Evaluation of the one-step nucleic acid amplification method for rapid detection of lymph node metastases in endometrial cancer: prospective, multicenter, comparative study

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ABSTRACT

Objective To evaluate the diagnostic performance of the one-step nucleic acid amplification (OSNA) method for the detection of sentinel lymph node (SLN) metastases in women with apparent early-stage endometrial cancer compared with standard ultrastaging.

Methods Prospective, multicentric, interventional study. Patients with apparent early-stage endometrial cancer who underwent primary surgical staging with SLN mapping were included. SLNs were serially sectioned with 2 mm slices perpendicular to the longest axis of the node: the odd slices were submitted to ultrastaging, whereas the even slices were submitted to the OSNA analysis. Diagnostic performance was calculated taking ultrastaging as referral standard.

Results Three-hundred and sixteen patients with 668 SLNs were included. OSNA assay detected 22 (3.3%) positive SLNs, of which 17 (2.5%) were micrometastases and 5 (0.7%) macrometastases, whereas ultrastaging detected 24 (3.6%) positive SLNs, of which 15 (2.2%) were micrometastases and 9 (1.3%) macrometastases (p=0.48). Regarding negative SLNs, OSNA detected 646 (96.7%) negative nodes, including 8 (1.2%) isolated tumor cells, while ultrastaging detected 644 (96.4%) negative nodes with 26 (3.9%) isolated tumor cells. Specificity of OSNA was 98.4% (95% CI 97.5 to 99.4), accuracy was 96.7% (95% CI 95.4 to 98.1), sensitivity was 50% (95% CI 30.0 to 70.0), while negative predictive value was 98.1% (95% CI 97.1 to 99.2). Discordant results were found in 22 SLNs (3.3%) corresponding to 20 patients (6.3%). These were 10 (1.5%) false-positive SLNs (all micrometastases): one (0.1%) of these was a benign epithelial inclusion at ultrastaging. There were 12 (1.8%) false-negative SLNs of OSNA, of which 9 (1.3%) were micrometastases and 3 (0.5%) macrometastases. Overall, 17/668 (2.5%) benign epithelial inclusions were detected at ultrastaging.

Conclusion The OSNA method had high specificity and high accuracy in detecting SLN metastasis in apparent early-stage endometrial cancer. The advantage of the OSNA method could be represented as the possibility to analyze the entire lymph node thus eliminating sampling bias.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ OSNA is a safe method to assess lymph node metastasis in endometrial cancer.

WHAT THIS STUDY ADDS

⇒ The specificity and accuracy of OSNA in detecting sentinel lymph node metastasis is high. The sensitivity is low. No difference between OSNA and ultrastaging in detecting macrometastases and micrometastases was evident in a population of apparent early-stage endometrial cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ OSNA could represent a methodology able to diagnose lymph node metastasis with a standardized analysis with reduced risk of protocol heterogeneity and sampling bias.

INTRODUCTION

Endometrial cancer is the most frequent gynecological cancer in developed countries.[1] The incidence of this malignancy has increased in the last years with an estimated increase of 1% every year.[2] It is known that lymph node metastasis represents a major prognostic factor, and it is an indication for adjuvant chemoradiotherapy.[3] Sentinel node (SLN) mapping is now widely used in the staging process for apparent uterine-confined endometrial cancer. This is supported by extensive literature,[4 5] and consequently, incorporated into international guidelines.[3 6] Accuracy and sensitivity of SLN in endometrial cancer has been shown to be promising in low and intermediate risk cases, and more recently, also in high-risk patients.[7 8] It is well known that the ultrastaging protocol of SLN allows the diagnosis of a higher number of low-volume metastases.[9] Nevertheless, there is a large heterogeneity in the literature regarding ultrastaging standardization with no universally accepted protocol.[10]

More recently, the one-step nucleic acid amplification (OSNA) method has been proposed for diagnosing lymph node metastasis. OSNA is a rapid assay, able to detect the presence of cytokeratin 19 mRNA in SLNs, consisting of a short homogenization followed by amplification of cytokeratin 19 mRNA directly from the lysate.[11] Different studies have described the use and the accuracy of OSNA for the detection of nodal metastases in endometrial cancer.[12–15] Nevertheless, the evidence supporting the use of OSNA in endometrial cancer is still poor, particularly in comparison with other malignancies.[16]

The aim of the present study was to establish the clinical performance (measured as specificity, sensitivity, and accuracy) of the OSNA method for the detection of SLN metastasis in patients with apparent early-stage endometrial cancer.

METHODS

Study Protocol

This study was a prospective, multicenter, interventional study approved by the institutional ethical committee (protocol number 49384/19, ID: 2891). All the patients received information about the study and signed a specific written consent. All clinical and pathological data were collected in the Redcap institutional electronic database. Consecutive patients with apparent International Federation of Gynecology and Obstetrics (FIGO) stage I–II endometrial cancer histologically diagnosed undergoing primary surgery between May 2020 and December 2021 were included. Patients were operated at the Fondazione Policlinico Agostino Gemelli IRCCS, Rome, Italy (principal investigator site) and at the Santa Maria Della Misericordia Hospital, University Health Agency, Friuli Centrale, Udine, Italy. Only patients with no evidence of enlarged (short axis >10 mm) pelvic or para-aortic lymph nodes underwent SLN mapping. Exclusion criteria included fertility sparing surgery; neoadjuvant treatment; no SLN mapping attempted; other previous or concomitant metastatic cancers; dedifferentiated/undifferentiated, endometrial stromal sarcoma, and carcinosarcoma histology.

All patients underwent pre-operative work-up, including hysteroscopic biopsy, transvaginal ultrasound scan, abdominal MRI scan and/or CT scan. Surgical staging consisted of total hysterectomy, bilateral salpingo-oophorectomy, and SLN mapping.

Surgical approach was laparoscopic or robot-assisted according to body mass index as per internal protocol (patients with body mass index >30 kg/m^2 underwent robotic approach).[17] Patients with intra-operative evidence of enlarged lymph nodes and/or with extra-uterine disease were not included in the final analysis but were considered in the intent to treat.

Sentinel Lymph Node Mapping

SLN mapping was performed according to previously reported standardized algorithm.[18] SLN was detected after 1 mL superficial and deep cervical injections of indocyanine green (diluted with sterile water at 1.25 mg/mL) at 3 and 9 o'clock (blue dye was used only in cases of referred allergy to iodine). Indocyanine green injection was performed with a 22-gage needle, after docking in the case of robotic surgery. About 10–15 min after the cervical injection, the retroperitoneal space was opened, and pelvic lymph nodes were assessed with a near infrared camera.

The number of SLNs, and their anatomical locations, were recorded. In cases of no pelvic and aortic mapping, a cervical re-injection was performed in the side of mapping failure. In cases of negative mapping after re-injection, side-specific pelvic lymphadenectomy was performed.

After removing the surrounding adipose tissue ex vivo, the SLNs were serially sectioned at 2 mm slices perpendicular to the longest nodal axis, and the slices were alternatively processed with the two methods in the operating room by the surgeon; the portions intended for OSNA analysis were immediately placed in refrigerated (2° to 8°C) containers for transport. The odd slices were submitted to ultrastaging, and the even slices were submitted to OSNA analysis. Lymph nodes ≤4 mm in the long axis were divided into halves with one half attributed to each method (Figure 1). Transport time between surgery and laboratory had to be less than 15 min to avoid RNA degradation. If OSNA could not be performed within 8 hours after resection, lymph nodes were immediately stored at −80°C.

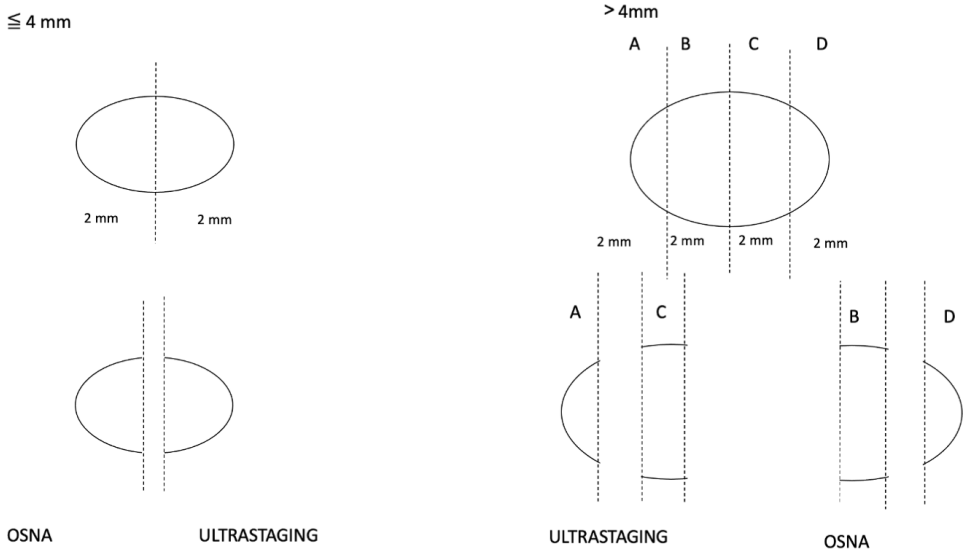

Figure 1 Methodology of sentinel lymph node assessment. OSNA, one-step nucleic acid amplification.

OSNA Evaluation

The even slices belonging to the same lymph node were processed together with the OSNA method.

Frozen samples were homogenized in 4 mL of lysing buffer for 90 s at 25 000 rpm using stainless steel blades or for 60 s at 10 000 rpm using LYNOPREP Blade Set and centrifuged for 1 min at 10 000 x g. Subsequently, cytokeratin 19 mRNA was amplified by reverse transcription loop-mediated isothermal amplification in the RD-210. According to the manufacturer's instructions, automated amplification with a ready-to-use reagent kit was performed directly from the sample lysate, no RNA purification required. Considering that the copy number, as determined by the OSNA assay, correlates well with the metastatic tumor volume,[19] lymph nodes were defined as 'negative' if fewer than 250 copies/µL were found; 'positive for micrometastasis' with levels of 250–5000 copies/µL and 'positive for macrometastasis' with levels above 5000 copies/µL. Isolated tumor cells were defined if a level between 160 and 250 copies/µL was detected.[14]

Ultrastaging Evaluation

The odd slices were fixed in 10% buffered formalin, embedded in paraffin, and submitted to a permanent section. Levels at 150 µm intervals were sliced from each paraffin block. At each level, adjacent sections were generated, the first of which was stained with hematoxylin and eosin. If no tumor was detected, then the second section was stained with immunohistochemistry using the anti-cytokeratin AE1:AE3. The size of lymph node metastasis was classified according to the volume of the tumor deposit: macrometastases (>2.0 mm); micrometastasis (>0.2–2.0 mm). Isolated tumor cells were defined as microscopic clusters and single cells measuring ≤0.2 mm.[20]

Statistical Analysis

Diagnostic performance of OSNA was calculated considering ultrastaging as the reference standard. Isolated tumor cells were not considered 'metastatic' lymph nodes for the performance analysis. Sensitivity, specificity, accuracy, positive and negative predictive values were reported with their 95% confidence intervals. Considering the expected low prevalence of positive nodes, we estimated positive and negative likelihood ratios; positive likelihood ratio is the true positive rate divided by the false-positive rate while the negative likelihood ratio is calculated as the ratio between the false-negative rate and the true-negative rate.

Likelihood ratios can be interpreted as changes in the likelihood of a positive node after doing the OSNA test: one meaning no change, values >10 large increase, and <0.1 large decrease in probability. Agreement between the two methods was evaluated with the Cohen's κ.

Quantitative variables were summarized using median and range while categorical items were reported as absolute counts and percentages. All statistical analyses were performed with IBM SPSS Statistics, version 27.0.

In accordance with the journal's guidelines, we will provide our data for independent analysis by a selected team by the editorial team for the purposes of additional data analysis or for the reproducibility of this study in other centers, if such is requested.

RESULTS

Patients Characteristics and SLN Findings

A total of 379 patients with early-stage endometrial cancer met the inclusion criteria at the pre-operative evaluation. Figure 2 demonstrates the flowchart with the inclusion and exclusion process.

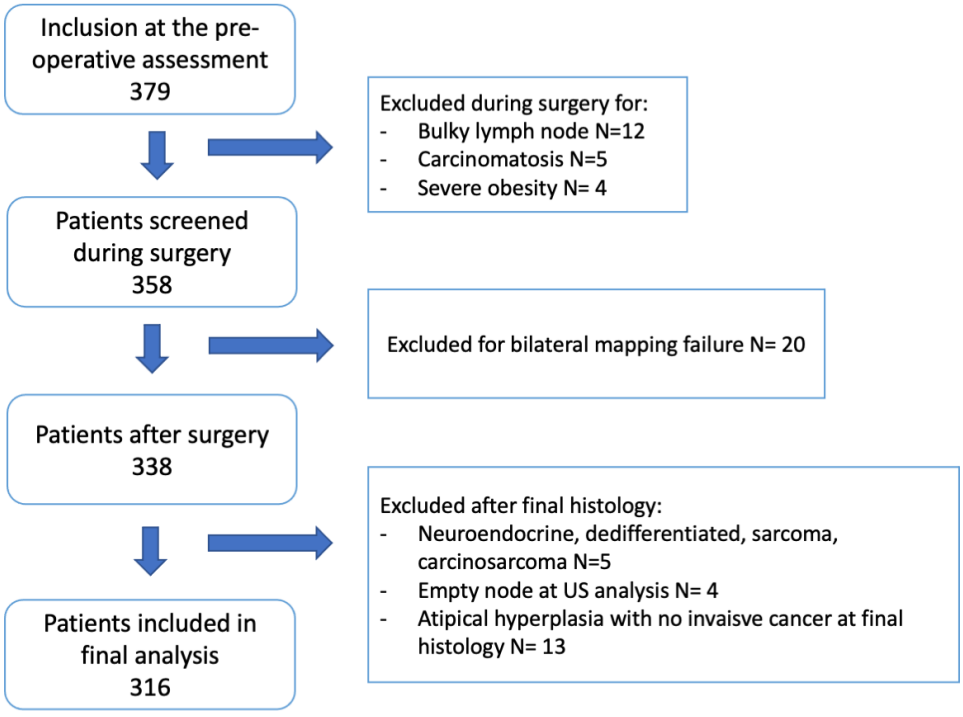

Figure 2 Flowchart of inclusion/exclusion criteria.

Table 1 Patients' characteristics

Characteristics	n=316
Age, median (range)	60 (28–75)
BMI, median (range)	28.0 (17.6–54.7)
Histology	
Endometrioid	274 (86.7%)
Serous	22 (7%)
Mixed	11 (3.4%)
Clear cell	5 (1.6%)
Adenocarcinoma NOS	4 (1.3%)
Grade	
G1	58 (18.4%)
G2	198 (62.6%)
G3	56 (17.7%)
Unknown	4 (1.3%)
Myometrial invasion	
<50%	151 (47.8%)
>50%	95 (30%)
No	70 (22.2%)
FIGO stage*	
IA	205 (64.9%)
IB	66 (20.9%)
II	12 (3.8%)
IIIA	3 (0.9%)
IIIB	1 (0.3%)
IIIC1	24 (7.6%)
IIIC2	1 (0.3%)
IVB	4 (1.3%)
LVSI	
No	264 (83.5%)
Yes	51 (16.2%)
Unknown	1 (0.3%)
Number of SLNs per pelvic side per patient. median (range)	1 (0–5)
SLN mapping	
Bilateral	263 (83.2%)
Unilateral	53 (16.8%)
Benign epithelial inclusions at ultrastaging	17/668 (2.5%)

*FIGO stage IIIC is reported according to lymph node positivity with both methods or by either method (isolated tumor cells considered as 'negative')
BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; NOS, not otherwise specified; SLN, sentinel lymph node.

Three hundred and sixteen patients were included in the final analysis. The baseline clinicopathological characteristics are reported in Table 1. The median age was 60 years (range 28–75). Median body mass index was 28.0 kg/m^2 (range 17.6–54.7). The majority of patients were diagnosed with endometrioid histology (n=274, 86.7%), grade 2 (n=198, 62.6%) without lymphovascular space involvement (n=264, 83.5%).

Table 2 Status of sentinel lymph nodes

SLN=668	OSNA	Ultrastaging	P value
Positive SLNs	22 (3.3%)	24 (3.6%)	0.482
Micrometastases	17 (2.5%)	15 (2.2%)	
Macrometastases	5 (0.7%)	9 (1.3%)	
Negative SLNs	646 (96.7%)	644 (96.4%)	
Negative	638 (95.5%)	618 (92.5%)	
Isolated tumor cells	8 (1.2%)	26 (3.9%)	

SLN, sentinel lymph node; OSNA, one-step nucleic acid amplification.

In 263 (83.2%) patients there was bilateral and in 53 (16.8%) patients unilateral SLN mapping. At least one SLN was identified in all patients with a total of 668 SLNs retrieved and analyzed with both methods (OSNA and ultrastaging). Overall, 25 (7.9%) patients were found to have micrometastatic or macrometastatic SLN (by both methods or by either method).

Comparison of OSNA and Ultrastaging

OSNA detected 22 (3.3%) metastatic SLNs, of which 17 (2.6%) were micrometastases, and 5 (0.7%) macrometastases. Ultrastaging detected 24 (3.6%) positive SLNs of which 15 (2.2%) were micrometastases and 9 (1.3%) macrometastases. Regarding negative SLNs, OSNA detected 646 (96.7%) nodes, including 8 (1.2%) isolated tumor cells, while ultrastaging detected 644 (96.4%) nodes with 26 (3.9%) isolated tumor cells (Table 2).

When performing analysis per patient, we found agreement between the two methods in 291 (92.1%) patients with negative SLNs and 9 (2.8%) patients with metastatic SLNs. Conversely, OSNA detected metastatic SLNs in 6 (1.9%) patients with the negative ultrastaging, whereas 10 (3.2%) patients with positive ultrastaging were classified as negative by OSNA (Online supplemental table 1).

Concerning the agreement per SLN between the two methods, we found 22 (3.3%) discordant results: 10 (1.5%) lymph nodes OSNA positive but ultrastaging negative (false positive) and 12 (1.8%) lymph nodes OSNA negative but ultrastaging positive (false negative) (Online supplemental table 2). Of these 12 OSNA negative, 9 (75%) lymph nodes had micrometastases and 3 (25%) lymph nodes had macrometastases at ultrastaging. False negativity of OSNA involved 12 lymph nodes in 11 patients as two lymph nodes were in the same patient. Moreover, one patient had a false-negative OSNA on one pelvic side (macrometastasis at ultrastaging) but a true-positive micrometastasis (therefore detected both with OSNA and ultrastaging) on the contralateral side. Details of false-negative and false-positive patients at OSNA are reported in Table 3 and Online supplemental table 3, respectively.

Only in one patient (0.3%) with OSNA-positive (micrometastasis – 480 copies/µL) and ultrastaging-negative SLN, was benign epithelial inclusion found with the ultrastaging evaluation. Overall, the rate of benign epithelial inclusions found at ultrastaging in this series was 17/668 (2.5%) lymph nodes.

Table 3 False-negative SLN with OSNA method (OSNA negative, ultrastaging positive)

Node	Age	Histology	Grade	LVSI	T (cm)	MI	OSNA	US (mm)
1	55	Endometrioid	3	No	2.8	>50%	Neg	MIC (1.7)
2	69	Serous	3	Yes	3.5	<50%	Neg	MAC (3)
3	56	Endometrioid	2	Yes	5	<50%	Neg	MIC (0.54) Epith incl
4	61	Endometrioid	2	Yes	3	<50%	Neg	MIC (NR)
5	58	Endometrioid	2	Yes	2.5	<50%	Neg	MAC (3)
6	62	Endometrioid	2	Yes	4	>50%	Neg	MIC (0.35)
7	62	Serous	3	Yes	2.2	<50%	Neg	MIC (NR)
8	37	Endometrioid	2	Yes	1.7	<50%	Neg	MIC (0.5)
9	63	Endometrioid	2	Yes	2.2	>50%	Neg	MIC (0.8)
10	69	Serous	3	Yes	3.5	<50%	Neg	MIC (0.8)
11	59	Endometrioid	2	Yes	6	>50%	Neg	MIC (0.9)
12	66	Endometrioid	2	Yes	7	>50%	Neg	MAC (2)

Epith incl, epithelial inclusion; LVSI, lymphovascular space invasion; MAC, macrometastasis; MI, myometrial invasion; MIC, micrometastasis; NR, not reported; OSNA, one-step nucleic acid amplification; T, tumor dimension; US, ultrastaging.

Online supplemental table 4 shows the details of the 11 SLNs with concordant positive results between the two methods. However, in two (18.2%) of these (numbers 1 and 4) there was a discrepancy in the volume of SLN metastasis as in both cases, this was reported to be micrometastasis at OSNA and macrometastasis at ultrastaging.

Diagnostic Performance

Analysis per lymph node demonstrated that the OSNA method had a specificity of 98.4% (95% CI 97.5% to 99.4%) and sensitivity of 50% (95% CI 30.0% to 70.0%), while accuracy was 96.7% (95% CI 95.4% to 98.1%). Cohen's κ showed a moderate agreement between the two methods (OSNA and ultrastaging) 0.51 (95% CI 0.33 to 0.69).

Positive predictive value was 54.5% (95% CI 33.7% to 75.4%), while negative predictive value was 98.1% (95% CI 97.1% to 99.2%). Considering the low prevalence of positive nodes (24 out of 668) that could result in a high negative predictive value, we calculated positive and negative likelihood ratios: the positive likelihood ratio was 32.2 (95% CI 11.7 to 88.9) suggesting that OSNA could be considered an informative test, while the negative likelihood ratio was equal to 0.51 (95% CI 0.18 to 1.40), suggesting a poor performance in predicting the negative status.

DISCUSSION

Summary of Main Results

We found that the specificity, sensitivity, and accuracy of OSNA compared with ultrastaging were 98.4%, 50%, and 96.7%, respectively. No difference in the rate of macrometastasis and micrometastasis was evident when OSNA was compared with ultrastaging.

Results in the Context of Published Literature

This is not the first study reporting the diagnostic performance of an OSNA assay in endometrial cancer.[12–15 21 22] In our study, unlike other studies, we focused the analysis on individual lymph nodes and not on patients, as we hypothesized that results per lymph node analyzed with the two methods might be different. We found 22/668 (3.3%) discordant lymph nodes, which seems to be in line with other studies: the multicenter ENDO-OSNA found 20 out of 147 discordant patients (13.6%).[15] Unlike this Spanish study, where only the central 1 mm portion was analyzed with ultrastaging, in our study 2 mm slices were alternatively analyzed with the two methods.

We found 12 (1.8%) false-negative SLNs. Of these lymph nodes, 9 (75%) were negative by the OSNA analysis but had micrometastases at the ultrastaging: given the size of the metastases in these lymph nodes (0.2–2 mm) and the size of the 2 mm sections to select for each method of analysis, we could ascribe the false negativity to a sampling bias, as already concluded in other studies.[12 13] As previously reported, OSNA offers multiple advantages over ultrastaging as it allows analysis of whole lymph nodal tissue, minimizing the risk of allocation bias conferring the possibility of standardization of the analysis across different institutions.[15] In fact, ultrastaging analysis is affected by a subjective evaluation, which is also strictly dependent on the type of protocol used. Confirming this, one systematic review tried to identify the ultrastaging method with the highest detection rate of lymph node metastases, but the comparative analysis was not possible due to the large heterogeneity of the included studies.[10]

Regarding the false-positive results, out of 10 SLNs, we found one with micrometastasis at OSNA and negative ultrastaging with endosalpingiosis foci. Reviewing our entire series, we found only 17/668 (2.5%) lymph nodes with benign epithelial glandular inclusion, in agreement with previously published results.[21] Therefore, we can confirm that the low rate of benign inclusion should not represent a limitation for the use of the OSNA method.[22]

Implications for Practice and Future Research

More and more studies are reporting the prognostic significance of micrometastases in endometrial cancer.[23] The ability of OSNA to detect micrometastasis in a rapid and standardized way can represent an important advantage over ultrastaging. Nevertheless, despite previously

published results[22] demonstrating that OSNA was able to identify more micrometastases and fewer macrometastases/isolated tumor cells, in our study no difference between micrometastases/macrometastases was found between the two methods. On the other hand, our results are in line with those of a very recently published study involving 77 patients with endometrial cancer, which concluded that the OSNA assay showed equally accurate detection of lymph node metastasis as the histopathological examination.[24]

The correct diagnosis of low-volume metastasis is clinically relevant. In fact, micrometastases are considered high risk of recurrence and treated with adjuvant therapy, while patients with isolated tumor cells are treated according to the risk factors of the primary tumor.[23 25] Currently, literature data on the management of low-volume metastases are influenced by the use of adjuvant therapy that may equalize the difference present between micrometastases and isolated tumor cells and by the short follow-up of the studies.[26 27] Further studies with prolonged follow-up and molecular tumor profiling are required to stratify these patients and understand who more than others may benefit from adjuvant therapy. It is possible that patients with low-volume disease and favorable molecular profiles do not need adjuvant treatment.[28] With the advent of molecular analysis in the stratification risk of endometrial cancer, we expect that future studies may help us to better understand how to modulate surgery according to the tumor's molecular characteristics, including sentinel lymph node biopsy.[29]

Strengths and Weaknesses

To the best of our knowledge, this is the largest prospective study investigating OSNA assay compared with ultrastaging, as we included 316 patients with 668 SLNs analyzed with both methods. The strength of our study is the prospective nature with a homogeneous population. In addition, it is the OSNA study with the largest sample size. One of the limitations of our study is that we analyzed with OSNA only portions of the entire lymph node with potential sampling bias, while it is known that one of the main advantages of the OSNA method is to analyze the entire nodal tissue. Another weakness is the low number of metastatic SLNs, with a potential limitation on accuracy analyses; on the other hand, the rate of positivity of SLNs is in line with previous reports.[30]

CONCLUSION

This study confirms that the OSNA method had high specificity and high diagnostic accuracy in detecting SLN metastasis in apparent early-stage endometrial cancer, in comparison with ultrastaging on the same lymph node. The sensitivity of the OSNA was low. The number of macrometastases and micrometastases detected by OSNA and ultrastaging was similar. The advantage of the OSNA method could be represented by the possibility to analyze the entire lymph node and thus eliminate sampling bias.

Pending a cost–benefit analysis of OSNA examination we can conclude that OSNA represents a valid and safe method to detect SLN metastasis in endometrial cancer.

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Supplementary Table 1. Comparison of OSNA and ultrastaging in sentinel lymph node performed per patient.

Patients	Ultrastaging Positive	Ultrastaging Negative	Total
OSNA Positive	9	6	15
OSNA Negative	10	291	301
Total	19	297	316

Supplementary Table 2. Comparison of OSNA and ultrastaging in sentinel lymph node performed per lymph node.

Sentinel lymph nodes (n=668)	Histopathological Ultrastaging		
	Positive	Negative	Total
OSNA assay positive	12 (1.8%)	10 (1.5%)	22 (3.3%)
OSNA assay negative	12 (1.8%)	634 (95%)	646 (96.7%)
Total	24 (3.6%)	644 (96.4%)	668 (100%)

Abbreviations: OSNA: one-step nucleic acid amplification.

Supplementary Table 3. False positive SLN with OSNA method (OSNA positive, US negative)

Node	**Age**	**Histology**	**Grade**	**LVSI**	**T (cm)**	**MI**	**OSNA**	**Copies (µl)**	**US**
1	69	Serous	3	No	2.5	>50%	MIC	480	Neg (Epith incl)
2	33	Endometrioid	1	No	/	0	MIC	540	Neg
3 4	62	Endometrioid	3	Yes	4	>50%	MIC	370 370	ITC ITC
5	47	Endometrioid	2	No	3	<50%	MIC	420	Neg
6	64	Endometrioid	2	No	3.7	>50%	MIC	3200	Neg
7	63	Serous	3	Yes	5.5	>50%	MIC	3000	Neg
8	58	Endometrioid	2	No	4.6	<50%	MIC	440	Neg
9	67	Mixed	3	Yes	3.2	>50%	MIC	290	Neg
10	68	Serous	3	No	0	0	MIC	340	Neg

LVSI: lymphovascular space invasion; T: dimension of tumor; MI: myometrial invasion; OSNA: one-step nucleic acid amplification. US: ultrastaging; ITC: isolated tumor cells; SLN: sentinel lymph node; MIC: micrometastasis; Epith incl. epithelial inclusion.

Supplementary Table 4. Details of concordant metastatic* lymph nodes (n=12)

Node	Histology	Grade	LVSI	OSNA	Copies (µl)	Ultrastaging
1	Mixed	3	YES	MIC	1200	MAC
2	Endometrioid	2	YES	MAC	7100	MAC
3	Endometiroid	2	YES	MAC	8400	MAC
4	Clear cell	3	YES	MIC	540	MAC
5	Endometrioid	2	YES	MIC	300	MIC
6	Endometrioid	2	YES	MAC	13900	MAC
7	Endometrioid	2	YES	MAC	32000	MAC
8	Endometrioid	3	YES	MIC	4700	MIC
9	Serous	3	YES	MIC	1100	MIC
10	Endometrioid	3	YES	MIC	1400	MIC
11	Endometrioid	2	YES	MIC	1600	MIC

LVSI: lymphovascular space invasion; OSNA: one-step nucleic acid amplification. US: ultrastaging; MIC: micrometastasis; MAC: macrometastasis.
*ITCs were not considered “metastatic” lymph nodes