SLAD group (27.1% *vs.* 10.8%; $p < 0.001$). Similar studies have been carried out in two Italian institutions (82) and totally 802 patients with early-stage EC were included. After 30-month median follow-up, there was no difference observed in DFS ($p=0.396$) and OS ($p=0.394$) between SLNM group and SLAD group. How et al. (85) recruited 275 SLNM + LAD patients and 197 LAD patients for study, which revealed that in clinical stage I patients, there was neither difference in the incidence and type of adjuvant therapy between the two groups, nor difference in RFS. The recurrence rate of pelvic wall in SLNM + LAD group was lower (31% *vs.* 71%). The former exhibited a reduced pelvic wall recurrence rate by 68% (HR 0.32, $p=0.007$). The authors suggested that SLNM may be superior to LAD due to the removal of lymph nodes at a higher risk of metastasis. However, this study can only prove that SLNM + LAD reduced the recurrence rate compared with LAD alone, for which it can hardly prove the advantages of SLNM alone. Imboden et al. (7) concluded that SLNM offered a considerable balance between oncologic safety and perioperative morbidity in 275 early-stage, G1 or G2 patients, especially for LVSI-positive. As shown in a meta-analysis recently published by Bogani et al. (2), compared with LAD, SLNM exhibited no difference in recurrence rate and PAL metastasis. In addition, A cohort study with 5546 patients published by Polcher et al. (86) indicated that LAD failed to improve DFS or OS compared with SLNM. On the contrary, it resulted in more complications in the high-risk histology type. The most recent multi-institutional retrospective study performed by Bogani et al. (87) compared the long-term oncologic results of SLNM, SLNM+LAD and LAD. The results found that there was no statistical difference between the three strategies in DFS ($p=0.570$) and OS ($p=0.911$); moreover, the survival outcome was similar in low risk, intermediate risk, and high-risk group. Kogan et al (88) compared 193 EC patients with LAD and 250 patients with SLN+LAD. They found that SLN may improve the oncologic outcome with a more favorable 6-year OS (HR 0.5, 95% CI 0.3-0.8, $p = 0.004$) and PFS (HR 0.6, 95% CI 0.4-0.9, $p = 0.03$). Also, SLN seemed to reduce the risk of recurrence in pelvis or lymph node region with a 6-year RFS of 95% compared to 90% ($p=0.04$) in LAD only group. Recently, Jayot et al. from France analyzed 248 EC patients between 2007 and 2018 undergone SLN procedure, as a result, the 3-year OS was 99% and 3-year RFS was 92% (89).

SLNM Reduced Intraoperative and Postoperative Complications

The most common complication of LAD was lymphedema, followed by lymph cysts, vascular and nerve injury, blood loss, and prolonged operation, etc. It seems that these risks can be reduced with the application of SLNM. Accorsi et al. (90) found that compared with TH, SLNM did not increases the incidence of intraoperative complications (p=1.0) and postoperative complications (p=0.782). While LAD laid more risk on intraoperative complications (HR, 14.25;95% CI, 1.85–19.63), postoperative complications (HR, 3.11; 95% CI, 1.62–5.98), and lower-extremity lymph edema (HR, 8.14; 95% CI, 1.01–65.27). Geppert et al. (91) drew comparison of the perioperative outcomes for TH + BSO, TH + BSO + SLN, and TH + BSO + LAD groups. The average operation time of SLN group and LAD group was found to be extended by 33 and 91 min, respectively. The incidence of lower limb lymphedema in SLN group was significantly lower than in LAD group (1.3% *vs.* 18.1%; p=0.0003). The same result was shown by Persson et al. (72, 92), in which SLNM reduced the risk of lower extremity lymphoedema by 14 times. In addition, Liu et al. (93) found that SLNM group significantly reduced the incidence of postoperative complications (5.2% *vs.* 13%; p=0.04), decreased intraoperative blood loss (56 ml *vs.* 80 ml; p=0.004), and shortened the operation time (137 min *vs.* 181 min; p <0.0001), meanwhile, the average number of lymph nodes dissected was significantly decreased (4 *vs.* 15; p <0.0001). When comes to lymphedema and lymph cyst, MSKCC concluded that SLN mapping was an independent factor in reducing patient reported lower-extremity lymphedema, while high BMI and adjuvant EBRT were associated with increased lymphedema (94). While another research stated that systemic LAD was the only factor that associated with the presence of lymphocele, the number of dissected nodes showed no impact. Compared with SLN+LAD group, SLN only group significantly decreased lymphocele rate from 14.1% to 3.4% (p=0.009) (95). Mayo Clinic analyzed 378 patients and found that SLN may significantly decrease the risk of lymphedema compared with LAD (26.0% *vs.* 49.4%, p<0.001) (96). Several meta-analyses included current retrospective and prospective studies presented similar conclusions, which was SLN resulted in less blood loss, lymphedema, and other complications, meanwhile, SLN detected more pelvic metastasis (97, 98). These results may indicate that SLNM is able to minimize the surgical risk and reduce the complications with no survival detriment in EC, which is of great value to improve the quality of life.

THE APPLICATION OF SLNM IN EARLY-STAGE HIGH-RISK EC

Nowadays, it is trending to carry on SLNM in early-stage high-risk EC, including high-risk histology (G3 endometroid, serous carcinoma, clear cell carcinoma, and carcinosarcoma), deep myometrial invasion, cervical involvement, and LVSI (+). Some institutions are making attempt to apply SLNM as routine surgical staging in all EC patients, except for patients with suspected lymph-node metastasis or failed mapping. Previous studies are typically performed on early-stage EC patients with mostly patients with lower-risk of recurrence and fewer higher-risk included. Recent studies attempted to evaluate the diagnostic accuracy and oncologic safety of SLNM in early-stage high-risk patients only. Though there are no RCTs published yet, existing evidence indicates that SLNM may be also efficient and safe in high-risk group, MSKCC has already established SLNM as a routine procedure for all candidate patients, including serous and carcinosarcoma type. However, it is essential to choose appropriate indication and strictly comply with SLN algorithm when using SLNM in high-risk patients (10).

The potential diagnostic value of the SLNM in high-risk patients has been proven in recent years. The DR ranges from 73% to 100%, the BDR varies from 56% to 95%, and NPV ranges from 93% to 100% (5, 42, 53, 72, 92, 99–107). Both SENTI-ENDO study and FIRES studies include low-risk and high-risk EC and present high DR and NPV (64, 83). There have been studies only including high-risk patients to evaluate the diagnostic value (**Table 1**). Frumovitz et al. performed study on 18 high-risk EC patients in 2007. The SLN DR was merely 45% (108), which may be the result of technique and surgeon experience. Then Torne et al. operated SLNM+LAD+PALAD on 74 high-risk patients in 2013, while the DR, sensitivity, and NPV were 74.3%, 92.3%, and 97.7% (53), respectively. Subsequently, in 2015, Farghali et al. showed a 73.1% DR, 94.4% sensitivity, and 100% specificity in 93 high-risk patients (100). In 2016, Ehrisman et al. demonstrated an increase from 92.3% to 100% in NPV by applying SLN algorithm to 36 high-risk EC patients (101). In 2017, plenty of constructive research results were obtained. For example, Soliman et al. performed SLNM+LAD+PALAD under 123 high-risk patients. Nineteen percent of the patients diagnosed with stage III exhibited DR, sensitivity, and FNR of 89%, 95%, and 4.3% (103), respectively. Baiocchi et al. included 236 high-risk EC patients. As a result, the SLN arm has a sensitivity of 90%, an NPV of 95.7%, and an FNR of 4.3%. Besides, the positive lymph node DR is significantly increased in SLN group compared with LAD group (26.7% vs. 14.3%, p=0.02) (106). In the same year, a multi-institutional research was conducted by Touhami et al., who performed SLNM+LAD +/−PALAD in 128 high-risk EC patients (including undifferentiated type). They found out that the sensitivity and NPV of SLNM were 95.8% and 98.2%, respectively (104). Furthermore, in 2018, Papadia et al. conducted analysis of 42 high-risk patients (including neuroendocrine cancer). They reported that the DR and BDR of SLN were 100% and 90.5%, respectively. Excitingly, the sensitivity and NPV were both 100% (42). Sweden teams performed robotic surgery on 257 stage I-II high-risk EC patients, resulting in a sensitivity of 100% and a NPV of 100%. The BDR was as high as 95%, and no adverse effect occurred (72). Wang et al. recently published their data and found a DR of 86.7% and FNR, NPV and sensitivity was 11.8%, 97.3% and 88.2% respectively. When considering SLN algorithm

TABLE 1 | The diagnostic value of SLNM in high-risk EC.

Author	Year of publish	Country	Study type	Study period	Number of pts	Histology	SLN method (dye and injection site)	Surgery approach	Overall DR	BDR	PASDR	Sensitivity	NPV	FNR
Burke et al. (30)	1996	USA	pilot	NA	15	EEC(G2,G3), CC, USC	BD; subserosal, myometrium	Lpt	67%	NA	NA	66.70%	87.50%	33.30%
Frumovitz et al. (108)	2007	USA	pro	2002-2004	18	EEC(G2,G3), CC, USC	BD, Tc; Fundus	Lps	45.00%	5.56%	22.22%	NA	NA	NA
Torne et al. (53)	2013	Spain	pro	2006.03-2011.03	74	EEC(G3),CC,USC,DM, CI	Tc; TUMIR	Lps	74.30%	14.00%	45.40%	92.30%	97.70%	7.70%
Perissinotti et al. (99)	2013	Spain	pro	2007.06-2010.12	44	EEC(G3),CC,USC, USM,DM	Tc; TUMIR	Lps	73.00%	NA	NA	NA	NA	NA
Farghali et al. (100)	2015	Egypt	pro	2007.05 -2011.05	93	EEC(G2,G3), CC, USC	BD; subserosal, myometrium	Lpt	73.10%	40.86%	0.00%	94.40%	98.90%	5.88%
Valha et al. (109)	2015	Czech	pro	2012.06-2014.02	18	stage I-II, intermediate and high-risk	BD; subserosal	Lpt	88.89%	NA	50.00%	NA	NA	NA
Ehrisman et al. (101)	2016	USA	retro	2012.08-2015.06	36	EEC(G3),CC,USC, CSM	BD,ICG;cervical	Lps,Rb	83.00%	56.00%	3.00%	77.80%	92.30%	22.22%
Baiocchi et al. (106)	2017	Spain	retro	2007.06-2017.02	236(75 SLN +LAD; 161 LAD)	EEC(G3),CC,USC, CSM,DM,LVSI	BD; cervical	Lps,Rb, Lpt	85.30%	60.00%	1.50%	90.90%	95.7%,	10.00%
Tanner et al.J (110)	2017	USA	retro	2012.12-2015.12	52	EEC(G3),CC,USC, CSM	BD,ICG;cervical	Lps,Rb	86.00%	59.60%	9.00%	77.80%	94.70%	22.20%
Soliman, PT (103)	2017	USA	pro	2013.04-2016.05	101	EEC(G3),CC,USC, CSM,DM,CI	ICG, BD, BD+Tc; cervical	Lps,Rb, Lpt	89.00%	58.00%	2.00%	95.80%	98.20%	5.00%
Touhami et al. (104).	2017	Canada	retro	2010.11-2016.11	128	EEC(G3),CC,USC, CSM,undifferentiated	BD, Tc, ICG; cervical	Lps,Rb, Lpt	89.80%	63.20%	5.00%	97.43%	98.80%	2.56%
Ducie et al. (107)	2017	USA	retro	2006–2013	120	EEC+any grade+DM; USC, CC	BD, ICG; cervical	NA	NA	NA	NA	96.40%	98.90%	3.60%
Buda et al. (111)	2018	Italy, Switzerland	retro	NA	171	ESMO high-intermediate and high risk	ICG, Tc+BD; cervical	NA	98.00%	80.1%(ICG); 65.7%(BD,Tc)	NA	85.2%; 91.2% for algorithm	93.4%;96% for algorithm	14.7%;8.8% for algorithm
Papadia et al. (42)	2018	Switzerland	retro	2012.12 - 2017.07	42	EEC(G3),CC,USC, CSM,NEC	ICG; cervical	Lps	100%	90.50%	NA	90%;100% for algorithm	97%;100% for algorithm	10%;0% for algorithm
Persson et al. (72)	2019	Sweden	pro	2014.06-2018.05	257	EEC(G3),non-EEC, DM, CI, non-diploid cell	ICG; cervical +/-reinjection	Rb	NA	82%; 94.8% after reinjection	NA	98%; 100% for algorithm	99.5%;100% for algorithm	3.7%;0% for algorithm
Wang et al. (105)	2019	China	retro	2016.08-2018.08	98	EEC(G3),CC,USC, CSM,EEC(G1,G2) +DM,CI	ICG; cervical	NA	95.92%	77.60%	NA	88.2%; 90.9% for algorithm	97.47%; 97.30% for algorithm	11.8%; 9.1% for algorithm
Ye et al. (112)	2019	China	pro	2016.07-2018.07	131 pts with 25 high-risk	EEC(G3),CC,USC, CSM,undifferentiated	ICG; cervical	Lps	100%	72%	NA	20%	83.30%	80%
Angeles et al. (76)	2020	Spain	pro	2006.03-2017.03	123	intermediate and high-risk EC	TUMIR	NA	70.70%	NA	NA	NA	NA	NA
Taskin et al. (113)	2020	Turkey	retro	2017.05-2018.11	38	high-risk (Mayo criteria)	ICG; cervical	Lps,Rb, Lpt	84.21%	68.40%	NA	80%	93.40%	NA

pts, patients; SLN, sentinel lymph node; LAD, lymphadenectomy; DR, detection rate; BDR, bilateral detection rate; PAS, para-aortic SLN; NPV, negative predictive value; FNR, false negative rate; NA, not applicable; EEC, endometrioid endometrial cancer; G, grade; CC, clear cell carcinoma; USC, uterine serous carcinoma; CSM, carcinosarcoma; DM, deep myometrial invasion; CI, cervical involvement; BD, blue dye; Tc, Technetium-99; TUMIR, transvaginal ultrasound-guided myometrial injection of radiotracer; Lpt, laparotomy; Lps, laparoscopic; Rb, robotic surgery; pro, prospective; retro, retrospective.

and surgical experience (over 30 cases), the FNR and NPV increased (105). Thus, SLNM seems to be feasible in high-risk context with an acceptable DR and diagnostic value. However, Ye et al analyzed 131 patients using ICG and SLNM followed by LAD (112). The sensitivity and NPV were unexpectedly as low as 20% and 83.3%, with a surprisingly high FNR of 80%. The author considered the risk of missing IPL of SLNM in high-risk patients may be the reason, a large-scale multicenter study was needed to clarify the result.

Moreover, prospective and retrospective studies indicated that SLNM appears to have no negative impact on oncologic outcomes in high-risk EC patients (**Table 2**). MSKCC conducted a retrospective analysis of 136 patients with uterine carcinosarcoma in 2016 (114). The result showed that there was no difference in PFS (23 *vs.* 23.2 months; p=0.7) and detection of metastatic lymph nodes (p=0.2) between SLN group and LAD group. Local recurrence rate was 15% in SLN cohort and 24% in LAD cohort, which is consistent with previous study conducted by How et al. (85). Subsequently, in 2017, MSKCC retrospectively evaluated 248 patients with uterine serous carcinoma. No difference was observed either in the diagnosis of stage III/IV, adjuvant therapy rate, and 2-year PFS between SLN group and LAD group. However, the incidence of local recurrence was 9.7% and 9.1% in SLN group and LAD group (115). The exact effect of SLNM and related adjuvant therapy on local recurrence control needs to be further investigated. The same histology type was further and thoroughly reviewed by MSKCC in a recent paper published by Basaran et al. (118). This time, they carefully categorized uterine serous carcinoma patients in January 1996 to December 31, 2017 into SLN only group (79) and LAD without SLN group (166). The two cohorts showed no survival difference in stage I to III uterine serous carcinoma as they yielded similar detection of nodal metastasis. Also, PALND did not show any survival benefit on OS. Moreover, MSKCC and Mayo Clinic investigated 176 deeply invasive endometrioid EC in 2018. When other factors were balanced, the PFS, OS, and recurrence rate exhibited no difference (117). Additionally, in 2018, Buda et al. reported an Italian multicenter study, which included 266 high-risk patients. The 3-year DFS and OS showed no difference in SLN group and SLAD group (116). In the same year, Buda et al. published data obtained from Italian and Swedish multicenter of 171 high-risk EC patients. The 5-year DFS indicated no difference among SLN group and SLAD group (111). The impact of SLNM on clear cell carcinoma was investigated by Mayo Clinic and MSKCC (119). The researcher included early stage serous or clear cell endometrial carcinoma with any degree of myometrial invasion. The results showed that SLNM cohort (118 patients) did not increase lymphatic recurrence and exhibit a similar OS (88% *vs.* 77%, p=0.06) with LAD cohort (96). However, in node-negative cases, SLNM group may be associated with decreased RFS (73% *vs.* 91%, p=0.05), despite the majority of SLNM patients received chemotherapy (84% *vs.* 40%, $p < 0.001$). Most recently, Bogani et al. compared SLN alone and SLN followed by LAD (121) in 196 high-risk patients (121). The two groups showed no difference in DFS (p = 0.416) and OS (p = 0.940) despite that LAD removes more positive nodes.

However, it is noted that only a few intuitions perform SLN-algorithm only in the SLN cohort for the comparison study, whereas others are more likely to perform LAD followed by SLNM, thus, the results are rather a comparison between SLN +LAD and LAD, which make the survival results less convincing and more complicated. The role of backup LAD for high-risk cases remains areas of investigation. Also, there are studies addressing the problem and comparing the oncologic outcomes between SLN and more extensive LAD with or without SLN, preliminary results suggested that there are no difference in these approaches (87, 118).

These results may indicate that the application of SLNM in high-risk EC patients is as efficient and safe as in the lower-risk type, for accurate staging, thus guiding adjuvant therapy, suggesting SLN may be an optimal choice for high-risk patients. However, the effect is attributed to the adjuvant therapy based on lymph node status or eradication of lymph node metastases directly is unclear, since earlier studies did show a survival benefit for patients did systemic LAD with an average of 12 lymph nodes moved (122), and the current favorable studies are limited by its prospective or retrospective nature. Lack of RCTs, long-term follow-up studies, standardized SLNM technique, and ultra-staging protocol, as well as adjuvant therapy are the primary concern. In an ideal clinical research, the patients should be randomly assigned into SLNM arm or LAD arm, and receive standard post-operative adjuvant therapy according to stage information (40). It is plausible to add LAD, particularly PALAD, in high-risk group before high-quality evidence is published.

CURRENT APPLICATION OF SLNM

SLNM is gaining widespread utilization for staging in EC. It was first written in the NCCN guideline since 2014. And for now, FIGO and NCCN all support the utilization of SLNM in apparent uterine-confined EC despite lack of RCTs. Studies have proven that SLNM with ultra-staging may be effective in providing prognostic information for regional lymph node, choosing adjuvant therapy, and reducing operation complications.

NCCN recommends the application of SLNM in EC patients with lesions apparently confined to the uterine cavity without any extra-uterine metastases on imaging examination. Meantime, NCCN also permits the potential use of SLNM in early-stage high-risk EC patients like serous carcinoma, clear cell carcinoma and carcinosarcoma (123). Surgeons must strictly follow the technical details and SLN algorithm in operation, including superficial and deep injection of cervix, thorough evaluation of abdominal and pelvic cavity, resection of all SLN and suspicious enlarged lymph nodes, additional LAD on unmapped side when SLN mapping failure occurs and ultra-staging pathology is performed in combination with routine H&E. Whether to perform PALAD is at the discretion of the

TABLE 2 | The oncologic outcomes of SLNM in high-risk EC.

Author	Year of publication	Country	Study type	Time period	Patient group (N)	Histology	LN positive rate	p value	DFS	p value	OS	p value	Distant recurrence rate	p value
Schiavone et al. (114)	2016	USA	retro	1998.01-2014.08	SLN-A(48) LAD(88)	USM	22.90% 21.59%	p=0.4	23m(2y) 23.2m	p=0.7	NA NA		70% 74%	NA
Ducie et al. (107)	2017	USA	retro	SLN (2006–2013) LAD (2004–2008)	SLN-A(120) SLAD(103)	EEC: any grade, MI>50%; USC/CC, any MI.	21.70% 19.40%	p=0.68	NA NA		NA NA		NA NA	
Schiavone et al. (115)	2017	USA	retro	2005.01-2015.07	SLN-A(153) LAD(95)	USC	31% 38%	p=0.3	77% 71%	p=0.3	NA NA		15.03% 23.16%	NA
Baiocchi et al. (106)	2017	Spain	retro	SLN (2007.06-2017.02) LAD (2012.11-2017.02)	SLN+LAD(75) LAD(161)	EEC(G3), CC, USC, CSM, DM, LVSI	26.70% 14.30%	p=0.02	NA NA		NA NA		NA NA	
Buda et al. (111)	2018	Italy, Switzerland	retro	NA	SLN-A(66) SLN+SLAD (105)	High-intermediate and high-risk	27.30% 32.40%	p=0.297	79.20% 81.60%	p=0.831	NA NA		0 0.95%	NA
Buda et al. (116)	2018	Italy	retro	2010.10-2014.02	SLN(61) LAD(139)	High-intermediate and high-risk	16.70% 7.30%	p=0.002	HR: 0.92 (3y)	p=0.646	HR: 0.92 (3y)	p=0.675	NA NA	
Schlappe et al. (117)	2018	USA		SLN (2005–2013) LND (2004–2008)	SLN-A(82) LAD(94)	DM EEC	33.30% 14.80%	p=0.005	adjusted HR:0.87	NA	adjusted HR:2.54	NA	20.80% 14.90%	NA
Basaran et al. (118)	2020	USA	retro	1996.01-2017.12	SLN alone(79) LND without SLN (166)	USC	26.50% 29.50%	p=0.6	58.8%(2y) 64.9%(2y)	p=0.478	89.1%(2y) 83.9%(2y)	p=0.9	36.7%* 40.9%*	p=0.524
Schlappe et al. (119)	2020	USA	retro	2006- 2013 2004- 2008	SLN(118) LND(96)	USC/CC with any MI	21.70% 20.50%	p=0.83	68.9%(3y) 80.3%(3y)	p=0.32	87.9%(3y) 76.8%(3y)	p=0.06	NA	
Nasioudis et al. (120)	2020	USA	retro	2012-2015	SLN(460) LND(920)	EEC(G3) and non-EEC	10.5% 13.30%	p=0.10 NA	NA NA		84.3%(3y) 86.8%(3y)	p=0.86	NA	
Bagoni et al. (121)	2021	Italy	retro	2009.01-2019.12	SLN(50) SLN+LAD (146)	EEC(G3) with MI >50% and non-EEC	28% 23.20%		NA NA	p=0.416	NA NA	p=0.940	16% 12%	0.413

The data refers to all types of recurrence.

N, number; LN, lymph node; DFS, disease-free survival; OS, overall survival; pro, prospective; retro, retrospective; SLN-A, SLN-algorithm; LAD, lymphadenectomy; EEC, endometrioid endometrial cancer; G, grade; CC, clear cell carcinoma; USC, uterine serous carcinoma; CSM, carcinosarcoma; MI, myometrial invasion; DM, deep myometrial invasion; LVSI, lympho-vascular invasion; m, months; y, year; NA, not applicable; HR, hazard ratio.

surgeon (48). While in the latest consensus and surgical assessment tool, which aims to standardize the surgical technique and quality of SLNM in EC, it also recommends cervical injection of ICG, however, when mapping failure occurs, it points out 4 choices: waiting and turning to contralateral side, exploring the uncommon regions like presacral, common iliac or para-aortic area, re-injection of tracer, or performing side specific LAD (61).

Moreover, the application of FS of SLN is in debate due to the low sensitivity, expensive price, and the propensity to neglect LVMD (10, 26). However, Tanner et al. (110) argued that it was plausible to add FS when SLN map failure occurs, which was called "reflux FS", as it could reduce the need for LAD based on uterine factors by decreasing the rate from 18.6% to 7.1%. Besides, they recommended a direct LAD instead a reflux FS for high-risk EC. Similar results were obtained by Sinno et al. (27) and Altin et al. (124). Thus, NCCN guideline suggests that secondary SLAD may be considered in the cases of failed SLN mapping (125). In addition, Renz et al. (126) from Stanford University and Bellaminutti et al. (127) from Switzerland found that adding intraoperative FS to SLN can find micrometastases with a good accuracy, and NPV, thus, may identify patients who are in need for a systemic LAD for dissecting additional lymph node metastases.

At present, the application of SLNM is gradually expanding, and more than 70% of patients may be suitable for SLNM (50). Recent surveys from ESGO and SGO confirmed that 50.2% (128) of European gynecological oncologists and 82.7% (129) of USA gynecologic oncologists adopted SLN in EC. In low-risk patients, who usually do not have to perform LAD, there are 2.4% lymph node metastatic potential (5), especially in LVSI positive patients (7). Additionally, LVMD is more likely to occur in low-risk patients (130). SLNM can remove fewer lymph nodes with sufficient staging information supporting adjuvant therapy, and will not cause the possibility of post-operative complications to increase compared with hysterectomy alone (7). In high-risk patients who should undergo LAD, approximately 80% (5, 107) of them do not have lymph node metastasis. Moreover, it is difficult for obese patients and patients with severe internal complications to tolerate LAD. Also, adjuvant therapy can eliminate obscured metastases that are not found in surgery theoretically as supported by many clinical trails (131), which showed that concurrent chemoradiotherapy can significantly extend PFS and OS in advanced EC patients. Moreover, SLNM improves the detection of metastases by identifying LVMD with assistance of ultra-staging and identifying lymph nodes in non-regular region, which is significant for accurate staging and choosing adjuvant therapy. Despite lack of RCTs and long-term follow-up studies, existing evidence advocate the utilization of SLNM in uterine-confined EC even in high-risk histology because of sufficient detection rate of SLN and nodal metastases, and similar survival outcome compared with conventional LAD. It is worth expecting long-term survival outcome, cost-performance, and complication incidence of SLNM in early-stage EC patients in ongoing randomized clinical trials.

CONTROVERSIAL ISSUES

Pathological Ultra-Staging and LVMD

Pathological assessment methods for lymph nodes include H&E and IHC staining. Ultra-staging is a combination of serial section and IHC (anti keratin AE1:AE3) to identify the LVMD (10, 15, 66). The standard set by SGO about LVMD is based on breast cancer guidelines published by AJCC (132): macro-metastasis (> 2 mm); low-volume metastases (LVM), including micro-metastasis (MM) (0.2–2 mm) and isolated tumor cells (ITCs) (< 0.2 mm). AJCC (133) set term pN0 (i+) for ITCs and pN1mi for MM in breast cancer. In NCCN guideline, pN0 (i+) is set for ITCs in EC patients (125). A more accurate staging may be needed to guide further personalized adjuvant therapy and evaluate prognosis.

There is no standardized protocol for ultra-staging yet. MSKCC (134) divides H&E-negative SLN into two levels (50 μm apart). Then, if the previous one remains H&E negative, two consecutive 5-μm thick sections are sliced at every level, one for H&E and the other for IHC (**Figure 2A**). M.D. Anderson Cancer Center (135) cut three serial 250-μm-thick sections for lymph node which has a negative H&E, with one repeating H&E. If it is still negative, the other two slices undergo IHC (**Figure 2B**). As indicated by reports, there is no difference between two kinds ultra-staging on the detection of SLN metastases for both high-risk and low-risk EC patients (26, 136).

The incidence of LVMD varies approximately from 3.8% to 19.7% (10, 62, 79, 130, 134, 137, 138) However, the LVMD

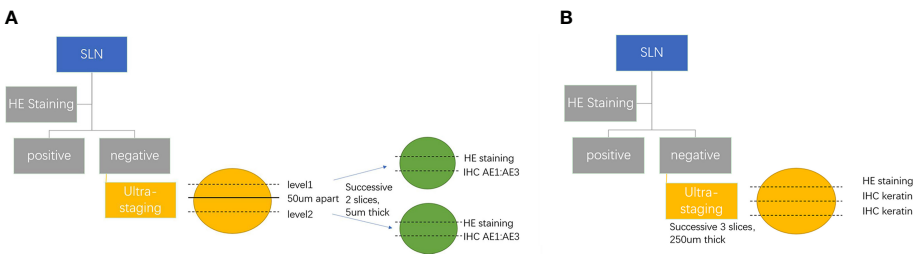

FIGURE 2 | **(A)** MSKCC SLN ultra-staging protocol. **(B)** M.D. Anderson Cancer Center SLN ultra-staging protocol.

detected by ultra-staging accounts for almost 50% of all lymph node metastases. The risk factors related with LVMD are LVSI, unfavorable histology, myometrial invasion, and so on. Yabushita et al. (139) figured out the relevance between LVSI and the positive expression of keratin in IHC staining. The author stated that keratin positive is the independent risk factor for recurrence. Todo et al analyzed 61 EC patients with intermediate risk for recurrence (140). The results showed a 14.8% incidence of LVMD and deep myometrial invasion was significantly associated with ITC/MM (p=0.028). Bogoni et al. (130) hold that LVMD is more likely to be detected in low-risk patients. However, research done by Mueller et al. concluded that ITC incidence increased with depth of myoinvasion. Twenty-five percent of deeply invasive G1/G2 and 18% of deeply invasive G3 tumors had ITCs compared to a rate lower than 1% in non-invasive endometroid EC patients. When coming to non-invasive serous type, the incidence for ITC goes up to 10% (141).

Though the clinical significance of LVMD remains under investigation, more stage IIIc patients are diagnosed by ultra-staging and 5% to 15% patients face upstaging (134). Whether MM or ITC need adjuvant therapy and indicate better or worse prognosis are conflicting. Recent data tend to consider patients with MM for a following adjuvant therapy, whereas patients with ITCs do not. Todo et al. (140) concluded that LVMD was an independent risk factor for extra-pelvic recurrence. Compared to node-negative patients, a noticeable 20% decrease was observed in 8-year OS and PFS in LVMD patients. However, no statistical difference was calculated. MSKCC (142) reported a large cohort study that 5.2% patients had LVMD and 5.6% patients found macrometastases. As a result, the LVMD group shows a significant increase in 3 year-RFS compared with the macro-metastases group (86 vs. 71%, p <0.001), as most LVMD receive adjuvant therapy. Plante et al. (143) published a single center prospective study involving 519 EC patients. The 3-year PFS was 95.5% for ITCs, which was similar to MM (85.5%) and lymph node negative (87.6%) and much better than macro-metastases (58.5%). Brugger et al. (50) found out that patients with ITC and MM received more adjuvant therapy and presented much better oncologic outcomes. A recent review published by Bogani et al. believed that the patients with MM detected in SLN should receive adjuvant therapy, whereas whether ITC undergoes adjuvant therapy depends on uterine factors (130). The similar conclusion was reached by Goebe et al, in which they sent 155 SLN negative patients tissue slides into IHC staining retrospectively (144). Even though 13.5% of SLN negative patients found ITCs, no recurrence was found in patients had previously undetected ITCs without receiving adjuvant therapy as well, suggesting that ITCs may not be relevant to recurrence risk. However, Sawicki et al. (145) stated that LVMD are independent of histology type, myometrial invasion, LVSI and cervical invasion and they does not affect prognosis. It is noted that in breast cancer, though LVMD is recorded in staging, they do not influence the treatment decision for they do not change survival (126).

In addition, ultra-staging improved the detection of nodal metastasis to two times compared with normal H&E, and interestingly half of positive lymph nodes are SLN (79, 81). SLN may have an advantage in identifying LVMD. Niikura et al. (146) obtained a 5% of LVMD in SLN, compared to merely 0.3% in non-SLN. FIRES study (64)also indicated that SLN is more likely to identify metastases than non-SLN (5% *vs.* 1%, p=0.0001). Moreover, compared with traditional LAD, which removes over 20 lymph nodes, SLNM, which removes less than four lymph nodes in most papers, reduces the workload and makes ultra-staging more feasible for pathologists. SLNM permits a possibility that pathologist could pay attention to fewer lymph nodes. However, it is noted that most institutions only perform ultra-staging on SLN but non-SLN due to many factors, which may underestimate the incidence of LVMD in non-SLN.

Nevertheless, ultra-staging owns such limits, which is time-consuming, that cannot be done intraoperatively, whereas intraoperative FS seems to be low sensitivity in identifying LVMD and the discrepancy between pathologists and institutions. OSNA, which is one-step nucleic acid amplification, comes to the researchers' eyes. It is a molecular-based method for the detection of metastatic lymph nodes in breast cancer or colorectal cancer patients using CK19 as a single marker. Mounting evidence has demonstrated good sensitivity and specificity of OSNA in identifying positive nodes, especially micro-metastasis, in endometrial cancer (147–150). Compared with ultra-staging, OSNA is much faster thus can be done intraoperatively; moreover, it identified more SLN involvement, resulting in 20.69% of patients upstaged as FIGO stage III (150). The technique is autonomous and quantifiable, which saves pathologist's work and makes results more comparable and less variable (151). Also, the use of the entire lymph node avoids insufficient analysis of pathology, thus increasing the identification of metastatic lesions. However, one limit is the risk of false-positive cases as CK19 can also be expressed in normal endometrium (152), so developing new specific markers may be necessary. Also, the method needs an entire node which makes morphologic observation of metastatic features unachievable (147), as well as future research for other molecular testing (153). Moreover, the cost is almost 10 times higher than the current pathology examination (147) and the best cutoff value for identifying macro-metastasis and LVMD, as well as predicting non-SLN involvement in EC may need further investigation (154).

Aortic Lymph Node Dissection

Anatomical study has proven that EC can directly metastasize into PAL through pelvic-infundibular ligament pathway. Currently, the dissection of para-aortic area is left to the surgeon's decision based on NCCN SLN algorithm. The possibility of missing occult PAL metastasis, especially IPL metastasis, is one of the primary concerns of SLNM. But existing evidence shown that the incidence of IPL metastasis is rare with approximately 0.5% to 3.8% (49, 51). Chiang et al. (155) summarized 18 papers and concluded that the incidence of IPL metastasis is as low as 1.7%. Kumar et al. (21) demonstrated that lymph node metastatic rate for para-aortic region and pelvic cavity is similar (12% *vs.* 17%). When pelvic lymph nodes are

positive, 51% have PAL metastases. Whereas when pelvic lymph nodes are negative, PAL metastases, namely IPL metastasis, are found in 3% patients. Usually, patients with positive pelvic lymph nodes would receive adjuvant therapy, which could eliminate the possible aortic lesions in theory, for SLNM with ultra-staging has an excellent ability to detect pelvic metastasis with high sensitivity and NPV. Also, researchers have developed strategies like "dual site injection (156)" or "reinjection (72)" to increase the detection of aortic SLN to reduce FNR. Researchers from Korea showed that a sequential administration of bilateral uterine cornus injection of ICG followed by cervical injection, improved the para-aortic SLN detection rate from 5.7% to 38.2% in upper para-aortic area ($p<0.001$) and 18.7% to 67.1% in lower para-aortic area ($p<0.001$), which in turn identified more metastatic SLN in aortic area (7.9% vs. 2.4%) ($p=0.070$) (157). Researchers from Italy and Turkey suggest the addition of preoperative PET-CT in favor of PALAD decision (111, 113, 158). Taskin included 38 high-risk patients. Though SLN algorithm had a 100% sensitivity and NPV in finding the pelvic metastases, the IPL metastases were only detected by PET-CT. Risk factors associated with PAL metastases are reported to be type II EC, pelvic lymph node metastases, deep myometrial invasion (≥1/2) and LVSI, thus, PALAD based on these risk factors may be reasonable choice. It is noted that the detection of metastatic PAL was similar between SLN group and LAD group even in high-risk histology type EC (106, 117), which indicates that SLNM does not compromise the detection of PAL metastases in high-risk patients.

Moreover, the survival benefit of PALAD remains controversial. SEPAL study indicated that PALAD failed to affect the prognosis in low-risk patients, despite a positive impact on intermediate and high-risk patients (12); however, CART analysis conducted by Barlin et al. (159) stated that PALAD bears no relation to OS in EC patients. Whether the oncologic outcome is influenced by removing metastases directly or by personalized adjuvant therapy like radiotherapy extent based on lymph node status is unclear. Some believe that PAL metastases may be eradicated by adjuvant therapy dependent on accurate staging, which has shown to be an advantage of SLNM, which was found to detect more stage IIIC patients despite fewer lymph nodes dissected than extensive LAD.

Non-SLN Metastasis

A major challenge in implementing SLNM lies in the potential of residual metastasis of non-SLN. Retrospective data have reported an incidence of 35% to 40% of non-SLN metastasis (64, 138). The risk of non-SLN metastasis is associated with the size of SLN metastasis and uterine higher-risk factors (160). Touhami et al. (138) found out that 60.8% of non-SLNs were positive when SLN was found to harbor macro-metastases. Otherwise, only 5% non-SLN was positive when SLN had LVMD. Similar results were reached by Biocchi, 54.5% macrometastasis and 15.4% micrometastasis were found non-SLN involvement, whereas in patients with ITCs in SLN, no metastasis was found in non-SLN (161). Turkish Gynecologic Oncology Group showed that one third SLN positive had non-SLN metastases, and the ratio increases to two thirds when SLN involvement was macrometastasis (162). Although non-SLN metastases could be controlled by adjuvant therapy and the promising results of high-risk EC patients support the hypothesis, the appropriate management of non-SLN is still worthy of further studies. Therefore, it is essential to strictly follow SLN algorithm, carefully evaluate non-SLN, and remove all suspicious enlarged lymph nodes. Further studies should be carried on to evaluate the effect of leaving metastatic non-SLNs in-situ.

FUTURE DIRECTIONS

In summary, quantities of studies indicated that SLNM may be a safe and effective alternative for lymph node assessment in apparently uterine-confined EC with a sufficient diagnostic accuracy and similar survival prognosis even in unfavorable histology types, thus it is gaining widespread acceptance to perform SLNM in EC patients. However, the lack of convinced evidence like RCTs and long-term follow-up data limit its utilization. Further investigations should be focused on the oncologic outcomes of SLNM and the clinical relevance of LVMD on adjuvant therapy. Better standardization of SLNM protocol, surgical training program, and ultra-staging technique are also needed. Besides, further improvement in the diagnostic accuracy and therapeutic safety of SLNM are in urgent need to provide more personal and minimal-invasive treatment for EC patients and make a difference to their prognosis.

AUTHOR CONTRIBUTIONS

LZ drafted the manuscript. LZ was responsible for the planning and carrying out the study. LZ and XZ reviewed the literature and summarized the data. MC carefully revised the manuscript. JW was responsible for the conceptualization and final review of this manuscript. All authors contributed to the article and approved the submitted version.

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Safety and Benefit Of Sentinel Lymph Nodes Biopsy Compared to Regional Lymph Node Dissection in Primary Vulvar Cancer Patients Without Distant Metastasis and Adjacent Organ Invasion: A Retrospective Population Study

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Background: The safety and benefit of sentinel lymph node biopsy (SLNB) compared with regional lymph node dissection (RLND) and no lymph nodes removed (NA) in patients with vulvar squamous cell cancer (VSCC) was not well studied.

Methods: A retrospective analysis on VSCC patients without distant metastasis and adjacent organ invasion from the Surveillance, Epidemiology, and End Results Program database between 2004 and 2016 was carried out. Within subgroups stratified by negative (LN–) or positive (LN+) regional lymph node findings, inverse probability weighting (IPW) adjusted multivariate Fine-Gray compete risk (CR) model and accelerated failure time (AFT) model was used to investigate the factors associated with and cancer-specific survival (CSS) and overall survival (OS).

Results: Of the 3,161 VSCC patients treated with surgery, 287 (9.1%) underwent SLNB, 1,716 (54.3%) underwent RLND, and 1,158 (36.6%) had no regional lymph nodes removed. As illustrated by IPW adjusted multivariate regressions, SLNB was significantly associated with prolonged CSS (LN–, adjusted sub-proportional hazard ratio [sHR] = 0.42; 95% confidence interval [CI], 0.19–0.93; P=0.032; LN+, adjusted sHR = 0.29; 95% CI, 0.16–0.54, P<0.001) and OS (LN–, adjusted time ratio [TR] = 1.38; 95% CI, 0.82–2.32; P=0.226; LN+, adjusted TR = 2.68; 95% CI, 1.73–4.14; P<0.001), although the effect of SLNB on OS was not significant within the LN– cohort. Moreover, SLNB led to improved CSS (adjusted sHR = 0.40; 95% CI, 0.23–0.70; P = 0.001) and OS (adjusted TR=1.15, 95% CI 0.76-1.73, P=0.279) compared with NA. Age was a significant prognostic factor of CSS and OS, whereas tumor size, surgery type, and invasion depth were not.

Conclusions: SLNB leads to significantly prolonged CSS and OS in VSCC surgery patients without distant metastasis and adjacent organ invasion than RLND, except for

the similar OS in the LN– cohort. SLNB could be carried out preferentially for VSCC surgery patients without distant metastasis and adjacent organ invasion, irrespective of tumor size, surgery type, invasion depth, and regional lymph nodes metastasis. Further prospective clinical trials are warranted to confirm the findings of this study.

Keywords: regional lymph node dissection, sentinel lymph node biopsy, surgery, tumor size, vulvar cancer, age, invasion depth

INTRODUCTION

Vulvar cancer is a rare malignancy that accounts for about 5% of all gynecologic cancer cases, with more than 6,100 newly diagnosed cases yearly in the United States, leading to nearly 1,400 deaths (1). Ninety percent of vulvar cancers are squamous cell carcinomas (VSCC) (2, 3). Currently, the primary treatment for VSCC is surgical resection and radiotherapy (with or without chemotherapy) if necessary (2, 4).

Vulvar cancer usually spreads to regional lymph nodes, such as the inguinal, femoral, or pelvic lymph nodes. The more the regional lymph nodes were involved, the worse the long-term survival was (5, 6). Therefore, regional lymph nodes dissection (RLND), covering inguinal and femoral lymph nodes, was usually performed to remove lymph nodes for work-up or therapy intent. However, RLND has a high probability of short- and long-term complications that are the leading cause of death after surgical treatment, such as wound breakdown, wound infection, lymphoceles, lymphedema, cellulitis, and erysipelas (7). After implementing several new surgical techniques of lymph nodes dissection procedure, the morbidity of complications after RLND decreased in recent years but remains high and clinically meaningful (8). Thus, sentinel lymph nodes biopsy (SLNB) was preferred to replace RLND for selected VSCC patients because of its less aggressiveness and lower probability of surgery complications (9). Moreover, SLNB has been proven to have high sensitivity of more than 95% to indicate positive regional lymph nodes and a specialty of as high as 100% (9, 10). However, more than 50% of VSCC patients still received RLND alone because of the limited application of SLNB (7), which was led to by the limited evidence on the safety and effectiveness of SLNB because of the rarity of vulvar carcinoma.

Thus, it is urgent to identify the safety and efficacy of SLNB in VSCC surgery patients, especially in those with negative regional lymph node findings. Therefore, we compared the long-term survival between patients who underwent SLNB and those who underwent RLND or NA in a large real-world cohort, controlling for several factors.

MATERIALS AND METHODS

Data Source and Study Population

The Surveillance, Epidemiology, and End Results (SEER) Program database of the National Cancer Institute was retrieved to identify patients with primary vulvar carcinoma from 2004 to 2016. Patients with the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3) primary site code of C51.0, C51.1, C51.2, C51.8 C51.9, and the ICD-O-3 histology codes of 8050-8084 (as squamous cell carcinoma) were enrolled (11).

Moreover, patients were excluded by the following criteria: 1) not squamous cell carcinoma; 2) not the first primary tumor; 3) survival months <1; 4) age at diagnosis < 18 or >80 years; 5) tumor size <1 millimeter; 6) no surgery performed; 7) debulking; 8) surgery not otherwise specified; 9) surgery performed unknown; 7) Distant metastasis; 8) Adjacent organ invasion; 9) AJCC pathologic staging criteria violation; 10) Lymph nodes removed unknown; 11) Positive lymph nodes without dissection. In addition, Debulking was excluded because it is performed for palliative rather than curative intent.

The region, insurance status, year of diagnosis, age at diagnosis, race, marital status, primary site, pathological grade, tumor size, invasion depth, surgery type, radiotherapy, lymph node size, and SLNB were derived from the corresponding fields of the SEER database. And then, they were included in regressions because they were found to be prognostic factors (12–15).

Outcomes

Vulvar cancer-specific survival (CSS) was the primary outcome calculated according to patients whose death were attributable to vulvar cancer. In contrast, patients who died of other causes rather than vulvar cancer were considered as compete-risks. Overall survival (OS) was the secondary outcome.

Statistical Analysis

The inverse probability weighting (IPW) was applied to adjust for the imbalance between groups. The region, insurance status, year of diagnosis, age, race, marital status, primary site, pathological grade, tumor size, invasion depth, surgery, radiotherapy, and lymph node size were all included in logit regression models to calculate the probability of the receipt of SLNB. Moreover, the IPW weights were calculated based on the pre-calculated logit models. We calculated IPW weights within each subgroup stratified by microscopically confirmed (positive histology) regional lymph nodes status—negative (LN–) or positive (LN+) regional lymph nodes findings. To assess the non-inferiority of SLNB compared with no lymph nodes removed (NA), regressions with IPW adjustment for SLNB *versus* NA were also performed. Sensitivity analysis on the missing value of lymph size was carried out to assess the consistency of the effect of SLNB *versus* RLND because clinical

lymph node status was an essential confounding factor associated with the choice of SLNB and RLND.

The Kaplan-Meier survival curves were plotted and compared by the Cox test because of IPW adjustment. Multivariate accelerated failure time (AFT) regression models and Fine-Gray compete-risk (CR) models were applied to calculate the time ratio (TR) and sub-distribution hazard ratio (sHR) and their corresponding 95% confidence intervals (95% CIs). A larger TR indicates a more prolonged survival. Univariate regression models were not performed because all the abovementioned factors were included in the multivariate model without stepwise variable filtering. After all, variable filtering based on *P* value was highly controversial. Multivariate Cox regression models were also carried out to evaluate OS, but the proportional hazard hypothesis was violated for some variables. Thus, a series of AFT models using either Gamma, Lognormal, or Weibull distribution was carried out. Finally, the AFT model with lognormal distribution was chosen because it is the simplest model that best fits the sample data set.

A two-tailed *P* value of less than 0.05 was considered statistically significant. All the statistical processes were performed in STATA 16.0 software (StataCorp, College Station, Texas).

RESULTS

Baseline Characteristics

Figure 1 shows the sample selection procedure. Of the 3,161 patients in this study, 287 (9.1%) underwent SLNB, 1,716 (54.3%) underwent RLND, and 1,158 (36.6%) had no regional lymph nodes removed. The median [interquartile range, IQR] follow-up of SLNB, RLND, and NA patients were 36 months [20–61 months], 52 months [22–92 months], and 56 months [28–94 months], respectively. The median [IQR] age of SLNB, RLND, and NA patients were 61 [51–69], 59 [50–70], and 58 [49–67], respectively. More SLNB patients were diagnosed after 2010 compared with RLND patients (79.4% *vs*. 54.1%) and have a tumor size of <2 cm (47.0% *vs*. 33.0%) and an invasion depth of >1 mm (77.0% *vs*. 67.2%). Patients who underwent SLNB had a higher percentage of being alive (86.8% *vs*. 70.6%) and negative lymph node findings (78.8% *vs*. 71.7%) compared with those who underwent RLND (**Table 1**).

Comparison of Overall Survival and Vulvar Cancer-Specific Survival Between the SLNB and RLND Groups

The IPW adjusted Kaplan-Meier curves of CSS and OS are summarized in **Figure 2**.

Patients who underwent SLNB had significantly improved CSS than those who underwent RLND both in the LN− cohort (unadjusted sHR=0.41; 95% CI, 0.18–0.96; *P*=0.041; adjusted sHR=0.42, 95% CI 0.19–0.93, *P*=0.032) and in the LN+ cohort (unadjusted sHR=0.56; 95% CI, 0.32–0.98, *P*=0.042; adjusted sHR=0.29; 95% CI, 0.16–0.54, *P*<0.001). Notably, patients who received radiotherapy had worse OS than those who did not (sHR=2.91; 95% CI, 1.32–6.42; *P*=0.008) in the LN− cohort (**Figure 3** and **Supplementary Tables 1**, **2**).

As for the OS, patients who underwent SLNB had prolonged OS than those who underwent RLND in the LN− cohort (unadjusted TR=1.51; 95% CI, 0.97–2.37; *P*=0.069; adjusted TR=1.38; 95% CI, 0.82–2.32; *P*=0.226) and in the LN+ cohort (unadjusted TR=1.21; 95% CI, 0.77–1.92; *P*=0.406; adjusted TR=2.68; 95% CI, 1.73–4.14; *P*<0.001), although the effect was not significant in the LN− cohort. (**Figure 4** and **Supplementary Tables 3**, **4**).

Older age was associated with a significant worse CSS (LN−: 18–49, reference; 60-69, adjusted sHR=3.15, *P*=0.005; 70–80, adjusted sHR=8.81, *P*<0.001; LN+: 18–49, reference; 70–80, adjusted sHR=2.19, *P*=0.006) and OS (LN−: 18–49, reference; 60–69, adjusted TR=0.32, *P*=0.002; 70-80, adjusted TR=0.19, *P*<0.001; LN+: 18–49, reference; 7–80, adjusted TR=0.32, *P*<0.001) (**Supplementary Tables 1**-**4**).

Comparison of Overall Survival and Vulvar Cancer-Specific Survival Between SLNB and NA Groups

To assess the non-inferiority of SLNB compared with NA, we conducted regressions of survival for SLNB and NA groups. Also, we found, compared with patients in the NA group, those who underwent SLNB had significantly prolonged CSS (unadjusted sHR=0.56; 95% CI, 0.31–1.00; *P*=0.049; adjusted sHR = 0.40; 95% CI, 0.23–0.70; *P*=0.001), but similar OS (unadjusted TR=1.44; 95% CI, 0.94–2.21, *P*=0.091; adjusted TR=1.15; 95% CI, 0.76–1.73; *P*=0.279) (**Figures 3**, **4** and **Supplementary Tables 5**, **6**).

Sensitive Analysis

Because clinical lymph node size was an important factor associated with the choice between SLNB and RLND, we carried out a sensitivity analysis on missing values of lymph node size. We assessed two extreme scenarios: all missing values of lymph node size were considered to be (1) < 5 mm and (2) ≥5 mm. Then, we performed IPW adjusted AFT and CR regressions for OS and CSS within the LN+ cohort and the overall cohort (combination of LN+ and LN−). Finally, the beneficial effect of SLNB on OS and CSS changed slightly but remain consistent (**Supplementary Table 7**).

DISCUSSION

This study's key findings were that SLNB led to significantly prolonged survival outcomes in VSCC surgery patients with no distant metastasis and adjacent organ invading compared to RLND and NA. To our knowledge, this study is the first retrospective study comparing SLNB with RLND in VSCC surgery patients who had no distant metastasis and adjacent organ invading, with 287 patients treated with SLNB and a sample size of 3,161 patients. This study controlled for diverse confounding factors, such as surgery type, tumor size, invasion

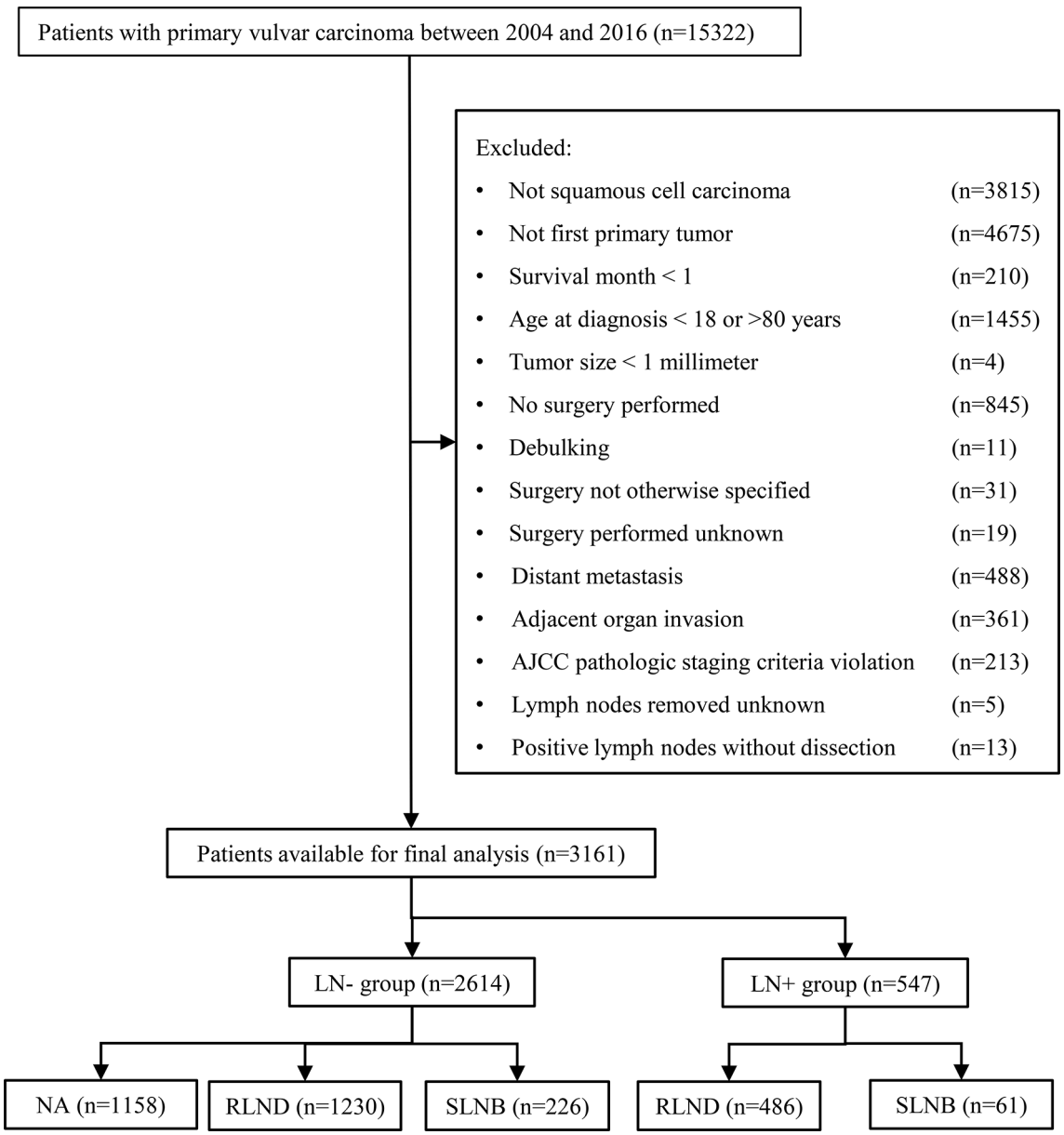

FIGURE 1 | Flowchart of the patient selection procedure. AJCC, American Joint Committee on Cancer; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; NA, no regional lymph node removed.

depth, radiotherapy, and positive lymph nodes findings. IPW adjustment was carried out to minimize the imbalance of variables between groups. This study adds to the supportive evidence of the beneficial effect of SLNB on the survival of VSCC surgery patients and extends the application scope of SLNB.

In this study, the beneficial effect of SLNB compared to RLND on CSS was larger in the LN+ cohort(sHR [95% CI]=0.29 [0.16–0.54]) than in the LN– cohort (sHR [95% CI]=0.42 [0.19–0.93]). Similar larger effect of SLNB was also present for OS in the LN+ cohort (TR [95% CI]=2.68 [1.73–4.14]) than in the LN– cohort (TR [95% CI]=1.38 [0.82–2.32]). Thereby, this study demonstrates that patients in the LN+ cohort could benefit more from SLNB than those in the LN– cohort. To explore the non-inferior effect of SLNB, we carried out a comparison between patients treated with SLNB with those with no regional lymph nodes removed, and the result was very promising. Patients treated with SLNB has significantly improved CSS (sHR [95% CI]=0.40 [0.23–0.70]) and similar OS (TR [95% CI]=1.15 [0.76–1.73]) compared with those in the NA group, which indicates that patients in the NA group might have missing detection of microscopic positive lymph nodes. So the extended application of SLNB was feasible.

TABLE 1 | Baseline characteristics.

Characteristics	NA No. (%)	RLND No. (%)	SLNB No. (%)
Total	1158	1716	287
Region			
East	599 (51.7)	812 (47.3)	96 (33.4)
Northern Plains	115 (9.9)	179 (10.4)	42 (14.6)
Pacific Coast	399 (34.5)	657 (38.3)	130 (45.3)
Southwest	45 (3.9)	68 (4.0)	19 (6.6)
Insurance status			
Insured	712 (61.5)	1038 (60.5)	227 (79.1)
Medicaid	145 (12.5)	237 (13.8)	29 (10.1)
Uninsured	36 (3.1)	80 (4.7)	2 (0.7)
Unknown	265 (22.9)	361 (21.0)	29 (10.1)
Year of diagnosis			
2004-2009	492 (42.5)	787 (45.9)	59 (20.6)
2010-2016	666 (57.5)	929 (54.1)	228 (79.4)
Age			
median age (IQR), year	58 (49–67)	59 (50–70)	61 (51–69)
18-49	317 (27.4)	419 (24.4)	60 (20.9)
50-59	326 (28.2)	445 (25.9)	73 (25.4)
60-69	289 (25.0)	410 (23.9)	84 (29.3)
70-80	226 (19.5)	442 (25.8)	70 (24.4)
Race			
White	985 (85.1)	1483 (86.4)	268 (93.4)
Black	135 (11.7)	169 (9.8)	9 (3.1)
Other	38 (3.3)	64 (3.7)	10 (3.5)
Marital status			
Married	505 (43.6)	777 (45.3)	149 (51.9)
Single	225 (19.4)	350 (20.4)	44 (15.3)
Divorced/separated/widowed	333 (28.8)	507 (29.5)	75 (26.1)
Unknown	95 (8.2)	82 (4.8)	19 (6.6)
Primary site			
Labium majus	83 (7.2)	158 (9.2)	23 (8.0)
Labium minus	55 (4.7)	87 (5.1)	19 (6.6)
Clitoris	10 (0.9)	50 (2.9)	5 (1.7)
Overlapping lesion	40 (3.5)	70 (4.1)	6 (2.1)
Vulva, NOS	970 (83.8)	1351 (78.7)	234 (81.5)
Pathology grade			
Grade I	373 (32.2)	460 (26.8)	88 (30.7)
Grade II	268 (23.1)	774 (45.1)	131 (45.6)
Grade III/IV	83 (7.2)	323 (18.8)	43 (15.0)
Unknown	434 (37.5)	159 (9.3)	25 (8.7)
Tumor size, cm			
<2	627 (54.1)	567 (33.0)	135 (47.0)
2-4	177 (15.3)	577 (33.6)	101 (35.2)
≥4	100 (8.6)	427 (24.9)	21 (7.3)
Unknown	254 (21.9)	145 (8.4)	30 (10.5)
Invasion depth, mm			
≤1	496 (42.8)	112 (6.5)	15 (5.2)
>1	278 (24.0)	1153 (67.2)	221 (77.0)
Unknown	384 (33.2)	451 (26.3)	51 (17.8)
Surgery			
LTE	387 (33.4)	109 (6.4)	21 (7.3)
SV	585 (50.5)	727 (42.4)	158 (55.1)
TV	87 (7.5)	314 (18.3)	38 (13.2)
RV	99 (8.5)	566 (33.0)	70 (24.4)
Radiotherapy			
No	1079 (93.2)	1279 (74.5)	232 (80.8)
Yes	79 (6.8)	437 (25.5)	55 (19.2)
Chemotherapy			
No	1125 (97.2)	1519 (88.5)	264 (92.0)
Yes	33 (2.8)	197 (11.5)	23 (8.0)
Lymph node size, mm			
<5	1158 (100.0)	1300 (75.8)	242 (84.3)
≥5	–	91 (5.3)	17 (5.9)
Unknown	–	325 (18.9)	28 (9.8)
Lymph node findings			
Negative	1158 (100.0)	1230 (71.7)	226 (78.8)
Positive	–	486 (28.3)	61 (21.2)
Follow-up time (IQR), month	56 (28–94)	52 (22–92)	36 (20–61)
Outcome			
Alive	972 (83.9)	1211 (70.6)	249 (86.8)
Dead from vulvar cancer	67 (5.8)	180 (10.5)	17 (5.9)
Not dead from vulvar cancer	115 (9.9)	316 (18.4)	20 (7.0)
Dead from unknown cause	4 (0.3)	9 (0.5)	1 (0.3)

LN–, negative regional lymph node findings; LN+, positive regional lymph node findings; NA, no regional lymph node removed; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; cm, centimeter; mm, millimeter; IQR, interquartile range.

Because of vulvar carcinoma's rareness, there have been no random control trials, which may not be feasible because of methodological and ethical issues, providing high-level evidence about the safety and efficacy of SLNB compared with that of RLND (16). This sizable retrospective study confirmed the significantly superior role of SLNB *versus* RLND in VSCC surgery patients within both the LN– and LN+ cohorts. This study found that surgery type, tumor size, and invasion depth did not limit the applying of SLNB, contrary to the previous study's finding that SLNB should be limited to tumors with size ≤4 cm and invasion depth >1 mm (17). Our study provides evidence for extending the indication of the application of SLNB patients with any tumor size and invasion depth, irrespective of surgery type and regional lymph nodes findings. In contrast, age should be taken into account because of its statistically significant association with survival. Together with the cost-efficacy of SLNB, more VSCC surgery patients will benefit from SLNB (17, 18).

The explanation of the promising survival outcome associate with SLNB may lie in that several innovation techniques of SLNB, including imaging tracer agent (ranging from blue dye to indocyanine green and Technetium-99m colloid albumin as well as their combination) and imaging equipment (from lymphoscintigraphy, single-photon emission computed tomography or computed tomography [SPECT/CT] to a fusion of SPECT/CT and ultrasound), have dramatically progressed the precision of lymph node localization (17, 19, 20). SPECT/CT could currently personalize lymphatic mapping and provide detailed information about the number and anatomical location of sentinel lymph nodes for adequate surgical planning in the groin (21). However, it is still essential to standardize the acquisition principles of SPECT/CT images and centralize SLNB performing in experienced centers for a personalized approach (17).

This study has some limitations. 1) Detailed information about surgery was not available in SEER, for example, hospital's care quality, imaging equipment, tracer agent for imaging, surgeon's professional experience. Thus, we could not profoundly control those factors' impact and handle the heterogeneity of those factors between groups. Moreover,

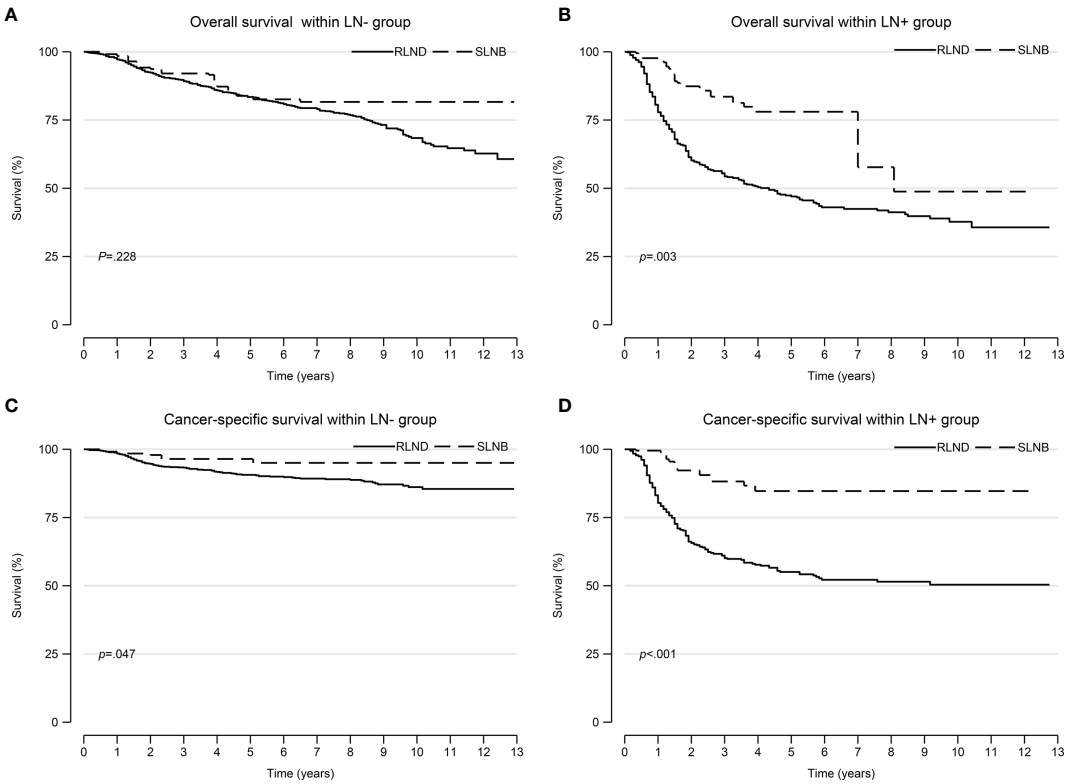

FIGURE 2 | Overall survival and cancer-specific survival curves after inverse probability weighting. **(A)** overall survival within the LN– cohort; **(B)** overall survival within the LN+ cohort; **(C)** cancer-specific survival within the LN– cohort; **(D)** cancer-specific survival within the LN+ cohort. LN–, negative regional lymph node findings; LN+, positive regional lymph node findings.

margin status was not reported in the SEER, so it could not be controlled, although margin status was proven to be not a significant prognostic factor of survival in early studies (22–25). 2) This study covered so long a period from 2004 to 2016 that some missing factors may bias the findings, despite the year of diagnosis was grouped into two intervals at 2010 and controlled in IPW calculation and multivariate regression, and no significant survival difference was found to be associated with the year of diagnosis. 3) As the retrospective study's nature, there might be missing confounders that may be important for analysis, which would lead to bias in our findings. For example, we did not know where the exact location of the tumors. Although we had adjusted for the primary site of the tumor, that might not be adequate to account for the bias caused

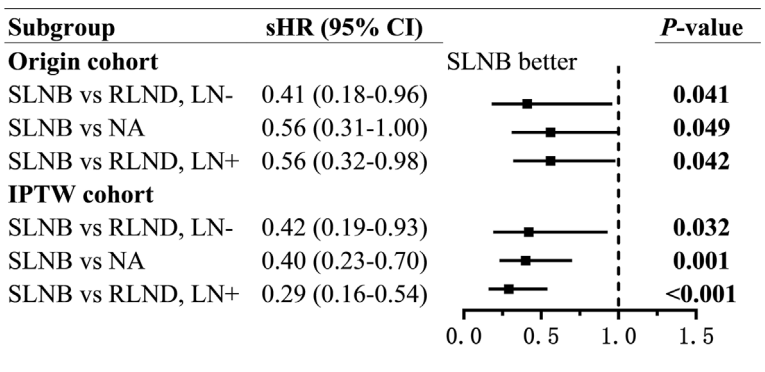

FIGURE 3 | Forest plot of compete-risk subdistribution hazard ratios (sHR) of cancer-specific survival. sHR, subdistribution hazard ratios; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; NA, no regional lymph node removed.

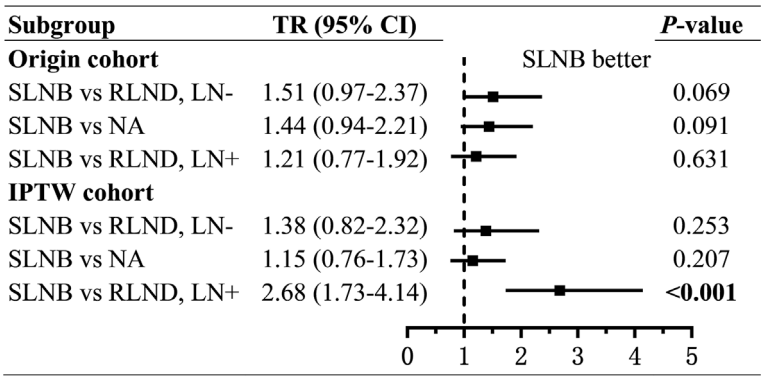

FIGURE 4 | Forest plot of time ratios (TR) of overall survival. TR, time ratio; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; NA, no regional lymph node removed.

by tumor location. 4) Despite IPW techniques, residual confounding may exist. 5) The pathological result of SLNB and RLND during the surgery process was unavailable in the SEER database, and only the final pathological histology results were given. Thus the false negatives and false positives that were of great interest could not be calculated.

Although our study had some limitations, it was the first retrospective study investigating SLNB in VSCC surgery patients without distant metastasis and adjacent organ involvement until now. Our study extends the scope of SLNB performing on this rare cancer. Our findings will make clinicians preferentially consider performing SLNB in VSCC surgery patients irrespective of surgery type, invasion depth, and positive lymph node findings so that more patients will benefit from SLNB.

CONCLUSIONS

SLNB results in significantly prolonged survival in VSCC surgery patients without distant metastasis and adjacent organ invading, irrespective of tumor size, surgery type, invasion depth, and positive lymph node findings. SLNB could be carried out preferentially in VSCC surgery patients. Further prospective clinical controlled trials are warranted to confirm the superior efficacy of SLNB.

DATA AVAILABILITY STATEMENT

Publicly available data sets were analyzed in this study. This data can be found here: https://seer.cancer.gov/.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WLZ: Conceptualization, Data curation, Formal analyses, Supervision, Writing-Original draft preparation, Writing-Reviewing, and Editing. YB: Conceptualization, Methodology, Software. YYY: Conceptualization, Formal analyses, Methodology, Software, Supervision, Visualization, Writing-Original draft preparation, Writing- Reviewing, and Editing. All authors contributed to the article and approved the submitted version.

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An ethical review process was not required for this study because the data were obtained from the Surveillance, Epidemiology, and End Results (SEER) database. The authors acknowledge the efforts of the SEER Program registries in the establishment of the SEER database. We have signed the Data-use Agreement for the SEER 1975–2016 Research Data File.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.676038/full#supplementary-material

Supplementary Table 1 | Multivariate compete-risk analysis of characteristics associated with cancer-specific survival in the LN– cohort for patients treated with SLNB and RLND. LN–, negative regional lymph node findings; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; IPW, inverse probability weighting; sHR, sub proportional hazard ratio; NOS, not otherwise specified; cm, centimeter; mm, millimeter.

Supplementary Table 2 | Multivariate compete-risk analysis of characteristics associated with cancer-specific survival in the LN+ cohort for patients treated with SLNB and RLND. LN+, positive regional lymph node findings; IPW, inverse probability weighting; sHR, sub proportional hazard ratio; NOS, not otherwise specified; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; cm, centimeter; mm, millimeter.

Supplementary Table 3 | Multivariate accelerate failure time analysis of characteristics associated with overall survival in the LN– cohort for patients treated with SLNB and RLND. LN–, negative regional lymph node findings; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; IPW, inverse probability weighting; TR, time ratio; NOS, not otherwise specified; cm, centimeter; mm, millimeter.

Supplementary Table 4 | Multivariate accelerate failure time analysis of characteristics associated with overall survival in the LN+ cohort for patients treated with SLNB and RLND. LN+, positive regional lymph node findings; SLNB, sentinel lymph node biopsy; RLND, regional lymph node dissection; IPW, inverse probability weighting; TR, time ratio; NOS, not otherwise specified; cm, centimeter; mm, millimeter.

Supplementary Table 5 | Multivariate compete-risk analysis of characteristics associated with cancer-specific survival for patients treated with SLNB and NA. LN–, negative regional lymph node findings; SLNB, sentinel lymph node biopsy; NA, no regional lymph node removed; IPW, inverse probability weighting; sHR, sub proportional hazard ratio; NOS, not otherwise specified; cm, centimeter; mm, millimeter.

Supplementary Table 6 | Multivariate accelerate failure time analysis of characteristics associated with overall survival for patients treated with SLNB and NA. LN–, negative regional lymph node findings; SLNB, sentinel lymph node biopsy; NA, no regional lymph node removed; IPW, inverse probability weighting; TR, time ratio; NOS, not otherwise specified; cm, centimeter; mm, millimeter.

Supplementary Table 7 | Effect of SLNB *versus* RLND from IPW adjusted multivariate AFT and CR models for sensitivity analysis about missing lymph node size. SLNB, sentinel lymph node biopsy; RLND, regional lymph node removed; IPW, inverse probability weighting; AFT, accelerate failure time; CR, compete-risk; TR, time ratio; sHR, sub proportional hazard ratio; LN+, positive regional lymph node findings; LN–, negative regional lymph node findings.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Circulating microRNAs in Early Breast Cancer Patients and Its Association With Lymph Node Metastases

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MicroRNAs have emerged as important regulators of the metastatic process. In addition, circulating miRNAs appear to be surprisingly stable in peripheral blood making them ideal noninvasive biomarkers for disease diagnosis. Here, we performed a proof-of-principle study to investigate the expression profile of circulating miRNAs and their association with the metastatic lymph node status in early breast cancer patients. Sentinel lymph node status was detected by one-step nucleic acid (OSNA) analysis. We performed RNA-sequencing in 16 plasma samples and validated the results by qPCR. Gene Ontology term enrichment and KEGG pathway analyses were carried out using DAVID tools. We found16 differentially expressed miRNAs ($q < 0.01$) in patients with positive SLNs. Fourteen miRNAs were down-regulated (miR-339-5p, miR-133a-3p, miR-326, miR-331-3p, miR-369-3p, miR-328-3p, miR-26a-3p, miR-139-3p, miR-493-3p, miR-664a-5p, miR-146a-5p, miR-323b-3p, miR-1307-3p and miR-423-3p) and 2 were up-regulated (miR-101-3pand miR-144-3p). Hierarchical clustering using differentially expressed miRNAs clearly distinguished patients according to their lymph node status. Gene ontology analysis showed a significant enrichment of biological processes associated with the regulation of the epithelial mesenchymal transition, cell proliferation and transcriptional regulation. Our results suggest the potential role of several circulating miRNAs as surrogate markers of lymph node metastases in early breast cancer patients. Further validation in a larger cohort of patients will be necessary to confirm our results.

Keywords: circulating microRNAs, breast cancer, lymph node, metastasis, surrogate biomarker

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INTRODUCTION

Breast cancer remains a common disease worldwide and the second cause of cancer death in the US (1). Early diagnosis, improvements in treatment and early onset of therapy are important factors determining the prognosis and management of patients with breast cancer. Various factors such as early age at menarche, late age at first birth and late age at menopause are related to breast cancer risk. However, lymph node (LN) affection remains the most important prognosis factor in breast cancer (2). There are a number of factors associated with metastases to the LN, including tumor size, presence of lymphovascular invasion, poor histological grade and age (3, 4). Nevertheless, for a significant number of early-breast cancer patients it is unclear whom will develop metastases. For instance, about 13% of patients with favorable prognostic factors at diagnose will develop metastasis and the percentage increases to 20-30% for LN-negative patients. In contrast, 20-30% of LN-positive patients will never metastasize (5), therefore it is unclear whether distant metastases arise in a sequential manner from LN metastases or in parallel through the blood stream and whether other factors such interactions between the tumor and the stroma favor locoregional metastases (6).

Most women diagnosed with breast cancer are initially treated with surgery to remove the tumor and to determine the presence of metastases in the sentinel LNs (SLNs). This is currently the recommended procedure for axillary staging of early breast cancer. Our institution use the one-step nucleic amplification (OSNA) assay (7) to accurately measure total metastatic volume in the SLN (8), as an alternative to intraoperative microscopy-based pathological assessment of the SLN. The OSNA assay is a rapid molecular detection of SLN metastasis based on the semi-quantification of cytokeratin 19 (CK19) mRNA copy numbers (7). Thus, only patients diagnosed with more than two macrometastatic SLN are further treated with axillary lymph node dissection (ALND) (9), the golden standard procedure for invasive breast cancer. However, ALND has been questioned in recent years because of inherent morbidity following the procedure without directly contributing to survival in primary breast cancer patients (10–12) and the recognition that not all patients with nodal disease may require extensive axillary surgery (13).

Elucidation of breast cancer's molecular biological features have had a dramatic effect on how patients are diagnosed and treated. However, effective management of breast cancer is still difficult because of the lack of sensitive and specific biomarkers for early detection and for diseases monitoring. Accumulating evidence in the last years has highlighted the potential use of peripheral blood circulating nucleic acids in breast cancer diagnosis, prognosis and for monitoring response to anticancer therapy. Among these, circulating microRNAs (miRNAs) are increasingly recognized as a promising non-invasive biomarker, given the ease with which miRNAs can be isolated and their structural stability under different conditions of sample processing and isolation (14–16).

MicroRNAs (miRNAs) are a small (19-25 nt) non-coding RNAs, expressed in a wide variety of organisms and highly conserved across species (17). MiRNAs regulate the expression of target genes by binding to complementary regions of messenger transcripts to repress their translation or regulate their degradation. MiRNAs are now recognized as novel post-transcriptional regulators targeting over 30% of the human genome (18). The overall emerging picture is that of a complex regulation level of gene expression, in which a single miRNA may control hundreds of targets (19). Many cellular pathways are affected by the regulatory function of miRNAs and several human pathologies, including cancers, have been associated with misregulation of the miRNAs (16) and their metastases (20). Numerous studies have identified widespread alterations in the expression of miRNAs related to human neoplasias. In breast cancer, analysis of miRNA expression classified the different breast cancer molecular subtypes and correlated these with various clinicopathological factors and numerous miRNAs have been shown to play a pivotal role in various steps of the metastatic process. In addition, circulating miRNAs are emerging as prognostic factors in breast cancer (14), but few studies have correlated their expression with the LN status, the occurrence of distant metastases and breast cancer recurrence. These studies have analyzed the expression of specific circulating miRNAs by qPCR and have shown promising results (21–24).

Herein, we sought to examine the miRNA content in plasma samples from early breast cancer patients with known SLN and axillary LN metastatic status. We designed a proof-of-principle study to profile the expression of miRNAs by RNA-sequencing using preoperative peripheral blood from patients with early breast cancer who were not previously treated. Our results are preliminary but support the hypothesis of the existence of a differential miRNA expression profile in the peripheral blood from breast cancer patients associated with the LN status of their tumors. Our data highlights the potential use of circulating miRNAs as surrogate markers of locoregional metastases in breast cancer. Further studies in a larger number of samples are warranted.

MATERIALS AND METHODS

Patients

We studied 16 patients with early breast cancer treated with surgery and diagnosed for positive SLNs. All patients had confirmed diagnosis based on histopathology of tumor biopsy. All tumors were invasive ductal carcinomas (IDCs) with or without *in situ* component. In 2 cases, tumors were mixed and show presence of invasive lobular carcinoma (ILC) component. Intraoperative SLN were evaluated using the OSNA assay (7). None of the patients had prior treatment with surgery, chemotherapy or radiation. All patients were hormone receptor (HR) positive, HER2 negative. We collected the following clinical and pathological parameters: age, menopausal status, personal and familiar disease precedents and clinical follow-up, tumor stage was be determined according to the AJCC/UICC system (25), histological grade was determined using the Elston-Ellis grading system (26),

histology (ductal, lobular, special types), presence of associated ductal or lobular carcinoma *in situ*, presence of vascular and lymphatic invasion, tumor infiltrating lymphocytes, type of invasion (expansive/infiltrating), tumor multifocality, tumor necrosis; proliferation of non-tumoral tissue (ductal hyperplasia, atypical ductal/lobular hyperplasia).

Blood Processing and Isolation of Plasma

Human plasma samples were collected prospectively from early breast cancer patients who have not received any previous treatment. Peripheral blood was withdrawn before surgery. Approximately, 10-15ml of peripheral blood was collected for plasma processing in EDTA tubes. Plasma tubes were processed within 2 hours of collection and spun at 1200xg for 10 minutes. Plasma was aliquot in 1.5 ml tubes and stored at -80C until further processing. All plasma samples used in this study were inspected for absence of hemolysis as previously described (27). Briefly, the hemolysis score (HS) was determined by ultraviolet-visible (UV-Vis) absorbance measurements using a NanoDrop® 2000 Spectrophotometer (Thermo Scientific, Barrington, IL, USA). Measurements were performed by applying 2 µl of plasma on the micro-volume pedestal after centrifugation at 1000 × g for 5 min at 4°C and using saline (PBS) as a blank. In addition, monitoring of hemolysis was conducted by qPCR for all samples by comparing the level of a highly expressed miRNA in red blood cells (hsa-miR-451a) with a miRNA unaffected by hemolysis (hsa-miR-23a-3p) as previously described (28). Samples with a $\Delta Ct > 7$ were discarded for further analyses.

RNA Isolation NGS Library Preparation and Next Generation Sequencing

RNA was isolated from 300µl of plasma samples with the miRNeasy serum/plasma advanced kit (Qiagen, Cat No/ID: 217204) according to the manufacturer's instructions. A range of spike-ins were added to the plasma samples prior to RNA isolation. A quality check was performed by qPCR previous to sequencing the samples. Sixteen samples were selected to perform NGS, including 12 positive SLNs (n = 6 macrometastasis and n= 6 micrometastasis) and 4 negative SLNs. Five µl of total RNA was used to construct the NGS libraries using the QIAseqmiRNA Library Kit (Qiagen, Cat. No: 331505). Briefly, after ligation of 3' and 5' adapters and Unique Molecular Identifier (UMIs) to miRNAs, complementary DNA libraries were constructed by reverse transcription followed by 22 cycles of PCR amplification and cDNA cleaned up using QMN beads. A library preparation quality check was performed using either Bioanalyzer 2100 (Agilent) or TapeStation 4200 (Agilent). Based on quality of the inserts and the concentration measurements, libraries were pooled in equimolar ratios and quantified using the qPCR ExiSEQ LNA™ Quant kit (Exiqon). The library pools were sequenced with a NextSeq500 platform (Illumina) using sequence runs of 75nt single-end reads with an average number of 10 million reads/sample. Raw data was demultiplexed and FASTQ files were generated using the bcl2fastq 2.18.0.12 software (Illumina) and files were checked using the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Genome Annotation and Quantification of miRNAs

Genome annotation was performed using the Exiqon/Xplore RNA pipeline. Following sequencing, Cutadapt (1.9.1) (29) was used to trimmed adaptor sequences. A quality check (QC) was performed to ensure Q-scores >30 (>99.9% correct) of our data (30). Reads with correct length were analyzed for the presence of UMIs using Cutadapt (1.9.1) and then collapsed by UMIs into FASTQ files. This approach eliminates library amplification bias and allows for true identification of the miRNAs. Bowtie2 software (2.2.6) was used for mapping the reads. The mapping criterion for aligning reads to spike-ins, abundant sequences and miRBase_20 was for reads to have perfect match to the reference sequences. To map the genome, one mismatch was allowed in the first 32 bases of the read. Small insertions and deletions (INDELs) were not allowed. The resulting sequences were annotated using the human assembly GRCh37 and the miRBase_20 database. IsomiR analysis was performed individually for each sample based on the occurrence of count variants for each detected miRNA. Read variants were merged onto a single count file with a consistent nomenclature across samples. Only isomiRs present at a level of 5% of total reads for a specific miRNA were retained. Transcripts per million (TPM) was used as a normalization procedure to correct for differences in sequencing depth and to quantified each RNA species.

Differential Expression Analysis

Differential expression analysis was performed using the EdgeR statistical software package (Bioconductor, http://www.bioconductor.org/). The analysis was performed using the trimmed mean of M-values normalization method (TMM) (31), based on the log-fold and absolute gene-wise changes in expression between samples. The TMM normalization compensates for sample specific effects caused by the variation in library size/sequencing depth between samples and also compensates for under- or over-sampling effects by trimming and scaling factors that minimize log fold changes between samples across the majority of the miRNAs. The isomiR analysis was done using Exiqon in-house scripts (exq_ngs_mircount). Predicted miRNAs analysis was performed based on the read count distribution using the exiqon_ngs_mirpred in house script and the secondary structure prediction according to the miRPara classification score (32). Volcano plots were constructed using R programming (33) by plotting the p value (-log10) on the y-axis and the expression fold change between the two experimental groups on the x-axis.

Principal Component Analysis and Heat Map and Unsupervised Clustering

Principal component analysis (PCA) was performed using R programming and TMM-normalized values as input. The same input was used to generate a heatmap and unsupervised hierarchical clustering by samples and gene expression profile with R scripts (33). We selected the top 50 miRNAs with the

largest coefficient of variation (% CV) across all samples to obtain a cluster of samples. The data was normalized to TMM and converted to log2 scale.

Gene Ontology Enrichment Analysis

Gene Ontology (GO) analyses (34, 35) were done with R TopGO package with experimentally verified targets of significantly differentially expressed miRNAs as input. Two different statistical tests were used and compared. First, a standard Fisher's test was used to investigate enrichment of terms between groups. Second, the Elim method (36) was used to incorporate the topology of the GO network and to compensate for local dependencies between GO that could mask significant GO terms. Comparisons from these two methods were used to highlight relevant GO terms.

Quantitative Real-Time RT–PCR Analysis

Quantitative real-time RT–PCR analysis was done with an ABI Prism 7500 Sequence Detection System using the miRCURY LNA™ Universal RT cDNA Synthesis Kit (Exiqon). The cDNA was diluted 50X and assayed in 10 µl PCR reactions according to the protocol for the miRCURY LNA™ Universal RT microRNA PCR System (Exiqon A/S); each microRNA was assayed twice by qPCR on the plasma Focus microRNA PCR panel. A no-template control (NTC) of water was purified with the samples and profiled like the samples. Analysis of the data was performed using the relative miRNA expression according to the comparative Ct (ΔΔCt) method using negative metastatic samples as reference. We used the geNorm (37) or the Normfinder algorithm (38) to select the best combination of two reference genes. Data from multiples plates were normalized using UniSp3 spike-in (Exiqon) as interplate calibrators.

Statistics

Differentially expressed miRNAs from RNA-sequencing data were detected by an exact test based on conditional maximum likelihood (CML) included in the R Bioconductor package edgeR (39). *P* values from RNA-sequencing were corrected (q-values) for multiple testing using the Benjamini-Hochberg procedure (40). A false discovery rate (FDR) $q < 0.05$ was considered significant. In all group comparisons missing expression values were treated as zero. Differences in total numbers of miRNAs between groups were analyzed by two-sided parametric t-tests. For analysis of clinicopathological parameters, quantitative variables between groups were compared using the Student's T-test and qualitative variables were compared using the X_2 or Fisher exact tests. A two-sided p-value ≤ 0.05 was considered significant.

RESULTS

A total of 25 patients were included in this study. However, only samples from 16 patients passed the pre-RNA-sequencing quality check (QC). The main clinicopathological characteristics of the patients are described in **Table 1**. A total of 12 (75%) patients had SLN-positive tumors (76%), of which 6 were OSNA-diagnosed as micrometastasis (38%) and 6 as macrometastasis (38%).

All samples passed the post-sequencing QC, which confirmed that the average read quality and base quality had a Q-score > 30 (99.9% correct) (30) and the expected read length distribution for miRNAs (**Supplementary Figure 1**). All samples were sequenced in one excellent run with a median 27.2 million read number. Following sequencing and trimming, all reads containing identical insert sequence and UMI sequence (insert-UMI pair) were collapsed into a single read and passed into the analysis pipeline. This allowed for true quantification of the miRNAs by eliminating library amplification bias and a better representation of the RNA molecules in the sample. We obtained an average of 1.8 million collapsed reads for each sample and good miRNA mapping reads with a very dominant miRNA peak in most of the samples, indicating a good sample/data quality (**Supplementary Table 1** and **Supplementary Figure 2**). Overall, we obtained an average genome mapping rate of 46.2% (**Figure 1A**), which are values well within the range for plasma samples. After mapping and counting to relevant entries in mirbase_20, the number of known miRNAs was calculated using TPM to measure expression. We found comparable numbers of identified miRNAs using either TPM > 1 (182 miRNAs) or TPM > 10 (125 miRNAs) (**Figure 1B**). We did not identify any sequences identical to those of known miRNAs in miRBase_20 for other organisms. However, we were able to predict 80 miRNAs based on the structural properties of the genome in the indicated locations resembling those of known miRNAs (**Supplementary Table 2**).

Next, we investigated whether the patients were assigned into biological groups based on their miRNA expression. We performed an unsupervised two-way hierarchical clustering of miRNAs and

TABLE 1 | Basic patient and tumor characteristics.

Variable		N (%)
Patients	Plasma	16 (100)
Age, years	Mean +SD	63 ± 13
	Median (range)	63 (46 - 89)
Tumor status	T1	10 (62)
	T2	2 (13)
	T3	4 (24)
Node status	Negative	4 (25)
	Micrometastasis	6 (38)
	Macrometastasis	6 (38)
Axillary Lymph Node Status	Negative	3 (50)
	Positive	3 (50)
Tumor grade	I	5 (31)
	II	9 (56)
	III	2 (13)
Estrogen receptor	Negative	0 (0)
	Positive	16 (100)
Progesterone receptor	Negative	1 (6)
	Positive	15 (94)
HER2 status	Negative	16 (100)
	Positive	0 (0)
Ki67	< 20%	15 (94)
	> 20%	1 (6)
Surgery	Mastectomy	5 (31)
	Lumpectomy	11 (69)
Lymphovascular invasion	Negative	12 (75)
	Positive	4 (25)

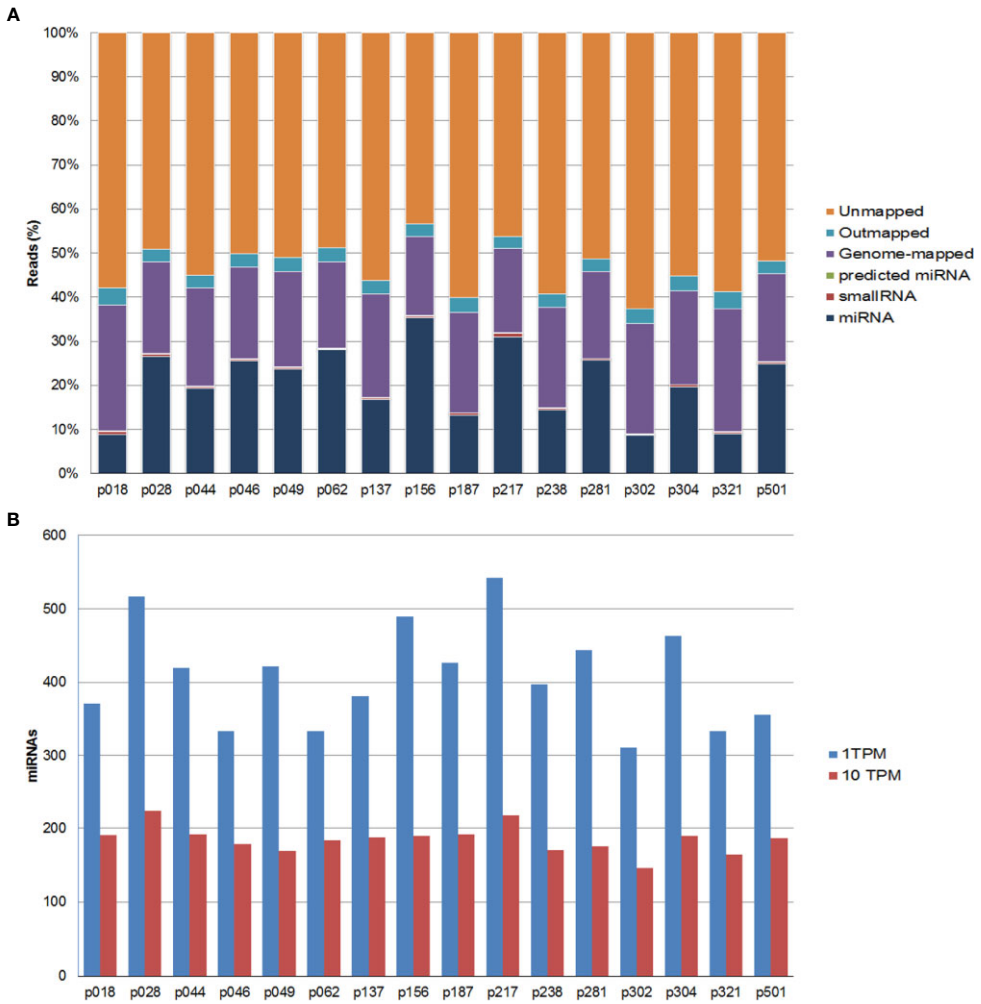

FIGURE 1 | Summary of the mapping results for all samples. **(A)** Percentage of sequencing reads for each sample. Reads are classified as miRNAs, small RNAs, genome-mapped, outmapped, high abundance (e.g. rRNA, polyA,mtRNA) and unmapped reads. **(B)** Number of identified known miRNAs with transcripts per million (TPM) normalized numbers of counts >1 (blue bars) or >10 (red bars).

samples using the 50 miRNAs with the largest coefficient of variation based on TMM counts (**Figure 2A**). Our results show that samples did not cluster according to the SLN outcome of the patients, suggesting that other clinicopathological factors are responsible for the variation on the samples. We obtained similar results using a principal component analysis. Interestingly, the 2 samples showing the greater variability (p18 and p62) corresponded to those patients whose tumors had a mixed pathological component (IDC and ILC) (**Figure 2B**).

Despite the unsupervised analysis did not group our samples according to the metastasis status of the patients, we identified differentially expressed miRNAs between groups based on the SLN outcome of the patients. First, we analyzed samples according to the positive (n=12) or negative (n=4) SLN metastasis status. We found 73 miRNAs with a significant differential expression ($p < 0.05$). However, only 16 miRNAs remained significant after correcting for multiple testing ($q < 0.05$) (**Table 2**). Fourteen miRNAs were down-regulated (miR-339-5p, miR-133a-3p, miR-326, miR-331-3p, miR-369-3p, miR-328-3p, miR-26a-3p, miR-139-3p, miR-493-3p, miR-664a-5p, miR-146a-5p, miR-323b-3pmiR-1307-3p and miR-423-3p) and 2 miRNAs were up-regulated (miR-101-3p and miR-144-3p) (**Figure 3A**). Next, we analyzed the data based on SLN metastasis status subgroups. When we compared patients with macrometastasis vs. negative SLNs, we found 42 miRNAs differentially expressed, but only miR-339-5p remained significant after FDR adjustment ($p < 0.0001$, $q = 0.0413$) (**Figure 3B** and **Table 2**). Similar results were obtained when we compared micrometastasis and negative SLNs, which yield 66 miRNAs differentially expressed, but only miR-376c-3p ($p = 0.0001$, $q = 0.046$), miR-326 ($p = 0.0003$, $q = 0.049$) and miR-323b-3p ($p = 0.0004$, $q = 0.049$) passed the FDR (**Figure 3C** and **Table 2**). Interestingly, we did not find any significantly differentially expressed circulating miRNAs between patients with

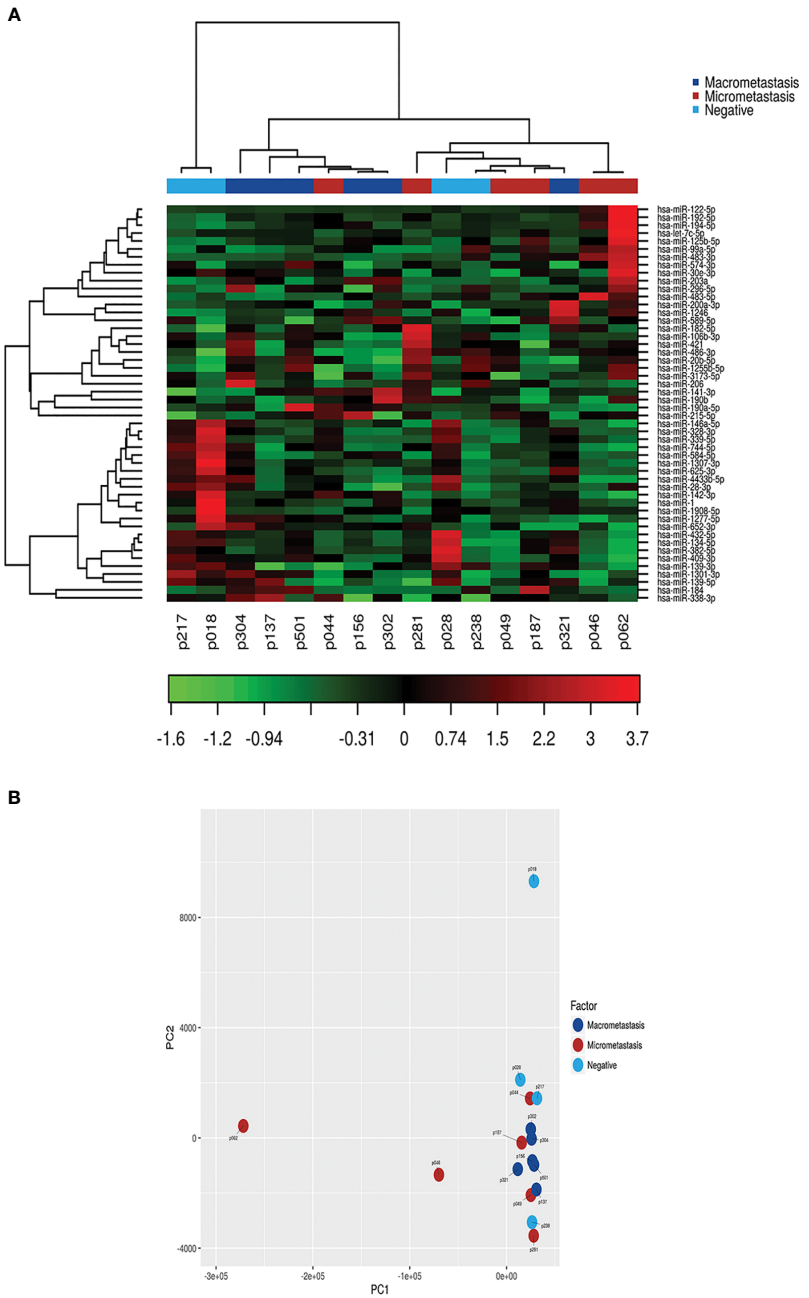

FIGURE 2 | Class discovery associated with the SLN metastatic status. The analysis was performed using the 50 miRNAs with the largest coefficient of variation based on trimmed mean of M-values (TMM counts). **(A)** Heat map and unsupervised hierarchical clustering. Each row represents one miRNA and each column represents one sample. The color represents the relative expression level of a miRNA across all samples. The color scale shows the expression level above (red) or below (green) the mean. **(B)** Principal Component Analysis (PCA) of samples according to the SLN metastatic status of patients.

macrometastasis or micrometastasis SLNs (**Figure 3D** and **Table 2**). In addition, we used the 16 significant differentially expressed miRNAs in patients with positive SLNs to build a heatmap and hierarchical clustering. Our results show that these miRNAs clearly separated patients with negative and positive SLNs (**Figure 3E**). Further validation on the same clinical samples was performed by specific qPCR assays. The down-regulation of 9 out of 14 miRNAs was confirmed in patients with positive SLNs, but we could not validate the up-regulation of miR-101-3p and miR-144-3p. Furthermore, the degree of down-regulation was higher for those patients that had additional metastases in their axillar lymph nodes (**Supplementary Figure 3**).

TABLE 2 | Differentially expressed miRNAs.

Names	Sequence (5' – 3')	TMM Positive	TMM Negative	logFC	p value	q value
hsa-miR-339-5p	TCCCTGTCCTCCAGGAGCTCACG	37.4	127.6	-1.8	< 0.0001	0.007
hsa-miR-133a-3p	TTTGGTCCCCTTCAACCAGCTG	6.1	28.4	-2.0	< 0.0001	0.008
hsa-miR-326	CCTCTGGGCCCTTCCTCCAG	10.6	50.0	-2.2	< 0.0001	0.008
hsa-miR-331-3p	GCCCCTGGGCCTATCCTAGAA	0.9	10.3	-2.8	0.0001	0.011
hsa-miR-369-3p	AATAATACATGGTTGATCTTT	7.9	26.0	-1.7	0.0005	0.031
hsa-miR-328-3p	CTGGCCCTCTCTGCCCTTCCGT	134.1	350.9	-1.4	0.0005	0.031
hsa-miR-26a-1-3p	CCTATTCTTGGTTACTTGCACG	2.1	10.9	-2.5	0.0007	0.034
hsa-miR-139-3p	TGGAGACGCGGCCCTGTTGGAGT	30.2	79.8	-1.4	0.0008	0.034
hsa-miR-493-3p	TGAAGGTCTACTGTGTGCCAGG	1.9	11.5	-2.1	0.0010	0.034
hsa-miR-664a-5p	ACTGGCTAGGGAAAATGATTGGAT	49.7	108.1	-1.1	0.0010	0.034
hsa-miR-101-3p	TACAGTACTGTGATAACTGAA	6070.1	3215.4	0.9	0.0011	0.034
hsa-miR-146a-5p	TGAGAACTGAATTCCATGGGTT	2960.5	6266.3	-1.1	0.0012	0.034
hsa-miR-144-3p	TACAGTATAGATGATGTACT	511.3	295.1	0.8	0.0013	0.035
hsa-miR-323b-3p	CCCAATACACGGTCGACCTCTT	10.5	29.9	-1.4	0.0016	0.040
hsa-miR-1307-3p	ACTCGGCGTGGCGTCGGTCGTG	150.3	337.4	-1.2	0.0017	0.040
hsa-miR-423-3p	AGCTCGGTCTGAGGCCCCTCAGT	353.7	649.1	-0.9	0.0023	0.050
hsa-miR-376c-3p	AACATAGAGGAAATTCCACGT	3.8	14.0	-1.8	0.0028	0.056
hsa-miR-1	TGGAATGTAAAGAAGTATGTAT	69.5	168.7	-1.3	0.0037	0.071
hsa-miR-1908-5p	CGGCGGGGACGGCGATTGGTC	23.1	59.1	-1.3	0.0042	0.073
hsa-miR-744-5p	TGCGGGGCTAGGGCTAACAGCA	138.5	298.1	-1.1	0.0042	0.073
hsa-miR-584-5p	TTATGGTTTGCCTGGGACTGAG	419.9	835.4	-1.0	0.0048	0.078
hsa-miR-6721-5p	TGGGCAGGGGCTTATTGTAGGAG	2.4	9.3	-1.9	0.0055	0.083
hsa-miR-432-5p	TCTTGGAGTAGGTCATTGGGTGG	130.1	432.4	-1.7	0.0055	0.083
hsa-miR-28-3p	CACTAGATTGTGAGCTCCTGGA	72.1	150.4	-1.0	0.0058	0.084
hsa-miR-29b-3p	TAGCACCATTTGAAATCAGTGTT	292	154	0.93	0.0068	0.094
	Macrometastasis		**Negative**			
hsa-miR-339-5p	TCCCTGTCCTCCAGGAGCTCACG	38.9	138.0	-1.8	0.0001	0.041
	Micrometastasis		**Negative**			
hsa-miR-376c-3p	AACATAGAGGAAATTCCACGT	1.5	13.7	-3.1	0.0001	0.046
hsa-miR-326	CCTCTGGGCCCTTCCTCCAG	9.8	49.2	-2.3	0.0003	0.049
hsa-miR-323b-3p	CCCAATACACGGTCGACCTCTT	7.1	29.5	-2.0	0.0004	0.049
	Macrometastasis		**Micrometastasis**			
hsa-miR-122-5p	TGGAGTGTGACAATGGTGTTTG	8948.2	76103.3	-3.1	0.0002	0.062
hsa-miR-125b-2-3p	TCACAAGTCAGGCTCTTGGGAC	0.3	8.9	-3.6	0.0006	0.090

Data shows the 25 most significant differentially expressed miRNAs according to the metastatic status of patients. The list includes the average trimmed mean of M-values (TMM) values, logarithmic fold change (logFC), raw p values and Benjamini-Hochberg FDR corrected q values.

Next, we sought to understand how our data is related to biological functions by performing a gene ontology (GO) analysis. Selecting *Homo sapiens* as the background of listed target genes, we obtained the GO term annotations and KEGG pathway analysis through the functional annotation summaries. The results are summarized in **Figure 4** and **Table 3**. The top 50 biological process GO terms ($p < 0.05$) associated with differentially expressed circulating miRNAs in patients with positive SLNs compared to the reference background (negative SLNs samples) are shown in **Figure 4A**. Our data shows that differentially expressed miRNAs associated with biological processes (BP) markedly focused on epigenetic gene expression regulation, the epithelial-mesenchymal transition (EMT), transcription, cell motility and proliferation processes ($p < 0.01$) (**Figure 4B**, **Table 3** and **Supplementary Data**). For instance, we found that positive regulation of mesenchymal cell proliferation term (GO: 0002053) was significantly enriched ($p < 0.0028$) as well as positive regulation of the histone H3-H4 methylation term (GO:0051571) ($p < 0.0017$). These two GO terms remained significant even when patients with positive SLNs were sub-classified as having macro- or micrometastasis in their SLNs. As for the cellular component (CC), the target miRNAs were significantly located in vesicle and membrane fractions ($p < 0.01$). Moreover, differentially expressed miRNAs were enriched in molecular function (MF) terms associated with transcription factors, G protein-related coupled peptide receptor activity, receptor regulator activity and microtubule motor activity ($p < 0.01$) (**Table 3**).

Our series include 16 patients with early breast cancer and we reported recurrence in 3 (19%) patients. The median follow-up time was 5.2 years (range 2.2 - 6.4 years). Two patients had secondary tumors in the colon and 1 patient in the liver. At last follow-up, two patients with recurrences in colon were reported alive with disease and we reported 3 deaths in patients due to complications related to the disease. We investigated whether the differential expressed miRNAs correlated with the patient's clinico-pathological parameters. The expression of miR-326, miR-26a-1-3p, miR-139-3p, miR-101-3p, miR-146a-5p and miR-144-3p was significantly lower associated in younger patients (< 60 years, $p < 0.05$), the expression of miR-328-3p and miR-144-3p was associated with further metastases in the aLNs ($p < 0.05$), miR-26a-1-3p,miR-144-3p and miR-323-3p

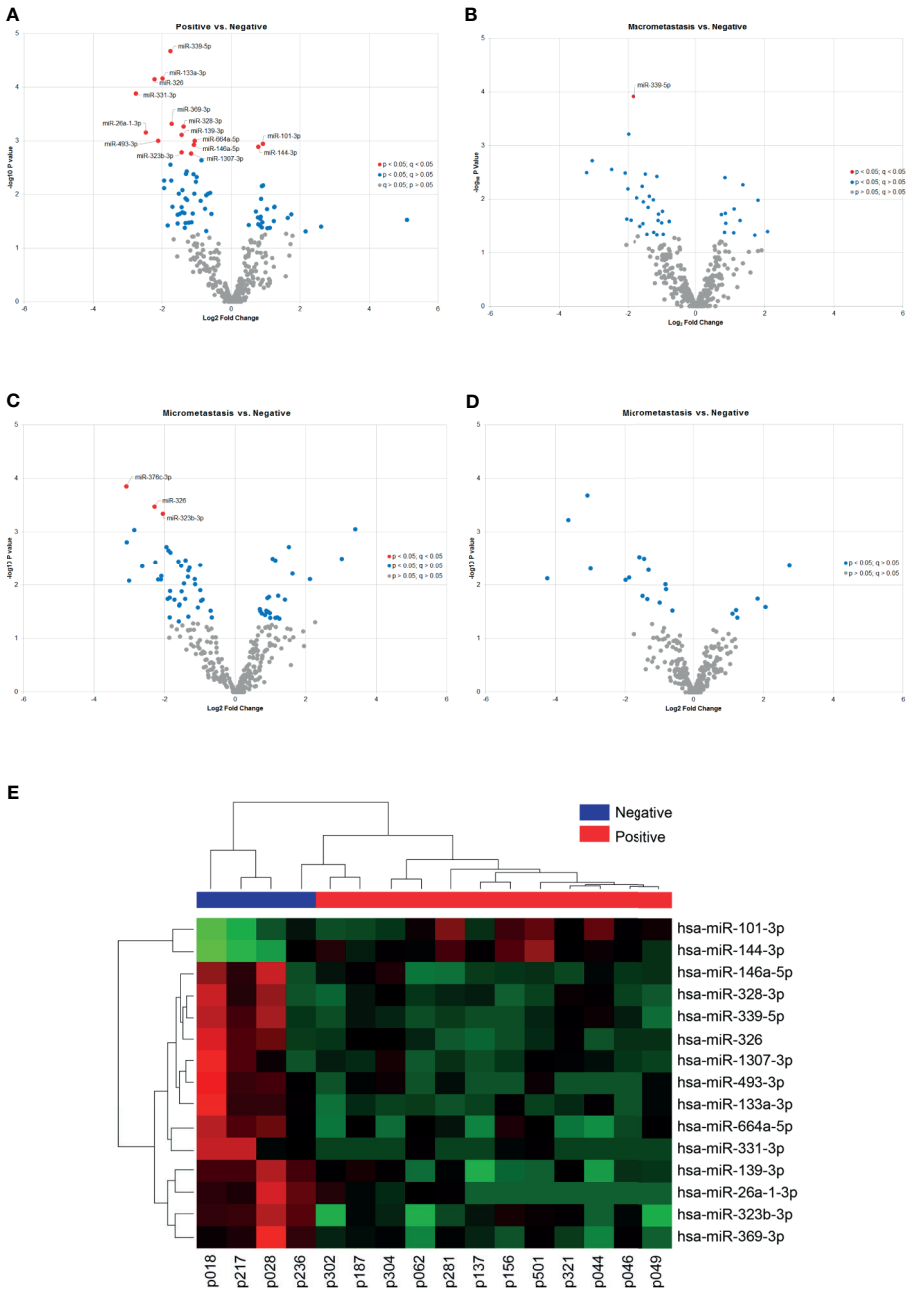

FIGURE 3 | Differentially expressed miRNAs according to the SLN status. **(A–D)** The volcano plots show differentially expressed miRNAs in plasma samples according to the patients' locoregional metastatic status as indicated. Only significant miRNAs with corrected q values < 0.05 are shown in the plots (red dots). The data show the relationship between non-adjusted p values (y-axis) and the fold change (x-axis) between the experimental groups. **(E)** Heat map and hierarchical clustering analyzed by samples and miRNAs. The analysis was performed using the 15 miRNAs differentially expressed between patients with positive and negative SLNs. Each row represents one miRNA and each column represents one sample. The color scale shows the expression level above (red) or below (green) the mean.

were associated with tumor stage ($p < 0.05$), miR-664a-5p and miR-323b-3p showed a non-significant association with tumor status ($p = 0.077$ and $p = 0.069$, respectively) and miR-26a-1-3p showed a non-significant correlation with the recurrence status ($p = 0.067$). We did not find any other significant association with other parameters. Due to the low number of events, we were unable to perform any survival analysis in our cohort of patients.

DISCUSSION

In recent years, miRNAs have emerged as important regulators of the various steps of the metastatic process (41). Currently, lymph node affection remains the most important prognosis factor in breast cancer (2) and the presence of metastasis in the SLNs is still currently the recommended procedure for axillary

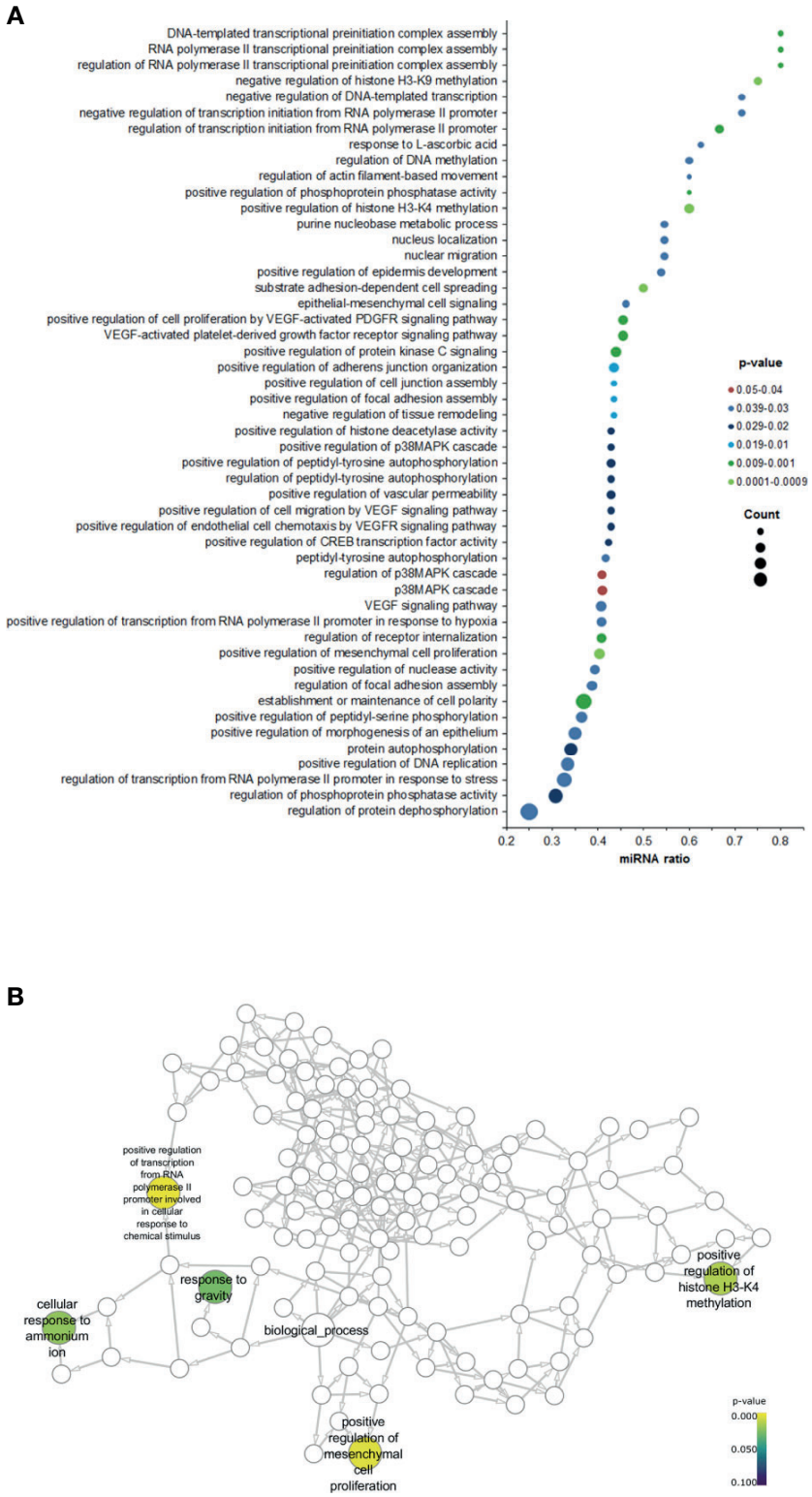

FIGURE 4 | Gene ontology (GO) enrichment analysis for the significant biological processes associated with positive SLNs. **(A)** Dot plot graph shows the 50 most significant biological process GO terms (y-axis) and the ratio between the number of expressed miRNAs associated to the GO term and the number of significantly differentially expressed genes associated to the GO term (x-axis). The color of the nodes indicates the p-value and the size of the nodes the number of miRNAs associated with a specific GO term. **(B)** Neural network shows the GO terms for the biological processes associated with patients with positive SLNs.

TABLE 3 | Gene Ontology (GO) analysis.

GO ID	GO Term	Counts	p value
BIOLOGICAL PROCESS			
GO:0051571	positive regulation of histone H3-K4 methylation	9/15	0.0017
GO:0042462	eye photoreceptor cell development	12/26	0.0017
GO:0070555	response to interleukin-1	17/40	0.0021
GO:0002053	positive regulation of mesenchymal cell proliferation	25/62	0.0028
GO:0071320	cellular response to cAMP	12/23	0.0034
GO:0002407	dendritic cell chemotaxis	4/5	0.0035
GO:0009629	response to gravity	6/8	0.0042
GO:0007097	nuclear migration	6/9	0.006
GO:0051573	negative regulation of histone H3-K9 methylation	6/8	0.0064
GO:0034446	substrate adhesion-dependent cell spreading	8/16	0.0067
CELLULAR COMPONENT			
GO:0031091	platelet alpha granule	18/44	0.0065
GO:0031983	vesicle lumen	17/46	0.0137
GO:0060205	cytoplasmic membrane-bounded vesicle lumen	17/46	0.0137
GO:0031093	platelet alpha granule lumen	16/41	0.0171
GO:0034774	secretory granule lumen	16/41	0.0171
GO:0044306	neuron projection terminus	9/20	0.0216
GO:1902495	transmembrane transporter complex	14/31	0.0218
GO:1990351	transporter complex	14/31	0.0218
GO:0015030	Cajal body	8/16	0.0239
GO:0034704	calcium channel complex	7/12	0.0245
MOLECULAR FUNCTION			
GO:0008528	G-protein coupled peptide receptor activity	9/15	0.0013
GO:0001618	virus receptor activity	6/13	0.0018
GO:0030955	potassium ion binding	5/6	0.0064
GO:0005161	platelet-derived growth factor receptor binding	17/39	0.0078
GO:0008798	beta-aspartyl-peptidase activity	4/7	0.0097
GO:0030545	receptor regulator activity	15/36	0.0097
GO:0003777	microtubule motor activity	11/20	0.0115
GO:0046625	sphingolipid binding	5/6	0.0128
GO:0008307	structural constituent of muscle	6/8	0.0131
GO:0017022	myosin binding	6/11	0.0144

Gene set enrichment analysis using GO categories (biological process, cellular component, molecular function) was applied to extract biological meaning from the identified differentially expressed transcripts and predicted mRNA targets. The top 10 GO categories associated with differentially expressed circulating miRNAs in patients with positive SLNs are shown. Counts refers to the ratio between the number of enriched differentially expressed miRNAs and the total number of miRNAs assigned to these terms. P values were calculated with a combination of the Elim method and the Fisher's exact method. GO terms with p values < 0.05 were considered enriched.

staging of early breast cancer. The accurate evaluation of patients with involved SLN determines further axillary lymph node dissection (ALND), the golden standard procedure for invasive breast cancer. However, ALND has been questioned in recent years because of inherent morbidity following the procedure without directly contributing to survival. In this study, we sought to gain a better understanding of the role of miRNAs in the metastatic process and whether specific expression patterns of miRNAs could predict SLN metastatic status in patients with early breast cancer. We performed a proof-of-principle study in plasma samples from 16 breast cancer patients with known SLN metastasis status. Importantly, plasma samples were collected prior to any treatment, thus the results using RNA-sequencing reflect the basal miRNA expression prior to any therapeutic intervention in these patients. Our results show a good quality sequencing data with mapping rates to miRNAs and comparable miRNA discovery across samples. Thus we are confident in the accuracy of the reported results.

Our data shows that 16 miRNAs were significantly differentially expressed in plasma samples from SLN-positive patients. Overall, we found a general down-regulation of miRNAs, with the exception of miR-101-3p and miR-144-3p that showed a 1.9- and 1.7-fold change up-regulation, respectively. However, we could not confirm the up-regulation of these 2 miRNAs and these results agree with the discrepancies on the direction of the dysregulation for both miRNAs. For instance, dysregulation miR-101-3p has been reported in several malignancies, including breast cancer (42, 43). While some reports indicate up-regulation of miR-101-3p, others indicated the opposite (42). This due to the fact that mature miR-101-3p originates from two different precursors located at different chromosomes. One precursor may be processed to 1 or 2 miRNAs and thus, the mature and precursor miRNA levels might not correlate, and this therefore will influence the clinical interpretation. The same study looked at putative miR-101-3p target genes were analyzed and the most predominant functions were transcription, metabolism, biosynthesis, proliferation, and transcription factor binding. This result indicated that candidate genes have a definitive impact on the pathogenesis of BC (42). Similar conflicting data has been reported for miR-144-3p. In several human cancers, the expression of miR-144-3p has been shown decreased (44), but in animal models repression of miR-144 significantly decreased cell proliferation, clonogenicity, migration and tumor formation in nude mice (45). Interestingly,

one report has shown that up-regulation of miR-144-3p was associated with families at high-risk for breast cancer (46). These data suggest that the role of miR-144-3p might differ by cancer type and tumor microenvironment.

Of those miRNAs down-regulated, miR-339-5p showed a 3.5-fold inhibition in patients with positive SLN metastasis. The expression of miR-339-5p remained significant when the analysis was performed in the subgroup of macrometastatic SLNs and we observed a non-significant trend towards significance for the subgroup of micrometastatic SLNs (q = 0.071). Our results agree with previous reports showing that reduced miR-339-5p expression in breast cancer is associated with increased metastasis to lymph nodes (47, 48), high clinical stages and worse clinical outcome (47). A similar association with positive LN has been reported in NSCLC patients (49). In addition, miR-339-5p expression is down-regulated in several human cancers including NSCLC (49), ovarian carcinoma (50), hepatocellular carcinoma (51), gliomas (52), colorectal cancer (53), osteosarcoma (54) and breast cancer (48). Mir-339-5p acts as a tumor suppressor gene and its expression is required to inhibit cell migration and invasion in breast cancer cells (47) in a mechanism that involves at least the B-cell lymphoma 6 (BCL6) protein. The authors showed that forced expression of BCL6 results in increased proliferation, anchorage-independent growth, migration, invasion and survival of breast cancer cell lines, whereas knockdown of BCL6 expression reduced these oncogenic properties of breast cancer cells (55). Interestingly, miR-339-5p has been shown to inhibit migration and invasion by targeting BCL6 in breast cancer (56), ovarian cancer cell lines (50) and in NSCLC (57). In addition, miR-339-5p down-regulation in NSCLC inhibits metastasis of NSCLC by regulating the epithelial-to-mesenchymal (EMT) transition *via* BCL6 (57). A recent report has shown that miR-339-5p regulates EMT through regulation of TGF-β (58) in osteosarcoma (54).

The EMT and the TGF-β pathways are two of the most important mechanisms underlying the metastatic ability of cancer cells (59, 60). We have previously shown the importance of the EMT in breast cancer (61) and here, we show that GO term analysis based on the DE miRNAs showed a significant association with the biological process "positive regulation of mesenchymal cell proliferation" (GO:0002053). Another pathway enriched was the positive regulation of H3 K4 methylation (GO:0051571), a mark that on a genome-wide scale is broadly associated with transcriptional regulation, and "negative regulation of H3K9 methylation" (GO:0051573). H3K9 methylation has been associated with the EMT through interactions of KDM1A (a H3K9 demethylase) with the members of the SNAI1 family of zinc finger transcription factors, including SNAI1 (SNAIL) and SNAI2 (SLUG). The expression of SNAI1 and E-cadherin is a hallmark of carcinoma development and metastasis (62). Our data suggest that that MiR-101 could be involved in the regulation of these pathways, as it has been shown to directly target the histone methyltransferase enhancer of zeste homologue 2 (EZH2), which could promote tumor proliferation and invasion (63).

Among the other circulating miRNAs that were down-regulated in our study, the expression of miR-133a-3p has been reported to be down-regulated in paired breast cancer tumor and serum samples (64), suggesting the tumor origin of miR-133a-3p. In contrast, miR-133a-3p has been found elevated in plasma samples from early-stage BC patients compared to healthy donors (65, 66) and similar results have been reported for circulating miR-1307-3p (67). On the other hand, down-regulation of miR-376c-3p (68) and miR-376c-3p have been linked to breast cancer recurrence (24). MiR-326 has been reported to target B7-H3 in breast cancer, an immunoregulatory protein that is overexpressed in several cancers and is often associated with metastasis and poor prognosis (69). Furthermore, its expression has been shown to inhibit tumorigenesis through direct targeting of Nin one binding protein (NOB1) and the MAPK pathway in glioma cells (52).

A main limitation of our study is the small number of samples analyzed since it was designed as a proof-of-principle study to assess the feasibility of using circulating miRNAs as potential surrogates of the lymph node metastatic status in breast cancer. Therefore, our results are preliminary and must be interpret with caution. Nonetheless, our data shows several circulating miRNAs that are significantly differentially expressed in relation to the SLN metastatic status of the patients. Moreover, we report an overall down-regulation of these miRNAs, which in most cases have been reported to be direct targets of proteins that promote metastasis. Further studies in a larger cohort of patients are warranted to validate these results and to unveil the molecular mechanisms of the miRNAs described here and the various steps of the locoregional metastasis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available at the Sequence Research Archive under ID PRJNA669408 and are available for download here http://www.ncbi.nlm.nih.gov/bioproject/669408.

ETHICS STATEMENT

This study was conducted according to the Declaration of Helsinki principles, with approval from the Clinical Research Ethics Committee at "Institut d'Investigació' Biomédica Sant Pau" (IIB Sant Pau). Written informed consent was obtained from all patients under institutional review board-approved protocols. All methods were performed in accordance with the relevant guidelines and regulations.

AUTHOR CONTRIBUTIONS

DE, LL-V, and AB were involved in the conceptualization of the project. DE, LL-V, OB, JM, IP, AM, CA, BGV, TR and EL were involved in resources, investigation and methodology. DE, LL-V, EL,

and AB were involved in analysis and interpretation of data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.627811/full#supplementary-material

Supplementary Figure 1 | Data quality checking. **(A)** Average read quality of the next generation sequencing (NGS) data. The average read Q-score is plotted on the x-axis and the number of reads on the y-axis. A Q-score above 30 is considered high quality data. **(B)** Base quality of the NGS data. The position in the read is plotted on the x-axis and the Q-score is plotted on the y-axis. The red line is the median Q-score and the dark blue is the mean value Q-score. A Q-score above 30 (>99.9% correct) is considered high quality data. **(C)** Read length distribution and adaptor trimming. miRNAs will appear as a peak around 18-23 nucleotides. **(D)** Radar plot showing relative spike-in signal for the samples. A range of spike-ins was added to the samples prior to RNA isolation. We observed an excellent correlation of counts corresponding to the spike-ins between the samples.

Supplementary Figure 2 | Summary of the mapping results for the samples. **(A)** Total number of reads for each sample sequenced. **(B)** Read length distribution for each class of RNAs. The peak around 18-23 nucleotides (red) corresponds to miRNAs.

Supplementary Figure 3 | RNA-sequencing validation. Relative gene expression was performed according to the comparative ddCt (ΔΔCt) method using negative metastatic samples as reference. The geNorm or the Normfinder algorithm were used to select the best combination of two reference genes. Data from multiples plates were normalized using UniSp3 spike-in as interplate calibrators. Each microRNA was assayed twice by qPCR on the Serum/plasma Focus microRNA PCR panel. **(A)** Data shows the comparison between patients with negative and positive SLNs. **(B)** Patients with positive SLNs were divided according to the presence or absence of further axillary lymph nodes (aLNs). Statistical analysis was performed using unpaired Student's t-test. All comparisons shown are statistically significant ($p < 0.05$).

Supplementary Table 1 | Mapping and yield results of the sequencing data. Reads are classified into the following classes: miRNA, maps to mirBase_20; small RNA, maps to sRNA database compiled by Exiqon; Predicted miRNA, predicted miRNAs using a prediction software; Genome, reads aligning to the reference genome, but not to miRNAs or sRNAs; Outmapped, maps corresponding to poly A and poly homopolymers as well as abundant rRNA and mtRNA; Unmapped, reads not aligning to reference genome. Median values for each type of reads are shown.

Supplementary Table 2 | Predicted miRNA results of the sequencing data. Predicted miRNAs for each sample sorted by total read count. The sequences reported do not match any known miRNA in the miRBase_20. The location is formatted as "chromosome: start-stop (strand)". Counts describes the number of reads which fall onto the location of the predicted miRNA (sum of all samples). Note that the name and numbering assigned here are project-specific and cannot be compared with the nomenclature found in the miRBase or other databases.

Supplementary Table 3 | Contributing miRNAs to GO terms for the categories biological process (BP), molecular function (MF) and cellular compartment (CC).

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REVIEW
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Adjuvant Treatment Recommendations in Early-Stage Endometrial Cancer: What Changes With the Introduction of The Integrated Molecular-Based Risk Assessment

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Adjuvant therapy recommendations for endometrial cancer were historically based on the individual patient's risk of disease recurrence using clinicopathologic factors such as age, stage, histologic subtype, tumor grade, and lymphovascular space invasion. Despite the excellent prognosis for early stages, considerable under- and overtreatment remains. Integrated genomic characterization by the Cancer Genome Atlas (TCGA) in 2013 defined four distinct endometrial cancer subgroups (POLE mutated, microsatellite instability, low copy number, and high copy number) with possible prognostic value. The validation of surrogate markers (p53, Mismatch repair deficiency, and POLE) to determine these subgroups and the addition of other molecular prognosticators (CTNNB1, L1CAM) resulted in a practical and clinically useful molecular classification tool. The incorporation of such molecular alterations into established clinicopathologic risk factors resulted in a refined, improved risk assessment. Thus, the ESGO/ESTRO/ESP consensus in 2020 defined for the first time different prognostic risk groups integrating molecular markers. Finally, the feasibility and clinical utility of molecular profiling for tailoring adjuvant therapy in the high-intermediate-risk group is currently under investigation (NCT03469674).

Keywords: endometrial cancer, molecular classification, adjuvant treatment, recommendations, risk factors

OVERVIEW

Endometrial cancer (EC) is the most common gynecological cancer in developed countries; the majority of cases are diagnosed at an early stage and addressed to surgical treatment (1). Traditionally, ECs have been categorized into two pathogenetic types based on clinical, metabolic, and endocrine characteristics: type I tumors (60–70%), associated with estrogen excess, obesity, hormone-receptor positivity, and endometrial hyperplasia, with favorable

outcomes, and type II tumors (30–40%), more common in non-obese women, associated with an atrophic endometrium, with aggressive clinical behavior and poor outcome (2).

Adjuvant therapy recommendations have traditionally been based on the individual patient's risk of disease recurrence using clinicopathologic factors such as age, stage, histologic subtype, tumor grade, and lymphovascular space invasion (LVSI) (3, 4). In particular, the ESMO/ESGO/ESTRO (European Society for Medical Oncology–European Society for Radiotherapy and Oncology–European Society of Gynaecological Oncology) consensus in 2016 proposed five risk groups to guide adjuvant therapy use (low, intermediate, high-intermediate, high, advanced/metastatic) (4).

Overall, risk-adapted treatments achieve excellent prognosis for early-stage type I ECs, with 10-year overall survival exceeding 80% (5, 6).

However, a small but substantial number of patients with favorable prognostic background unexpectedly experience recurrence of disease and poor survival (5–8), and it has been calculated that up to 10% of them will experience distant metastasis (7).

On the other hand, a not-negligible number of patients with unfavorable prognostic factors that are usually treated will never experience recurrence: in particular, seven high-intermediate-risk patients need to undergo vaginal brachytherapy (EBRT) to prevent one recurrence (7).

In 2013, the Cancer Genome Atlas (TCGA) defined four distinct EC subgroups (POLE mutated, microsatellite instability, low copy number, and high copy number) with possible prognostic value, and many others confirmed these data in external cohorts (3–5, 7, 8). Molecular risk classes are not completely superimposable with clinicopathological categories, but the combination of both models has been shown to perform better in terms of prognosis than the single ones by themselves (7).

The most recent ESGO/ESTRO and the European Society of Pathology (ESP) ESP recommendations, published at the end of 2020, incorporated molecular and clinicopathological features in an integrated classification system in order to guide adjuvant treatment choices (9).

A COMPREHENSIVE GENOMIC AND TRANSCRIPTOMIC ANALYSIS THROUGH NEXT-GENERATION SEQUENCING (NGS): A GENOMIC CLASSIFICATION

In 2013, the Cancer Genome Atlas Research Network (TCGA) has reported a comprehensive genomic and transcriptomic analysis of 373 EC cases, mainly endometrioid (307) (8).

This characterization categorized EC tumors into four genomic classes with different molecular and prognostic profiles. Such molecular analyses were proven feasible (>96%) and highly reproducible in external cohorts of patients (7, 10, 11). The distribution of histologic subtypes into the four molecular classes is reported in **Figure 1**.

POLEmut

This molecular class is defined by pathogenic mutations in the exonuclease domain of DNA polymerase epsilon (10).

This gene encodes a catalytic subunit of DNA polymerase epsilon involved in nuclear DNA replication and repair. The most common alterations in POLE detected in EC samples are hotspot mutations at P286R, V411L, S297F, A456P, and S459F (10, 12–14).

Overall, this genomic class is associated with excellent prognosis. It accounts for less than 10% of all EC cases, and it is associated with low copy-number aberrations and a very high mutational burden (232×10^{-6} mutations/Mb).

The associated morphological characteristics of this subgroup include high rate of tumor-infiltrating lymphocytes and/or peritumoral lymphocytes, morphologic heterogeneity/ambiguity, bizarre/giant tumor cell nuclei, endometrioid histotype but also clear cell carcinomas, undifferentiated carcinomas, and carcinosarcomas (13, 15).

Approximately 65% of this molecular class is associated with intermediate and high-risk phenotype according to ESMO 2013 classification (16). In particular, it is frequently associated with grade 3 endometrioid cancers. Given the favorable prognosis of this subgroup, no adjuvant treatment could be suggested, reducing the possible overtreatment, particularly in the high-intermediate- and high-risk group. The PORTEC-4a trial (ISRCTN11659025) will answer whether this strategy is safe and efficient in the high-intermediate-risk subgroup (17).

Microsatellite Unstable (MSI Hypermutated)

This molecular class is characterized by the presence of microsatellite instability.

MSI represents the phenotypic evidence that DNA mismatch repair (MMR) is not functioning normally. The MMR is a system for recognizing and repairing erroneous insertion, deletion, and misincorporation of bases that can arise during DNA replication and recombination, as well as repairing other forms of DNA damage.

The most common alteration in MMR detected in EC samples is MLH1 promoter methylation (8).

Overall, this genomic class is associated with intermediate prognosis.

It encounters for 25–30% of all EC cases and is characterized by low copy-number aberrations and high mutational burden (18×10^{-6} mutations/Mb).

The associated morphological characteristics of this class include lower uterine segment location, mainly endometrioid histology, mucinous differentiation, tumor-infiltrating lymphocytes, peritumoral lymphocytes, and with lymphovascular space invasion, mainly substantial (18–20).

Approximately 30% of this molecular class is associated with the low-risk phenotype according to ESMO 2013 classification (16).

Around 10–14% of EC MMRd patients are estimated to have a Lynch syndrome. In particular, chances are higher in case of MSH2−/MSH6− or PMS2− and lower in case of MLH1− (40 and 67% *vs* 2%) (21, 22).

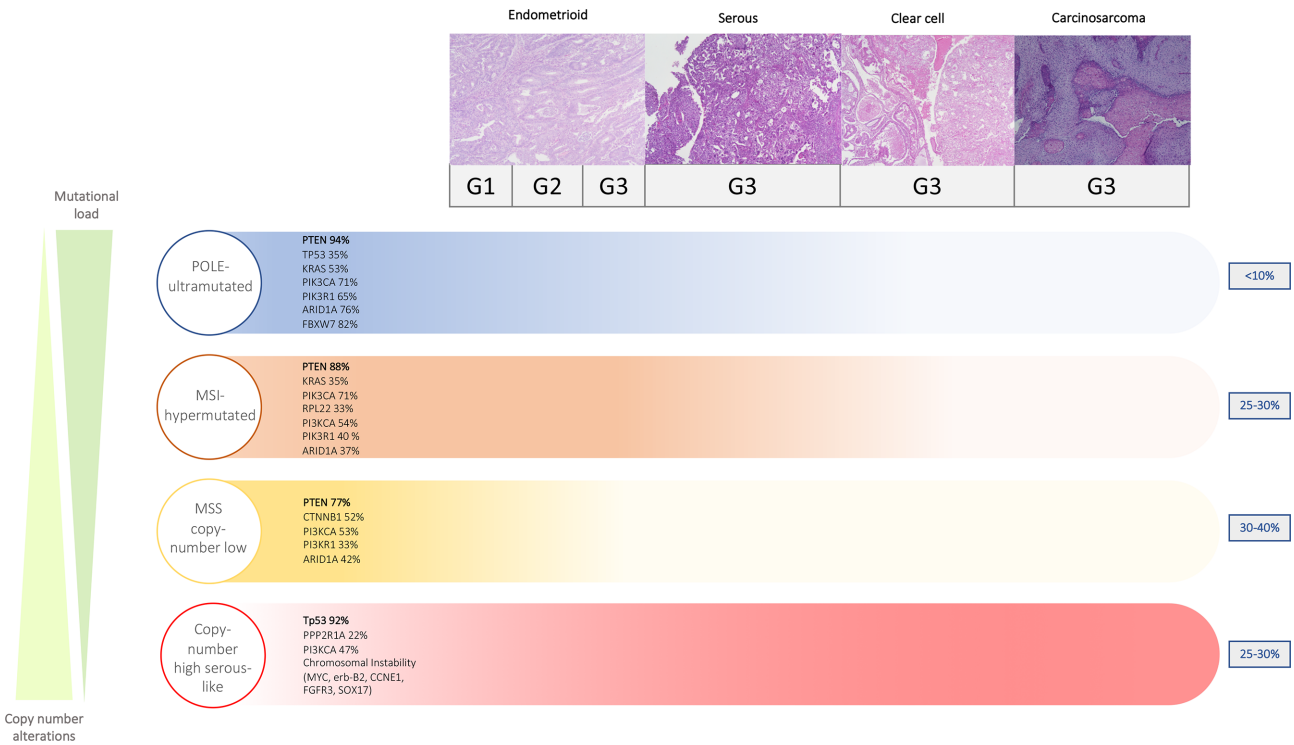

FIGURE 1 | Distribution of molecular features across different EC histotypes.

Copy-Number High (Serous-Like)

This genomic class is defined mainly by TP53 mutations (8).

P53 gene encodes for p53 protein (TP53) mainly involved in cell cycle arrest, DNA repair, senescence, apoptosis induction, but also cell metabolism regulation and cell response to oxidative stress (23).

Overall, this genomic class is associated with unfavorable prognosis.

It encounters for 25–30% of all EC cases and is characterized by a low mutation rate ($2{\cdot}3 \times 10^{-6}$ mutations/Mb) and high copy-number aberrations.

The associated morphological characteristics of this class include serous, endometrioid, and mixed serous and endometrioid histology, grade 3, poor inflammatory stroma (23).

Approximately 25% of this molecular class is associated with low- and intermediate-risk phenotypes according to ESMO 2013 classification (16). About 4% of EC are classified as multiple classifier at molecular profiling, and when POLE and p53abn coexist, the prognosis is driven by POLE. In the same way, when MMR-d and p53abn coexist, the prognosis is driven by MMR-d (24).

Copy-Number Low

This genomic class comprises mainly microsatellite-stable cancers characterized by frequent CTNNB1 mutations (8).

Overall, this genomic class is associated with intermediate prognosis.

It encounters for 30–40% of all EC cases and is characterized by a low mutational burden (2.9×10^{-6} mutations/Mb) and low copy-number aberrations.

The associated morphological characteristics of this class include endometrioid histology, grade 1–2, very poor inflammatory stroma (25).

Approximately 50% of this molecular class is associated with the low-risk phenotype according to ESMO 2013 classification (16).

FROM NGS TO IMMUNOHISTOCHEMISTRY (IHC): THE MOLECULAR EC CLASSIFICATION

Methodologies used for the TCGA study are costly, complex, and unsuitable for wider implementation in clinical practice. In 2015, a pragmatic molecular classifier based on surrogate immunohistochemistry assays was developed and validated in internal and external cohorts (10, 11, 26, 27). It was aimed at replicating and replacing the TCGA classification, which relied on whole-exome sequencing (WES). This approach was tested by multiple study groups, which makes evidences concerning its feasibility and reliability especially robust (28).

The "MSI hypermutated" group was identified as MMR deficient (MMRd) using MMR IHC testing (MLH1, MSH2,

MSH6, and PMS2), and it showed high concordance with MSI assay by NGS.

The "high copy number" group was identified as p53-abnormal (p53abn) determining p53 status by IHC testing; the subgroup obtained, however, was not completely equivalent to the TCGA one.

No surrogate was found for POLEmut detection; thus, NGS was maintained.

The "low copy number" group was determined by exclusion and called non-specific molecular profile (NSMP).

INTEGRATED CLINICOPATHOLOGIC AND MOLECULAR CLASSIFICATION: THE ESGO/ESTRO/ESP 2020 RISK CLASSIFICATION AND ADJUVANT TREATMENT RECOMMENDATIONS

The integration of molecular and clinicopathological factors in early-stage ECs in various PORTEC trials cohorts resulted in a stronger model with improved risk prognostication (7).

In particular, the AUC of the integrated molecular risk assessment showed a substantial improvement in predicting locoregional recurrence, distant recurrence, and overall survival compared to clinicopathological classification alone.

Its main implication is to guide clinicians' choices in terms of fertility-sparing treatments, surgery, adjuvant therapy, and surveillance in order to improve outcomes for women with EC.

In the light of available evidences, the ESGO/ESTRO/ESP decided to jointly update EC management evidence-based guidelines, implementing the use of molecular classification.

Risk group classification includes both cases that undergo molecular profiling and cases who did not. If molecular classification tools are not available, traditional pathologic features are used to classify EC patients. The main characteristics of the large trials included in the consensus, which guided treatment decision making, are summarized in **Table 1**.

Clinicopathological factors include the following:

- age
- International Federation of Gynecology and Obstetrics (FIGO) stage 2009
- depth of myometrial invasion
- tumor differentiation grade
- tumor type (endometrioid *vs* non-endometrioid)
- lymphovascular space involvement (LVSI)

Molecular features include the following:

- POLE mutation analysis by DNA sequencing
- p53 assessed by IHC
- MLH1, MSH2, MSH6, and PMS2 assessed by IHC

A consensus definition for LVSI in the literature is lacking. It reported good inter-observer agreement in discriminating "true LVSI" from "LVSI mimics" and in grading the extent of LVSI through a semiquantitative system (33). Nevertheless, some problematic cases exist. In addition, substantial LVSI in EC seems to have a stronger prognostic significance than focal LVSI (34–36).

Overall, the new ESTRO/ESGO/ESP guidelines published in 2020 integrate molecular into clinical classification and encouraged molecular classification in all EC especially high-grade tumors with only POLE mutation analysis possibly omitted in low-risk and intermediate-risk carcinoma with low-grade histology. Based on this, p53abn tumors with myometrial invasion are considered and treated as high-risk patients with chemotherapy or the combination of chemotherapy and radiotherapy. Stage I–II POLEmut ECs without residual disease are considered low-risk patients for which no adjuvant treatment is recommended.

Low-Risk Class

This risk class includes patients with one of the following conditions:

- FIGO 2009 stage IA (<50% myometrial invasion), endometrioid histology, grade 1, LVSI negative
- POLEmut in FIGO 2009 stage I–II EC without residual disease
- MMRd/NSMP in FIGO 2009 stage IA G1, LVSI negative or focal

Routine lymphadenectomy for nodal staging purposes is generally not recommended for this group (37, 38). Sentinel lymph node biopsy can be considered for staging purposes, but it can be omitted in cases without myometrial invasion (38, 39). The incidence of recurrence after surgery alone is <5% (40). No adjuvant treatment is recommended for this group.

Intermediate-Risk Class

This risk class includes patients with one of the following conditions:

- FIGO 2009 stage IB (<50% myometrial invasion), endometrioid histology, grade 1–2, LVSI negative or focal
- FIGO 2009 Stage IA endometrioid, grade 3, LVSI negative or focal
- FIGO 2009 Stage IA non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) without myometrial invasion
- MMRd/NSMP in FIGO 2009 stage IB, endometrioid histology, grade 1–2, LVSI negative or focal
- MMRd/NSMP in FIGO 2009 stage IA, endometrioid, G3, + high-grade, LVSI negative or focal
- p53abn in FIGO 2009 stage IA without myometrial invasion

Lymphadenectomy can be considered as a staging procedure to better tailor adjuvant treatment (37, 41).

The incidence of recurrence after surgery alone is between 5 and 10% (29, 32, 42, 43).

EBRT is recommended to decrease vaginal recurrence since it has been shown to reduce the risk of local relapse (29, 32, 42, 43). Observation is an option, especially for patients aged <60 years (44).

TABLE 1 | Relevant clinical trials for the ESGO/ESTRO/ESP consensus.

Clinical trial	Reference	Years	Numberof patients enrolled	Inclusion criteria	Study design	Treatments	Conclusions	Note
PORTEC-1	Creutzberg et al. (29) Lancet	1990–1997	714	• Stage IC grade 1–2 • Stage IB Grade 2–3 • Endometrial adenocarcinoma	RCT 1:1	EBRT (46 Gy using 2 Gy daily fractions) *vs* NAT	EBRT significantly reduced the risk of locoregional recurrence, without survival benefit.	• Routine lymphadenectomy not performed
PORTEC-2	Nout et al. (30) JCO	2002–2006	427	• Age >60, stage 1 grade 1–2 • Age >60, stage 1 grade 3 • Any age and stage 2A grade 1–2 or grade 3 with <50% invasion	RCT 1:1	Pelvic EBRT (46 Gy in 23 fractions) *vs* VBT (21 Gy HDR in 3 fractions, or 30 Gy LDR)	VBT is effective in preventing vaginal recurrence.	• Routine lymphadenectomy not performed
PORTEC-3	De Boer et al. (31) Lancet	2006–2013	660	• Stage 1A endometrioid grade 3, LVSI+ • Stage IB endometrioid grade 3 • Stage II endometrioid • Stage IIIA, IIIB IIIC endometrioid Serous EC with invasion), IB, II, or III. • Clear-cell EC with stages IA (with invasion), IB, II, or III. stages IA (with	RCT 1:1	EBRT (48·6 Gy in 1,8 Gy fractions given on 5 days per week) *vs* radiotherapy and chemotherapy (consisting of two cycles of cisplatin 50 mg/m2 given during radiotherapy, followed by four cycles of carboplatin AUC5 and paclitaxel 175 mg/m^2)	EBRT+CHT for high-risk endometrial cancer did not significantly improve overall survival but improved 5-year failure-free survival compared with EBRT alone.	• Routine lymphadenectomy not performed
GOG-99	Keys et al. (32) Gyn Oncol	1987–1995	392	• IB • IC • IIA (occult) • IIB [occult]	RCT 1:1	EBRT 50.40 Gy given more than 28 fractions of 180 cGy *vs* NAT	EBRT decreases the risk of recurrence, but should be limited to high-intermediate-risk patients.	cycles of carboplatin AUC5 and paclitaxel 175 mg/m^2)Selective bilateral pelvic, and para-aortic lymphadenectomy
ASTEC/EN5	ASTEC/EN.5 Study Group, Lancet 2009	1996–2005	905	• FIGO stage IA G3 • IB grade 3 • IC all grades • Papillary serous all stages and grades • Clear-cell histology all stages and grades	RCT 1:1	EBRT (40– 46 Gy in 20–25 daily fractions) *vs* NAT	EBRT did not improve overall survival compared to observation.	• Lymphadenectomy as part of surgical staging was not a requirement

RCT, randomized control trials; EBRT, external beam radiotherapy; VBT, vaginal brachytherapy; CHT, chemotherapy; FIGO, International Federation of Gynecology and Obstetrics 1999; NAT, non-adjuvant treatment.

For p53abn FIGO 2009 stage IA without myometrial invasion cases, adjuvant treatment should be discussed on a case-by-case basis since specific data are missing.

High-Intermediate-Risk Class

This risk class includes patients with one of the following conditions:

- FIGO 2009 stage IA, regardless of grade or depth of invasion with LVSI unequivocally positive
- FIGO 2009 stage IB, grade 3, regardless of LVSI status
- FIGO 2009 Stage II

Lymphadenectomy for nodal staging purposes can be considered (45).

The incidence of recurrence after surgery alone is between 12 and 14% (29, 32).

For those patients who underwent surgical nodal staging documenting negative nodes, VBRT is recommended to decrease vaginal recurrence, but no adjuvant therapy with close follow-up is an alternative acceptable option (4). In the case of substantial LVSI, EBRT can be considered in order to reduce the risk of pelvic and para-aortic nodal relapse (46). Similarly, cases displaying grade 3 tumors and/or substantial LVSI could benefit from adjuvant chemotherapy (31).

In patients for which lymph nodal status is unknown, VBRT is recommended for those patients who have LVSI negative, while EBRT is recommended for LVSI unequivocally positive to decrease pelvic recurrence (31, 46). Systemic therapy is considered of uncertain benefit (31).

High-Risk Class

This risk class includes patients with the following characteristics:

- FIGO 2009 stage I non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) with myometrial invasion, and with no residual disease

- FIGO 2009 stage I p53abn endometrial carcinoma with myometrial invasion, with no residual disease.

There is no agreement on the role of lymphadenectomy in this risk class (4).

The 5-year incidence of recurrence (vaginal, pelvic, or distant) is around 41% (29, 31, 46).

For this class, EBRT with concurrent and adjuvant chemotherapy or alternatively sequential chemotherapy and radiotherapy is recommended (29, 31, 46–48). However, chemotherapy additional benefit is unclear for patients with clear-cell carcinomas. Chemotherapy alone can be an alternative option (49).

ADDITIONAL FEATURES

Additional prognostic risk factors such as L1CAM and mutations in exon 3 of CTNNB1 later emerged and demonstrated to better mark differences in terms of prognosis among the four classes (16, 34, 50–52). Overall, three different prognostic profiles were delineated (see **Table 2**).

These additional features and, as a consequence, these profiles were not included in the most recent guidelines but were adopted in PORTEC-4a study to assign adjuvant treatment in the experimental arm (17).

CTNNB1

CTNNB1 gene encodes β-catenin protein, involved in regulation and coordination of cell adhesion and cell signaling.

In particular, within the copy number low group, CTNNB1 exon 3 mutation status was found prognostic for distant recurrence in EC (7).

Although nuclear expression of β-catenin could be an IHC surrogate of CTNNB1 exon 3 mutations, NGS testing remains the gold standard (52–55).

CTNNB1 status helped distinguishing, within this class, a favorable group (CTNNB1-wild type) with a similar prognosis to POLEmut tumors, from an unfavorable group (CTNNB1-mutant), with a similar prognosis to MMRd.

L1CAM

L1CAM is a 200 to 220 kDa membrane glycoprotein of the immunoglobulin superfamily and is crucially involved in processes of neurogenesis (56).

The established ≥10% threshold for positivity was based on the cutoff that best correlated with prognosis (57). It has been shown that patients bearing L1CAM-positive cancers have poorer disease-free and overall survival (51).

L1CAM positivity was mainly, but not exclusively, found in intermediate- and high-risk cancers (13.2 *vs* 25.8% in low and intermediate, respectively) (51). Moreover, it was associated with histopathological high grade and increasing depth of myometrium infiltration (58).

Given the association with an overwhelming increase in the likelihood of distal or local recurrence and poor overall survival, its presence indicates the need for adjuvant treatment (51, 59).

CONCLUSIONS

The traditional dualistic histopathologic classification that split EC into two groups, type I and type II cancer, is not more adherent to practical necessity of the clinicians. In recent years it has become increasingly clear that the traditional classification lacks reproducibility and yields heterogeneous molecular groups, hampering advances and implementation of precision medicine. This is particularly problematic for future clinical trials with targeted approaches that will demand inclusion of cancers with molecular similarities. The endometrial cancer classification proposed by TCGA would serve this purpose well, as it is based upon the combination of somatic mutational burden and somatic copy number alterations. Moreover, several publications on large and clinically well annotated (trial) cohorts have shown that surrogate IHC markers can be utilized for a TCGA-inspired molecular classification in routine surgical pathology, without the need for extensive sequencing. These surrogate markers have been extensively studied and show good performance. The prognostic value has been well established, with POLEmut EC having an excellent outcome and p53abn EC having the poorest clinical outcome, independent of risk group, type of adjuvant treatment, tumor type, or grade. This implies that de-escalation of adjuvant treatment for POLEmut EC patients should be explored, as is currently being done in the clinical PORTEC4a trial. Furthermore, recent data strongly suggest that the benefit for the addition of chemotherapy in the adjuvant treatment is limited to p53mut EC, which includes most serous cancers but also a significant portion of other histologic subtypes such as carcinosarcomas, thus suggesting an escalation of adjuvant treatment with chemotherapy combined with radiation when p53 mutation is detected. The implementation of molecular classification into clinical classification has the potential to serve in improving patient management by reducing over- and undertreatment (60). The use of this novel classification in routine clinical practice and future trial designs should be encouraged. Currently, one trial (PORTEC-4a) is ongoing to determine whether adjuvant treatment can be based on a molecular-integrated risk profile rather than standard

TABLE 2 | Three different prognostic profiles in FIGO 2009 Stage I EC, delineated including additional molecular factors.

	FAVORABLE	INTERMEDIATE	UNFAVORABLE
Characteristics	• POLE mut OR • NSMP CTNNB1 WT	• NSMP CTNNB1 mut • MMRd	• P53abn • LVSI substantial • >10% L1CAM

clinicopathological risk factors in high-intermediate-risk EC patients. The preliminary report of the first 50 patients enrolled showed that molecular assessment is feasible, but patients' acceptance rate was not completely satisfactory (around 35%) (17).

Nevertheless, possible technical limits such as the need of assay harmonization as well as the lengthening of reporting times should be addressed.

AUTHOR CONTRIBUTIONS

CN: Conceptualization; Metholodology; Data curation; Roles/ Writing - original draft; FC: Data curation; AP: Data curation; SD: Data curation; GD: Supervision; Writing - review & editing. GS: Supervision; Writing - review & editing. DL: Funding acquisition; Supervision; Writing - review & editing. All authors contributed to the article and approved the submitted version.

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REVIEW
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Prediction Models for Complete Resection in Secondary Cytoreductive Surgery of Patients With Recurrent Ovarian Cancer

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The most advanced epithelial ovarian cancer develops recurrent disease despite maximal surgical cytoreduction and adjuvant platinum-based chemotherapy. Treatment with secondary cytoreductive surgery (SCS) combined with chemotherapy or with chemotherapy alone for patients with platinum-sensitive recurrent ovarian cancer (ROC) is currently under heated discussion. Encouragingly, the results of the AGO DESKTOP III Study and the SOC1/SGOG-OV2 trial, which have been published recently, showed a striking advantage in terms of overall survival (OS) and progression-free survival (PFS) of ROC patients undergoing SCS compared to chemotherapy alone; moreover, a benefit of SCS exclusively for patients with complete gross resection (CGR) was particularly highlighted. CGR is considered the ultimate goal of SCS, on condition that the balance between maximal survival gain and minimal operative morbidity is maintained. Several models have been proposed to predict the rate of CGR, such as the MSK criteria, the AGO score, and the Tian model, over the last 15 years. This summary is mainly about the several previously published prediction models for CGR in SCS of ROC patients and discusses the effectiveness and limitations of these prediction models.

Keywords: complete gross resection, recurrent ovarian cancer (ROC), MSK criteria, AGO score, Tian model, secondary cytoreductive surgery (SCS)

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INTRODUCTION

The Vital Significance of CGR in SCS

Ovarian cancer is the leading cause of death and the second most common gynecological cancer (1). Primary debulking surgery followed by platinum-based chemotherapy with or without first-line maintenance therapy with bevacizumab or emerging targeted drugs remains the standard treatment of advanced epithelial ovarian cancer (2). Despite the fact that 80% of patients attain clinical complete remission *via* initial therapy, unfortunately, about 80% of patients can relapse within 3 years, including platinum-resistant and platinum-sensitive recurrence. The average 5-year survival rate following recurrence is less than 10% (3). Surgery and medical treatment are the cornerstones of

recurrent ovarian cancer (ROC) therapy. For patients with platinum-resistant ROC, secondary cytoreductive surgery (SCS) is usually not indicated due to the limited life expectancy and surgical morbidity/mortality, while patients with platinum-sensitive ROC can be treated with SCS combined with chemotherapy (platinum-based) or with chemotherapy alone (4). SCS is defined as an operation performed on patients who have either persistent disease at the completion of a planned course of chemotherapy or who subsequently experience clinical relapse, and the survival benefits of surgery need to be weighed against the risks of morbidity and mortality (5, 6). As for platinum-sensitive ROC, the role of SCS in ROC has so far not been fully confirmed by prospective randomized surgical trials, although SCS has been listed as an optional treatment in the National Comprehensive Cancer Network (NCCN) guidelines, which is primarily based on the results of a few single-center and multicenter retrospective case studies and limited meta-analyses (6–8).

In fact, the biggest limitation of these studies is the inherent patient selection bias, which is difficult to avoid in the absence of randomized clinical trials. Stirringly, the final results of the AGO DESKTOP III trial (NCT01166737) were announced in an oral presentation at the 2020 ASCO Annual Meeting (Abstract 6000). The results showed that patients undergoing SCS combined with chemotherapy benefited in terms of median overall survival (mOS = 53.7 *vs.* 46.0 months) and median progression-free survival (mPFS = 18.4 *vs.* 14.0 months) compared with those undergoing chemotherapy alone without increased surgical morbidity/mortality. More importantly, the study confirmed that complete gross resection (CGR) of macroscopic disease was the key point and that patients with any residual disease (even optimal) did not benefit from SCS (mOS = 61.9 *vs.* 28.8 months), even worse than those having chemotherapy alone (mOS = 61.9 *vs.* 46.0 months) (9). Simultaneously, the results of the SOC1/SGOG-OV2 trial (NCT01611766) were presented at the meeting (Abstract 6001) and subsequently published online by Zang et al. (10, 11). Compared with chemotherapy alone, both PFS (17.4 *vs.* 11.9 months) and the median time to start of the first subsequent therapy (TFST = 18.1 *vs.* 13.6 months) were in favor of the patients accepting SCS combined with chemotherapy. Moreover, the interim OS analysis showed that mOS was 58.1 months (95% CI not estimable to not estimable) in the surgery group and 53.9 (42.2–65.5) months in the no-surgery group (hazard ratio 0.82, 95% CI 0.57–1.19). Besides, the median accumulating treatment-free survival (TFSa) rates were 46.8 months in the surgery group and 42.4 months in the no-surgery group. Mature data on OS and TFSa are still awaited. Combining the previous subgroup analysis results of the GOG213 Study in 2019, it was shown that 150 patients with CGR after SCS, compared with those with residual tumor after surgery (89 patients), had longer OS (56.0 *vs.* 37.8 months) and longer PFS (22.4 *vs.* 13.1 months) (12, 13) (**Table 1**). In summary, all three randomized clinical trials (RCTs) showed a significant statistical advantage in PFS in the SCS group, with an even more significant difference in patients with CGR (about a 7-month increase in PFS). Data on OS are different in these two completed trials. With respect to the inconsistent results, a large amount of discussion focuses on issues such as platinum-free interval, pattern of recurrence, BRCA (breast cancer gene) status, and the use of bevacizumab and/or poly-ADP ribose polymerase (PARP) inhibitors (14, 15). Recently, a meta-analysis encompassed the above three RCTs and showed that SCS was superior to chemotherapy alone in terms of PFS, and particularly with PFS and OS benefits in the CGR subpopulation (8).

Based on the three RCTs mentioned above, a point that draws our attention the most is that CGR has been robustly confirmed

TABLE 1 | Comparisons between the GOG213, AGO DESKTOP III, and SOC1/SGOG-OV2 trials.

		GOG213	AGO DESKTOP III	SOC1/SGOG-OV2
No. of patients		485	408	357
Year		2007–2011	2010–2014	2012–2019
Age (years)		57	60.5	54
Primary FIGO III–IV		86%	74.6%	82%
Selection criteria		CGR (individuation)	AGO score	iMODEL+ PET-CT
Histology (serous)		86%	85%	81%
Platinum-free interval (months)		19.7	19.9	16.1
CGR		67%	74.5%	76.7%
Mortality rate		30 days: 0.4%	90 days: 0.5%	60 days: 0%
Chemotherapy (platinum-based)		100%	89%	97%/96%
Bevacizumab (second-line)		84%	23%	1.1%
PARPi (second-line maintenance)		NA	3.9%	10.1%
Surgery *vs.* no surgery, *n* (HR, 95%CI)	mOS	50.6 *vs.* 64.7 (1.29, 0.97–1.72)	53.7 *vs.* 46.2 (0.76, 0.59–0.97)	58.1 *vs.* 53.9 (0.82, 0.57–1.19)[a]
	mPFS	18.9 *vs.* 16.2 (0.82, 0.66–1.01)	18.4 *vs.* 14.0 (0.66, 0.54–0.82)	17.4 *vs.* 11.9 (0.58, 0.45–0.74)
	TFST	NA	17.9 *vs.* 13.7 (0.65, 0.52–0.81)	18.1 *vs.* 13.6 (0.59, 0.46–0.76)
CGR *vs.* incomplete resection, *n* (HR, 95%CI)	mOS	56.0 *vs.* 37.8 (0.61, 0.40–0.93)	60.7 *vs.*28.8 (0.40, 0.28–0.59)	Pending
	mPFS	22.4 *vs.* 13.1 (0.51, 0.36–0.71)	21.2 *vs.* 13.7 (0.98, 0.71–1.35)	Pending
CGR *vs.* no surgery, *n* (HR, 95%CI)	mOS	56.0 *vs.* 64.7 (1.03, 0.74–1.46)	60.7 *vs.* 46.2 (0.57, 0.43–0.76)	Pending
	mPFS	22.4 *vs.* 16.2 (0.62, 0.48–0.80)	21.2 *vs.* 14.0 (0.56, 0.43–0.72)	Pending

FIGO, International Federation of Gynecology and Obstetrics; CGR, complete gross resection; PARPi, poly-ADP ribose polymerase inhibitor; HR, hazard ratio; TFST, time to start of the first subsequent therapy; NA, not applicable; mOS, median overall survival; mPFS, median progression-free survival.

[a]*Results of the interim overall survival analysis.*

as the most crucial survival determinant in ROC. The ultimate goal of SCS should be the removal of all visible tumors. This is consistent with previous studies. A meta-analysis on the role of SCS for ROC reported that each 10% increase of complete resection rate translates into a 3-month increase of OS (16). In addition, various studies on SCS have shown that achieving CGR in SCS was the most vital factor associated with survival benefit (8, 17). Therefore, identifying valid prediction models for CGR in SCS is an urgent need, for two reasons: one is for the selection of patients most appropriate for surgery and the other is for avoiding surgical burden on the part of patients of both limited benefit from the procedure and limited overall life expectancy.

PREDICTION MODELS FOR PROPER PATIENT SELECTION TO ACHIEVE CGR IN SCS

Almost all of the evidence indicated a benefit of SCS exclusively in patients with CGR. However, not every patient is suitable for complete resection surgery in consideration of the accompanying surgical morbidity and mortality rates. Over the last 15 years, several models have been developed for predicting surgical outcomes, PFS, or OS on the basis of the clinical and pathological data available at the primary diagnosis and recurrence (3). Among them, only the Memorial Sloan Kettering (MSK) criteria, the AGO (Arbeitsgemeinschaft Gynäkologische Onkologie) score, and the Tian model are the most often cited models with international validity, while others have not been externally verified. The models are introduced as follows.

MSK Criteria

As early as 1998, the Second Ovarian Cancer Consensus Conference demonstrated the factors for the identification of optimal candidates for SCS: progression-free interval (PFI) >12 months, response to primary chemotherapy, good performance status, and feasible complete resection based on preoperative evaluation (3). Then, a large retrospective single-institution study of 153 patients (from 1987 to 2001) undergoing SCS was conducted by the MSK Cancer Center. This study suggested that the goal of SCS should be to achieve residual disease ≤0.5 cm. Then, a prediction model was established based on disease-free interval (DFI), the number of recurrence sites, and evidence of carcinomatosis with a CGR rate of 41% (**Table 2**) (18).

TABLE 2 | MSK criteria.

Disease-free interval	Single site	Multiple sites: no carcinomatosis	Carcinomatosis[a]
6–12 months	Offer SCS	Consider SCS	No SCS
12–30 months	Offer SCS	Offer SCS	Consider SCS
>30 months	Offer SCS	Offer SCS	Offer SCS

MSK, Memorial Sloan Kettering; SCS, secondary cytoreductive surgery.
[a]Carcinomatosis was defined as the presence of 20 tumor nodules noted at the time of surgery.

AGO Score

In about the same period as the MSK study, a series of AGO-DESKTOP OVAR trials on surgery in ROC were carried out. Firstly, a retrospective multicenter study (DESKTOP I trial) of 267 patients (from 2000 to 2003) found that CGR was associated with prolonged survival in ROC and developed a hypothesis for a predictive score to identify patients who had complete resection during SCS. Different from the MSK criteria, the AGO score consists of a good performance status, absence of ascites, and outcome of primary surgery/initial FIGO (International Federation of Gynecology and Obstetrics) stage (**Table 3**) (18). Secondly, the score model was subsequently verified in a multicenter trial (DESKTOP II trial) of 516 patients, which was the first prospectively validated study to positively predict surgical outcomes in ROC with a CGR rate of 76%. However, the negative predictive value was 38% and the specificity was low (53%), which could not be ignored either (19). Finally, the AGO DESKTOP III stood as a phase III prospective randomized controlled trial, as we have mentioned above—the AGO score was widely used in clinical practices (9).

Tian Model

To better assess the parameters associated with CGR in SCS, Tian et al. conducted a large retrospective multicenter international study on 1,075 patients (before 2009) with ROC undergoing SCS by collecting raw data from nine previously published studies including the MSK and AGO data. Besides, additional data on 117 patients (from 2007 to 2009) who were not included in the development of the model were used for external validation and to assess the discrimination of the model. CGR was achieved in 40% of the population, with rates ranging from 8.3% to 65.9%. After an analysis of the factors impacting the surgical outcomes of SCS, six significant parameters were identified *via* multivariate logistic regression, and each of them obtained a risk score based on the beta coefficient. According to the sum of the risk scores, patients would be categorized into the low-risk group (≤4.7) and the high-risk group (>4.7). The proportion of CGR in the low-risk group was 53.4%, while that in the high-risk group was 20.1% (**Table 4**). External validation of the Tian model showed sensitivity and specificity values of 83.3% and 57.6%, respectively. The area under the receiver operating characteristic curve for predicting CGR was 0.68 (20, 21).

Other Prediction Models

Due to the accumulated data confirming that CGR during SCS is associated with the largest survival benefit, whereas surgery with

TABLE 3 | AGO score.

Predictive parameters of CGR
Platinum-sensitive ROC
Good performance status (ECOG 0)
No residual disease after primary surgery (or, alternatively, FIGO I/II)
Absence of ascites in preoperative imaging (<500 ml)

AGO, Arbeitsgemeinschaft Gynäkologische Onkologie; CGR, complete gross resection; ROC, recurrent ovarian cancer; FIGO, International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group.

TABLE 4 | Tian model.

Impact factors	Scoring					
	0	0.8	1.5	1.8	2.4	3.0
FIGO stage	I/II	III/IV				
RD after primary surgery	0		>0			
PFI (months)	≥16				<16	
ECOG performance status	0–1				2–3	
CA_{125} at recurrence (U/ml)	≤105			>105		
Ascites at recurrence	Absent					Present

FIGO, International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group; RD, residual disease; PFI, progression-free interval.

large tumor bulks of 1 cm diameter or more left does not alter the prognosis significantly, relevant studies have focused on the search for a prediction model for CGR to select the appropriate patients.

A single-center retrospective study analyzed 135 patients (from 2009 to 2013) with ROC and came up with an equation that allowed calculation of the SeC-score value. This study found with internal validation that the preoperative variables such as CA_{125}, HE_4, ascites, and residual disease (RD) at primary surgery were all involved in the risk of optimal SCS, with sensitivity and specificity of 82% and 83%, respectively (22).

A similar single-center retrospective study analyzed 80 patients (from 1982 to 2012) with ROC undergoing SCS using the grouping model. A total of four favorable prognostic factors were independently associated with better survival: treatment-free interval (TFI) >12 months, absent distant metastasis, solitary disease, and performance status (PS) = 0. Patients with three to four of these factors had better survival and higher CGR rates (79% *vs*. 40% *vs*. 33%) than those with two or none or only one factor. Therefore, SCS for patients with three to four of the above favorable factors at ROC was strongly recommended. SCS may be considered in patients with two factors (the Minaguchi criteria) if CGR is expected to be achieved, although prospective studies were warranted to validate the results (23, 24).

A few studies have conducted some exploration to select suitable patients with ROC for successful SCS by laparoscopy. Fanfani et al. (25) reported that this could be effective for the evaluation of candidates for CGR using PET-CT and a staging laparoscopy (S-LPS)-based method. This method had been validated with an overall accuracy rate for primary debulking ranging between 77.3% and 100%. At a total predictive index value (PIV) ≥8, the probability of optimal primary resection at laparotomy was equal to 0, and the rate of unnecessary exploratory laparotomy was 40.5% (26, 27). However, the subjective evaluation of PET-CT images and S-LPS in this study rather than a scoring standard limited its application and promotion. A similar limitation existed in the study of Yang et al. (28). The selection criteria were developed using a laparoscopic-based PIV score combined with assessment of the multidisciplinary team (MDT), but lacked quantification of the MDT.

Bogani et al. (29) reported an innovative method using artificial intelligence (AI), which was useful in weighing the importance of the clinical variables predicting CGR. As a result, three main factors—DFI (importance = 0.231), retroperitoneal recurrence (importance = 0.178), and RD at primary surgical treatment (importance = 0.138), were proposed to predict CGR using artificial neuronal network (ANN) analysis. However, these predictors have not yet been modeled and lack validity.

DIFFERENCES AMONG THE THREE PREDICTION MODELS (MSK, AGO, AND TIAN MODEL)

As mentioned above, only the MSK criteria, AGO score, and the Tian model have been externally validated in clinical studies. This review focuses on discussing the strengths and limitations of these main prediction models. In terms of the number of populations included in these studies, retrospective case data in the MSK criteria were limited by a single institution, while it was more comprehensive in the Tian model, with case data from a larger international multicenter. With respect to variables, the three models have a common point: that PFI or platinum-sensitive recurrent is considered as the most important predictive factor, without doubt, which shows a positive correlation with complete resection. Unlike the AGO score and the Tian model, the number of recurrence sites and peritoneal carcinomatosis are considered as negative predictors in the MSK criteria. This was confirmed by the DESKTOP I trial, demonstrating that patients with and without peritoneal carcinomatosis had complete resection rates of 26% and 74%, respectively ($p < 0.0001$). Peritoneal carcinomatosis was a negative predictor for complete resection, but had no effect on prognosis if complete resection is achieved. In the case of complete resection of peritoneal carcinomatosis, there was no difference in OS when compared with complete resection without peritoneal carcinomatosis (30). Another study also confirmed this viewpoint: that patients who have multisite recurrence tend to have shorter PFS, but that there is no difference in OS (31). In the development of the AGO score, peritoneal carcinomatosis and CA_{125} in preoperative diagnostics were not included in multivariate analysis because of their correlation with ascites; stepwise analysis with elimination of one of these three variables showed ascites being the most useful one (32). Based on this, we were inclined to think that the Tian model is quite similar to the AGO score, with only one additional factor, CA_{125}, in the Tian model compared to the AGO score (21).

EVALUATION AND EXTERNAL VERIFICATION OF THE THREE PREDICTION MODELS

Recently, as valid selection criteria, both the AGO score and the Tian model have been prospectively validated in the form of increased PFS in DESKTOP III and SOC1/SGOG-OV2, respectively, while the MSK criteria lacked prospective

validation up until now (9, 11). In the last decade, a numbers of retrospective studies demonstrated that the three prediction models (the MSK criteria, AGO score, and the Tian model) were widely applied in clinical practice to help inform decision-making for ROC patients. Harter et al. (33) performed an exploratory analysis to evaluate the decision effectiveness of the AGO score in 217 patients with SCS in ROC from 1999 to 2013, before and after introduction of the AGO score. The results showed that the AGO score could identify suitable candidates for SCS, with CGR being 89.3% and 66.7% in positive and negative AGO scores, respectively, indicating that the AGO score did not present a very good negative predictive value. The authors held the view that the selection criteria for surgery in patients with negative AGO score were not standardized, owing to the time span of the study. Nevertheless, it should be noted that 38% of the patients with a negative AGO score achieved absent residual tumor after SCS and that the PFS was comparable with that of patients with a positive AGO score. This aspect showed that the AGO score does not affect a patient's inoperability. Therefore, further studies should be carried out to evaluate the predictive and prognostic impact of a negative score (4). Cowan et al. (34) conducted a population-based retrospective study of the MSK Cancer Center to compare the predictive value of the MSK with that of the validated Tian model and the AGO score. The results showed good concordance between the Tian model and MSK, with accuracy rates of 88% and 86%, respectively, in predicting CGR, while that of the AGO score was 49%. In addition, the MSK criteria were more user-friendly because fewer variables were involved. The Tian criteria may be applied to intermediate MSK cases for further stratification. The AGO score and the MSK criteria were retrospectively applied to 194 patients in another study to assess the probability of achieving CGR in SCS. The results showed that both models contributed to identifying patients undergoing SCS, while 63.4% of patients with a negative AGO score achieved CGR. Moreover, the concordance indices of two separate nomograms based on the AGO score and the MSK criteria (C-index values of 0.5900 and 0.5989, respectively) were also not high. Therefore, the authors implied that these models might be too strict that they exclude patients from the chance of a successful ROC surgery (35). Besides, several retrospective studies have argued that the AGO score and the Tian model show high positive predictive values for complete SCS, 80.0%–84.3% and 73%–80.3%, respectively, but also relatively high false negative rates of 61%–68.5% and 55.6%–70%, respectively. We would still highlight that both scores identify a subset of patients who could achieve CGR, but do not select patients who are suitable candidates for surgery compared to chemotherapy. Further studies and discussion are warranted so as not to prohibit patients from having potential life-extending surgery (36–38). In addition, preoperative imaging is an essential tool in making the right decision (4). Several studies have suggested that additional refinement of the score, such as with whole-body MRI or PET-CT, is needed to exclude women from SCS. Overall, the selection criteria and potential beneficial subpopulation of CGR could be ultimately refined in future clinical practice (38).

LIMITATIONS OF THE CURRENT PREDICTION MODELS

In view of the similar CGR and OS achieved in the three foregoing phase III randomized clinical trials, this provides proof that the AGO score and the Tian model are validated scoring criteria used to select appropriate patients to achieve CGR. Not only are patient criteria important, but there are also several limitations that lead to an unfavorable influence on the accuracy of the prediction models. Firstly, none of the models incorporates the surgeon's own surgical ability as an evaluation parameter on the condition of distinct surgical experience of SCS. Patients with the same prediction scores undergoing SCS performed by a gynecologic oncologist or a general gynecologist may obtain different CGR rates. Secondly, differences in the clinical resources have not been included in the models in terms of the comprehensive capabilities of the MDT teams, which vary in different hospitals. As is known, some surgical operations such as those requiring intestinal and urological skills are involved during the SCS process and usually entail cooperation with MDT teams. Thirdly, most of the models lack preoperative imaging diagnosis to exclude inoperable patients with distant metastasis, such as in the lung or brain. As a result, adequate preoperative evaluations with whole-body MRI or PET-CT are necessary. Fourthly, so far, except for the three mainstay prediction models, the predictive value of the others has not been externally validated. Fifthly, recently, several retrospective studies have evaluated the impact of biological features, such as the BRCA status and the use of PARP inhibitors, with some controversial results on the benefits of SCS (39). Fagotti et al. found that patients with *BRCA1*/*BRCA2* wild type benefited from SCS, while for patients with *BRCA1*/*BRCA2* mutations, the benefit was not as obvious. Subsequently, their further study demonstrated that SCS increases the TFST and post-recurrence survival in platinum-sensitive ROC patients with $BRCA^{mut}$ candidates for olaparib maintenance after platinum-based chemotherapy (40, 41). Conversely, another study showed a benefit of SCS irrespective of the *BRCA1*/*BRCA2* status among patients mostly not treated with PARP inhibitors (42). Besides, a phase II multicenter RCT (SGOG SOC-3 Study, NCT03983226) was conducted to answer the question of whether patients can benefit from a potential CGR combined with niraparib maintenance in platinum-sensitive secondary recurrent patients. The results are awaited (43). Further research should be conducted to investigate the benefits of SCS in relation to the molecular characteristics (*BRCA* or homologous recombination deficiency status) and the use of PARP inhibitors and/or bevacizumab and to identify individualized surgical strategies, accordingly optimizing the prediction model for CGR in SCS for ROC patients.

CONCLUSION

Adequate selection of ROC patients for surgery is crucial due to the primary goal of SCS of achieving CGR. In view of the above

discussion, each of the three mainstay models—the MSK criteria, the AGO score, and the Tian model—has its strengths and limitations. They can be efficiently applied to clinical practice to help inform decision-making for ROC patients, but with relatively high false-negative rates, while other models need to be externally validated. Further prospective randomized surgical studies are warranted to compare the prediction accuracy and the advantages and disadvantages of those models. To sum up, choosing the right patient, right clinic, and right surgeon may be the key point to achieve good outcomes from SCS. We await an enhanced prediction model that integrates detailed clinical data of patients (such as preoperative imaging, molecular characteristics, and the use of bevacizumab and/or PARP inhibitors), the surgeon's surgical ability, and the capability of the MDT team for achieving maximum CGR in SCS for ROC patients.

AUTHOR CONTRIBUTIONS

CJ wrote the manuscript. ZL provided practical suggestions and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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