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Sentinel lymph node pathological ultrastaging: Final outcome of the Sentix prospective international study in patients with early-stage cervical cancer

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HIGHLIGHTS

- Pathological ultrastaging is a key component of sentinel lymph node (SLN) biopsy in cervical cancer.
- 43% of cases with N1 (macrometastasis, micrometastasis) are detected by pathological ultrastaging of SLN.
- The detection rate of SLN metastatic involvement correlates with the number of levels assessed by ultrastaging.
- Four levels should become the international standard, detecting over 90% of N1 cases.

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ABSTRACT

Objective. To report the outcome of SLN staging in the SENTIX international prospective trial of SLN biopsy in patients with cervical cancer with an intensive ultrastaging protocol and central quality control and to evaluate how the intensity of pathological assessment correlates with metastatic detection rate in SLNs.

Methods. Eligible were patients with stages T1a1/LVSI+ to T1b2 (<4 cm, ≤2 cm for fertility sparing), common tumor types, no suspicious lymph nodes on imaging, and bilateral SLN detection. SLNs were examined

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intraoperatively and processed by an intensive protocol for ultrastaging (paraffin blocks sectioned completely in 150-μm intervals/levels). SLNs from each site were submitted for central quality control.

Results. In the SENTIX SLN study, 647 out of 733 enrolled patients underwent SLN ultrastaging, identifying 12.5% (81/647) with node positive, N1 cases. Intraoperative detection revealed metastases in 56.8% (46/81) of these cases, categorized into macrometastases (83.7%), micrometastases (26.3%), and isolated tumor cells (9.1%). Ultrastaging identified additional metastatic involvement in 43.2% (35/81) of patients, with detailed sectioning revealing metastases (MAC/MIC) at first level in 20 cases (24.7%), at levels 2–4 in 9 cases (11.1%), and at level ≥5 in 6 cases (7.4%).

Conclusion. SLN ultrastaging detects additional 43% of N1 (MAC/MIC) in patients with negative LNs by imaging and intraoperative pathological assessment. The detection rate of positive SLN correlates with the intensity (number of levels) of ultrastaging. Examination of four levels from paraffin blocks, which detects >90% of patients with N1, is a reasonable compromise for an international standard for ultrastaging.

Study registration: NCT02494063 (ClinicalTrials.gov).

1. Introduction

In patients with cervical cancer, sentinel lymph node (SLN) biopsy was developed to preserve other lymph nodes and lymphatic vessels, thereby reducing postoperative morbidity such as lower limb lymphedema or symptomatic lymphoceles. This technique not only lowers postoperative complication rates but also allows a detailed pathological examination of a small number of critical lymph nodes —those with the highest risk of metastatic involvement—through ultrastaging [1]. Compared to intraoperative frozen section examination and standard lymph node processing, SLN ultrastaging in cervical cancer has been shown to potentially increase the detection rate of macrometastasis (MAC) and micrometastasis (MIC) [2,3].

The protocol for SLN pathological ultrastaging includes five key parameters, three of which determine how much SLN tissue remains unprocessed, such as gross processing, the number of levels in paraffin blocks and the distance between levels. Currently, no internationally accepted standard for pathological ultrastaging exists, presenting a significant limitation. So far, there have been no recommendations on the number of levels to examine. However, SENTIX SLN study has helped to improve the international European guidelines by incorporating such information [4]. This development aims to reduce variability in the intensity of SLN ultrastaging and improve the sensitivity of surgical lymph node staging in cervical cancer patients.

Previous studies on SLN biopsy in cervical cancer have suffered from serious limitations, particularly the limited number of paraffin block levels processed, leaving much tissue unexamined. The review of studies published until 2019 revealed that most studies examined a maximum of 2 levels, and only 6% of studies processed the entire SLN without unexamined residual tissue [5]. Furthermore, no study has evaluated the specific paraffin block section levels where metastases were detected, making it impossible to correlate metastatic detection rates with the intensity of ultrastaging protocol [6].

The SENTIX trial was designed to evaluate the safety of the SLN biopsy in patients with early-stage cervical cancer [5]. Trial enrollment has been completed, with survival outcome data expected to be published in 2024. SLN biopsy was performed as the first step of surgical management with mandatory intraoperative frozen section examination of SLNs [2,7–11]. Patients with intraoperatively detected lymph node involvement were excluded from the SLN study and did not undergo radical hysterectomy [12,13].

The SENTIX trial features an intensive ultrastaging protocol and central quality control of pathological processing. Each SLN was grossly processed in 2-mm thick slices, paraffin-embedded, and sectioned at 150-μm intervals until no SLN tissue left unexamined. At each level two sections were taken; one stained with hematoxylin and eosin and the other immunohistochemically. The SENTIX SLN ultrastaging protocol stands out from the others because it ensures that no SLN tissue remained unexamined. This comprehensive approach ensured the detection of all patients with nodal involvement, particularly those with MAC and most with MIC. Additionally, samples from all sites underwent central quality control of the ultrastaging protocol [5].

The aim of this SLN study is to report the outcomes of SLN surgical staging in the SENTIX trial and to assess the intensity of pathological ultrastaging required to detect most of metastases (MAC and MIC).

2. Materials and methods

2.1. Overview

SENTIX is a prospective observational multicenter trial focusing on the oncological safety of SLN biopsy without further systematic lymphadenectomy in patients with early-stage cervical cancer (T1a1/LVSI+ to T1b2). The primary endpoint is the recurrence rate at 24 months after primary surgery [7]. This article describes an ad hoc analysis of a cohort that underwent pathological assessment of SLNs, using both frozen section and ultrastaging techniques.

The protocol was approved by the Institutional Review board of the leading institution (General University Hospital in Prague, Czech Republic) in 2015. Institutional review board approval of all participating sites was a prerequisite for participation. All participating patients signed informed consent before study enrollment. The study was performed in accordance with the Declaration of Helsinki. The protocol and trial design were published soon after the start of the study [7].

2.2. Patients

Patients scheduled to undergo primary surgery for stage IA1 with lymphovascular space invasion (LVSI) to IB1 cervical cancer (FIGO 2009) were eligible if their tumor was <4 cm in size or <2 cm if fertility sparing management was planned. Patients were included in this SENTIX substudy irrespective of the final tumor histological type. Successful bilateral SLN detection and its negativity on frozen section pathological examination were mandatory for continued enrollment in the SENTIX trial after surgery.

Patients with intraoperatively detected parametrial invasion, bulky lymphadenopathy, or distant cancer spread were excluded from the study. Neoadjuvant chemotherapy and history of pelvic or abdominal radiotherapy were not allowed. Patients with HIV infection or AIDS were excluded.

If the SLN was not detected or found only unilaterally, systematic pelvic lymphadenectomy was performed, and these patients were switched to a control group. All patients with intraoperatively diagnosed MAC or MIC were excluded from the SENTIX trial and postoperatively treated according to local institutional guidelines, but they were analyzed in this SLN ultrastaging substudy. All SLNs without intraoperatively detected MAC were postoperatively examined using the SENTIX protocol for ultrastaging.

2.3. Study protocol

The main features of the protocol that are particularly relevant to this article are as follows. Only patients with negative lymph nodes on imaging, no signs of extrauterine disease, and a tumor size <4 cm on imaging were preoperatively registered in the study. All surgical approaches (open and minimally invasive) and all tracers for SLN detection (blue dye, radiocolloid, or indocyanine green; or combinations) were eligible. Following publication of the Laparoscopic Approach to Cervical Cancer trial during the course of the SENTIX trial, the participating centers were advised to prefer the open surgical approach [14]. The mandatory SENTIX intensive pathological ultrastaging protocol was used and the quality assessment of SLN ultrastaging was centrally controlled. The central quality control of ultrastaging involved having all SLNs (slides and blocks) from two randomly selected patients from each center checked in the central pathology laboratory in Prague. In case of significant deviation from the SENTIX protocol for ultrastaging, all patients from the given center had their SLNs controlled and, if necessary, ultrastaging was completed according to the required parameters. The results of the central control were published in a separate paper [15].

2.4. Trial timeline

The first patient was enrolled into the SENTIX trial in June 2016. By October 2020, 733 patients had been enrolled across 47 centers in 18 countries, predominantly in Europe; 3 centers were in South America and 1 in South Africa. All patients had completed active follow-up by the end of 2022, and all have reached a milestone of ≥2 years since primary surgery. None of the interim analyses showed any safety risks.

2.5. SLN pathological processing

2.5.1. Intraoperative SLN examination (frozen section)

Intraoperatively, all SLNs were grossly evaluated by an experienced pathologist. SLNs were examined by preparing frozen sections of the suspected foci. All non-suspicious SLNs were examined by frozen sections in at least one additional slice, optimally from an area without lipomatous atrophy. Both equatorial and longitudinal protocols were allowed. Additional sections from larger SLNs were examined at the pathologist's discretion after considering the risk of tissue loss for ultrastaging.

If MAC was detected, the SLN was not further investigated by ultrastaging, and the patient was staged as pN1 (MAC). All SLNs that were intraoperatively negative or found to have MIC or isolated tumor cells (ITC) were processed by final ultrastaging. Ultrastaging of the SLN was performed in cases of intraoperative MIC findings to avoid overlooking MAC in the SLN, ensuring the patient received the correct pN1 category (MIC/MAC). Both MIC and MAC negatively impact prognosis and warrant adjuvant treatment.

2.5.2. Ultrastaging protocol

All SLNs were fixed in 10% buffered formalin, cut at 2-mm intervals, embedded in paraffin, and fully examined. The protocol involved preparing pairs of consecutive sections (each 4-μm-thick), at 150-μm-intervals between the pairs of sections, from each paraffin block until no lymph node tissue remained. The first section was stained with H&E and the second section was examined immunohistochemically using anti-cytokeratin antibodies (AE1/AE3) (Fig. 1) [7,8].

2.6. Classification of metastases

The type of metastasis was classified according to the TNM I (i.e., MAC >2 mm, MIC >0.2 and ≤2 mm). ITC was defined as single tumor cells, small clusters of cells <0.2 mm in diameter, or <200 cells. This classification was based on the American Joint Committee on Cancer (AJCC) recommendations for breast cancer staging that have not been specifically validated for cervical cancer [16].

2.7. Statistical analysis

We used standard descriptive statistical analyses, including determining absolute and relative frequencies for categorical variables and the median with the 5th to 95th percentile range for continuous variables. All analyses were performed using SPSS version 25.0.0.1 (IBM Corporation, 2019).

3. Results

3.1. Patients

A total of 733 patients were enrolled in the SENTIX trial (from June 2016 to October 2020), of whom 647 were eligible for the current analyses as shown in the flow chart in Fig. 2. Of 722 patients who underwent primary surgery, 11 patients were excluded preoperatively due to withdrawal of consent ($n = 3$), cancelled surgery ($n = 4$) or critical deviations from the study protocol at one prematurely closed center ($n = 4$). Based on intraoperative findings, patients with only unilateral detection and failed SLN mapping ($n = 57$) and patients with findings of more advanced disease ($n = 12$) were excluded. Postoperatively,

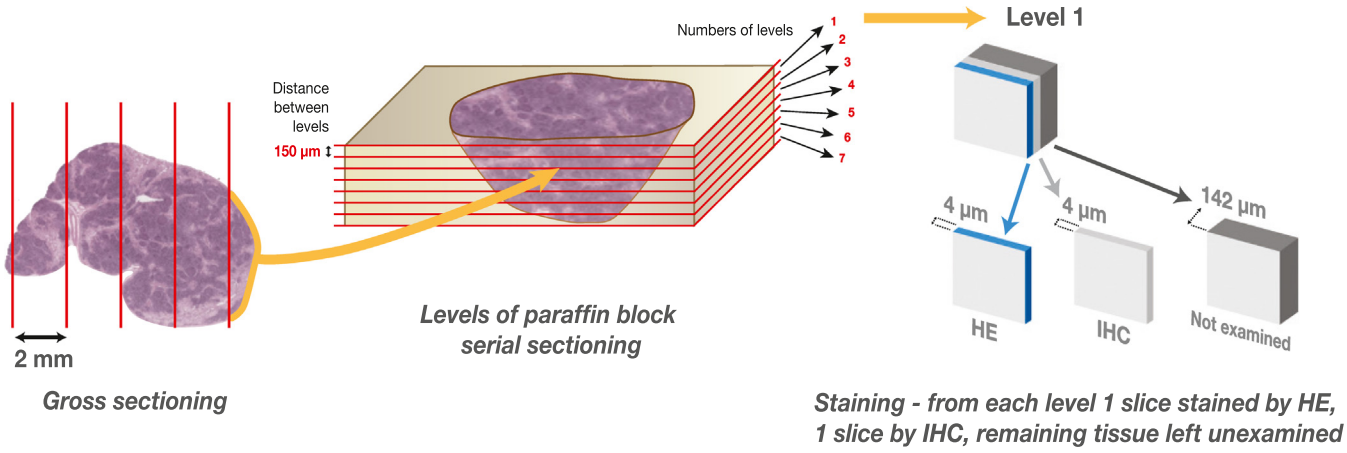

Fig. 1. SENTIX protocol for SLN ultrastaging.
HE, hematoxylin & eosin; *IHC*, immunohistochemistry.

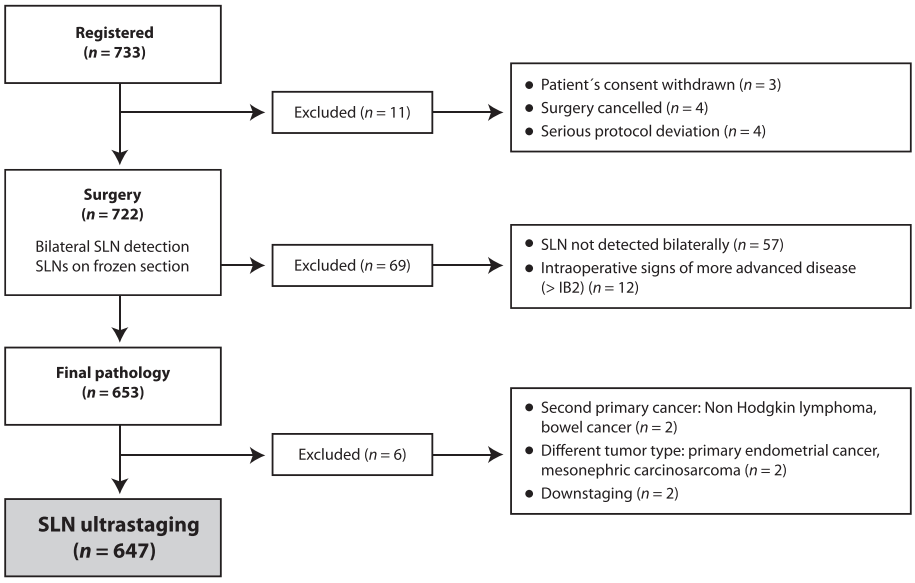

Fig. 2. Patient flow.
SLN, sentinel lymph node.

patients with a different histological tumor type ($n = 2$) or secondary primary tumor ($n = 2$) and patients down-staged to precancerous lesion only ($n = 2$) were excluded.

The clinical and surgical characteristics of the patients are shown in Table 1. Nearly two-thirds of patients (63.6%) had a tumor of ≤2 cm in size. The predominant histological type was squamous cell carcinoma (69.3%), and most patients (71.8%) were recruited at sites with >20 enrolled patients. A median of three SLNs was removed per patient during surgery with a 92% bilateral SLN detection rate.

Table 1
Patient characteristics ($n = 733$)[a].

Characteristics		*n* (%)/median (5th, 95th percentile)
Site size by enrollment	≤10	126 (17.2%)
	11–20	81 (11.1%)
	>20	526 (71.8%)
Age (years)	≤40	294 (40.1%)
	41–60	339 (46.2%)
	>60	100 (13.6%)
BMI (kg/m^2)		25.4/24.2 (18.8; 36.1)
Diagnostic method	Biopsy	331 (45.2%)
	Conization	399 (54.4%)
	na	3 (0.4%)
Tumor stage (FIGO 2009)	IA1 L1	32 (4.4%)
	IA2	54 (7.4%)
	IB1	640 (87.3%)
	na	7 (1.0%)
Tumor size	≤2 cm	466 (63.6%)
	2–4 cm	257 (35.1%)
	na	10 (1.3%)
Grade	1	179 (24.4%)
	2	373 (50.9%)
	3	157 (21.4%)
	na	24 (3.3%)
Tumor type	SCC	508 (69.3%)
	Adenocarcinoma usual type	210 (28.6%)
	Adenosquamous	9 (1.2%)
	na	6 (0.9%)
LVSI	Yes	211 (28.8%)
	No	470 (64.1%)
	Na	52 (7.1%)
Surgical approach	Laparotomy	296 (40.4%)
	Laparoscopy/robotic	437 (59.6%)
Bilateral SLN detection	Yes	659 (92.0%)
	No	57 (8.0)

LVSI, lymphovascular space invasion; *na*, not available; *SCC*, squamous cell carcinoma, *SLN*, sentinel lymph node.
[a] ($n = 716$) for bilateral SLN detection/detection method.

3.2. SLN pathological assessment

SLNs from all 647 eligible patients were processed first by intraoperative assessment and later by the intensive SENTIX protocol for ultrastaging except for cases with intraoperative detection of MACs. A total of 43 patients were diagnosed with MAC (6.6%), 38 with MIC (6.0%), and 22 (3.4%) with ITC. Overall, 12.5% (81/647) of patients were classified as pN1 (Table 2).

Most MACs (83.7%, 36/43) were detected intraoperatively with frozen section, while only 26.3% (10/38) of MICs were found this way. Nearly half (43%) of patients with positive nodes (pN1) were diagnosed after surgery through ultrastaging. The number of cases with metastatic involvement (MAC/MIC) increased with the intensity of SLN ultrastaging (i.e., number of levels examined). MAC/MIC was detected at level 1 in 20 patients (24.7%), at levels 2–4 in 9 patients (11.1%), and at levels ≥5 in 6 patients (7.4%) (Fig. 3). The majority (92.6%) of all SLN-positive cases (MAC or MIC) were identified within the first 4 levels of SLN examination by deep serial sectioning with a regular interval of 150 μm between the levels. No patient with MAC was identified beyond level 4 (Table 2). The median size of the MAC and MIC that were detected by ultrastaging, but not on frozen sections, was 3.3 mm (IQR = 3.0) and 1.0 mm (IQR = 0.6), respectively.

3.3. Comment

3.3.1. Principal findings

Our results show that the accuracy of LN staging is substantially improved by an intensive ultrastaging protocol for SLN pathological evaluation. Half of N1 cases are detected by serial sectioning, and the majority of these would not have been identified without SLN biopsy. The number of detected metastases correlates with the number of levels assessed from paraffin blocks. Four levels allow to detect >90% of cases with pN1, including MAC and MIC.

Our findings are based on prospective multicenter study on 647 patients whose SLNs were processed according to the intensive SENTIX

Table 2
SLN metastasis detection by frozen section and definitive ultrastaging ($n = 647$).

	Frozen section	Ultrastaging			Total[a]
		Level 1	Levels 2–4	Level ≥5	
MAC	36 (83.7%)	6 (14.0%)	1 (2.3%)	0 (0%)	43 (6.6%)
MIC	10 (26.3%)	14 (36.8%)	8 (21.1%)	6 (15.8%)	38 (6.0%)
ITC	2 (9.1%)	6 (27.3%)	10 (45.4%)	4 (18.2%)	22 (3.4%)
pN1 (MAC + MIC)	46 (56.8%)	20 (24.7%)	9 (11.1%)	6 (7.4%)	81 (12.5%)

Values are *n* (%) of the total number of patients per row.
ITC, isolated tumor cells; *MAC*, macrometastases; *MIC*, micrometastases.
[a] Percentages of all patients.

protocol for ultrastaging. All SLNs were completely processed until no residual lymphatic tissue was left unexamined, and adherence to SLN ultrastaging protocol was monitored centrally. To our knowledge, no prior prospective multicenter study has utilized such an intensive ultrastaging protocol. An excellent bilateral detection rate of 92.0% was achieved with a median of 3 nodes per patient.

There are several key features characterizing the SLN ultrastaging protocol, including gross processing, the number of levels (series) examined, the distance between levels (thickness of tissue cut out) and the use of immunohistochemistry staining [1,5,15,17].

A recent review of the SLN pathological protocols used in 127 studies, with >9000 patients, revealed that the quality of SLN processing varied considerably in all aspects of the procedure, including cut thickness of the gross lymph node (1–5 mm), the number of levels (range 0–cut out until no tissue remains), the distance between levels (40–1000 μm), and the number of sections per level [1–5]. Of note, the key parameter, the number of levels examined, showed the greatest variability among the studies [5]. Only eight of the studies (6%) used an ultrastaging protocol with full SLN processing, similar to the protocol used in the SENTIX study [5]. Such heterogeneity inevitably impacts the outcome of LN staging. If large parts of the nodes are not examined, the chance of small metastasis being missed increases.

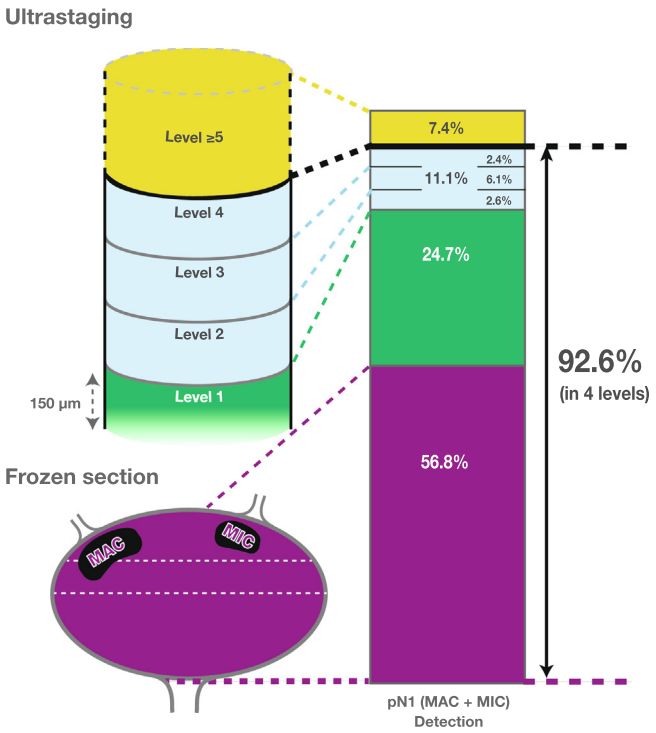

Fig. 3. Detection of metastatic SLN according to the number of levels examined.
MAC, macrometastases; *MIC*, micrometastases.

In two previous prospective studies, one single-arm using SLN biopsy followed by pelvic lymphadenectomy (Senticol I) and subsequent one (Senticol II) with two arms, comparing SLN biopsy alone and SLN biopsy followed by lymphadenectomy, a total of 345 (139 + 206) cases combined from both studies were analyzed for the sensitivity and negative predictive value of SLN biopsy [18,19]. SLN ultrastaging consisted of examining the SLN at 200-μm intervals with routine immunohistochemical staining without specification of the number of examined levels [2,18–20]. Ultrastaging increased the proportion of patients with positive SLN detected by intraoperative assessment (MAC/MIC/ITC) from 7% to 16.6%, similar to the outcome in the SENTIX study (from 7.4% to 16.0%). Definitive SLN ultrastaging allowed the identification of 43.6% of patients with pN1 status (MAC/MIC) versus 43.2% in our study. Final SLN ultrastaging in Senticol I + II detected 25.9% of patients with MAC, 83.3% of patients with MIC, and all patients with ITC who were not identified intraoperatively versus 16.3%, 73.7%, and 90.9% in our study, respectively [2]. A similar result was reported in a previous retrospective study at our center, in which 16.7% of MACs were missed intraoperatively and only detected at final ultrastaging [10].

3.4. Clinical implications

Nodal involvement (MAC and MIC) is the most important prognostic factor in early-stage cervical cancer and guides adjuvant treatment. Failure to detect lymph node involvement could lead to omitting adjuvant radiotherapy in otherwise low or intermediate-risk tumors, thereby increasing the risk of disease recurrence [21,22]. Our study on a large prospective cohort confirms that the main benefit of SLN biopsy is the improvement of LN staging accuracy through intensive pathological processing of a few selected SLNs.

To our knowledge, no study to date has examined the relationship between the intensity of pathological processing and the detection rate of SLN involvement [21,22]. The protocol for pathological ultrastaging should be designed to detect the majority of pN1 cases. According to the outcome of this study, examining four levels obtained from paraffin blocks represent a reasonable compromise, as it allows for the detection of >90% of MAC and MIC cases. This is level of accuracy justifies the recommendation to adopt the examination of four levels as a standard practice, ensuring comprehensive detection and improved patient outcomes.

3.5. Strengths and limitations

The SENTIX trial was unique for its evaluation of the SLN pathological assessment for several reasons. Firstly, SENTIX protocol for serial sectioning was very intensive and aimed to detect all MAC and MIC. SLN tissue was completely processed both at initial gross sectioning and also at the second step, where the number of levels obtained from paraffin blocks was not limited, but blocks were sectioned completely until no tissue was left. Secondly, the levels at which metastases were detected were reported by pathologists, enabling the correlation of metastatic detection rate with the number of levels. Thirdly, the quality of pathology was assessed by central reading. Finally, mandatory SLN

pathological processing included both intraoperative frozen section and ultrastaging, allowing the analysis of the proportion of pN1 cases identified at both steps of the SLN pathological assessment.

A limitation was that besides the number of levels, other parameters characterizing SLN ultrastaging were not tested for its impact in metastatic detection rate, mainly gross processing, and distance between levels. Both parallel (longitudinal) and perpendicular to the long axis SLN gross slicing was allowed. However, evidence that one method is superior to the other is lacking [23]. By processing the entire SLN in paraffin blocks, it is unlikely that different types of gross processing can influence metastatic detection rate. Distance between levels was standardized to 150 μm to maximize the detection of MIC. SLN ultrastaging adds value far exceeding the other parameters.

The selection of centers with previous experience in SLN biopsy might constitute both a strength and a limitation, as the study results might not be generalizable to less experienced centers.

4. Conclusion

There are two main conclusions that can be reached from this analysis of the SENTIX trial. First, >40% of patients with lymph node metastasis larger than 0.2 mm (MIC/MAC) and almost all patients with metastasis <0.2 mm (ITC) are only identified with definitive pathologic examination using an ultrastaging protocol. Second, the detection rate of positive SLN directly correlates with the intensity of ultrastaging. A minimum of four levels examined from paraffin blocks should become an international standard that has the potential to detect >90% of all patients with positive SLNs.

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CRediT authorship contribution statement

Roman Kocian: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Christhardt Kohler:** Writing – review & editing, Investigation. **Sylva Bajsova:** Writing – review & editing, Investigation. **Jiri Jarkovsky:** Software, Project administration, Formal analysis, Data curation. **Ignacio Zapardiel:** Writing – review & editing, Investigation. **Giampaolo Di Martino:** Writing – review & editing, Investigation. **Luc van Lonkhuijzen:** Writing – review & editing, Investigation. **Borek Sehnal:** Writing – review & editing, Investigation. **Octavio Arencibia Sanchez:** Writing – review & editing, Investigation. **Blanca Gil-Ibanez:** Writing – review & editing, Investigation. **Fabio Martinelli:** Writing – review & editing, Investigation. **Jiri Presl:** Writing – review & editing, Investigation. **Lubos Minar:** Writing – review & editing, Investigation. **Radovan Pilka:** Writing – review & editing, Investigation. **Peter Kascak:** Writing – review & editing, Investigation. **Pavel Havelka:** Writing – review & editing, Investigation. **Martin Michal:** Writing – review & editing, Investigation. **Toon van Gorp:** Writing – review & editing, Investigation. **Kristyna Nemejcova:** Writing – review & editing, Investigation. **Pavel Dundr:** Writing – review & editing, Investigation, Funding acquisition. **David Cibula:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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