Gynecologic Oncology 165 (2022) 257–263

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Value of routine cytokeratin immunohistochemistry in detecting low volume disease in cervical cancer

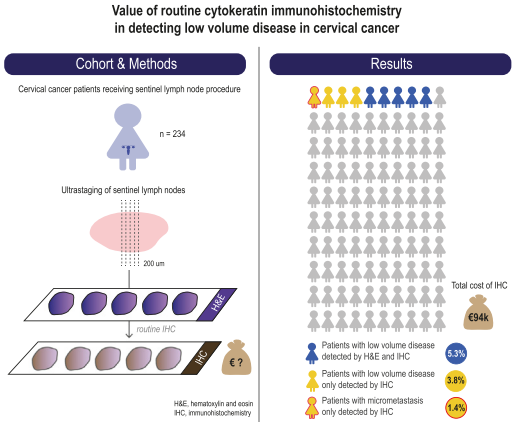
I.G.T. Baeten [a,*], J.P. Hoogendam [a], G.N. Jonges [b], I.M. Jürgenliemk-Schulz [c], A.J.A.T. Braat [c], P.J. van Diest [b], C.G. Gerestein [a], R.P. Zweemer [a]

[a] Department of Gynecologic Oncology, Division of Imaging and Oncology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[b] Department of Pathology, Division of Laboratory, Pharmacy and Biomedical Genetics, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[c] Department of Radiology and Nuclear Medicine, Division of Imaging and Oncology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

HIGHLIGHTS

- We evaluated routine immunohistochemistry as part of the ultrastaging protocol for sentinel lymph nodes in cervical cancer.
- Immunohistochemistry increases detection of low volume disease in sentinel lymph nodes.
- Immunohistochemistry impacts therapeutic strategy-decisions in 1.4% of early-stage cervical cancer patients.
- The added clinical value of routine immunohistochemistry comes at high associated costs.
- Selective use of immunohistochemistry based on risk factors should be considered.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 December 2021
Received in revised form 15 February 2022
Accepted 16 February 2022
Available online 24 February 2022

Keywords:
Cervical cancer
Sentinel lymph node
Ultrastaging
Immunohistochemistry
Low volume disease

ABSTRACT

Objective. In cervical cancer, sentinel lymph nodes (SLNs) are processed according to the pathological ultrastaging protocol. According to current guidelines, immunohistochemistry with pancytokeratin antibodies is performed in addition to step sectioning with hematoxylin and eosin (H&E), aiding the detection of low volume disease (micrometastasis and isolated tumor cells (ITC)). We studied the added clinical value, and costs, of routine immunohistochemistry (IHC).

Methods. We retrospectively included all FIGO stage IA-IIA1 cervical cancer patients who had undergone SLN procedures at UMC Utrecht from 2008 to 2020. Pathological data were derived from the Dutch Pathology Registry (PALGA) including SLN tumor status and number of slides stained with IHC.

Results. In total 234 cervical cancer patients were included. In the 516 surgically resected SLN specimens, 630 SLNs were discovered by the pathologist. Hereof, 579 SLNs from 211 patients were routinely processed with IHC. IHC identified three patients with micrometastasis and five patients with ITC undetected with H&E staining. Thereby, IHC significantly increased the number of patients with low volume disease from 11 (5.3%) to 19 patients (9.1%) ($p = 0.04$). To achieve this, 3791 slides were stained with IHC at an estimated additional cost of €94,775. In 1.4% (95% CI 0.3%–4.3%) of patients routine use of IHC adjusted the adjuvant treatment.

Conclusions. Routine use of IHC increases detection of low volume disease in cervical cancer SLNs compared to

* Corresponding author at: Department of Gynecologic Oncology, Division of Imaging and Oncology, University Medical Center Utrecht, PO Box 85500, Utrecht, 3508 GA, the Netherlands.
E-mail address: i.g.t.baeten@umcutrecht.nl (I.G.T. Baeten).

https://doi.org/10.1016/j.ygyno.2022.02.011

step sectioning with H&E alone by nearly 4%, with an impact on therapeutic strategy-decisions in about 1% of patients. In view of the high associated costs, cost-effectiveness of routine IHC is questionable.

1. Introduction

Lymph node involvement is the strongest prognostic factor for survival in cervical cancer and impacts therapeutic strategy for patients [1]. To assess nodal stage accurately and efficiently, the sentinel lymph node (SLN) procedure in cervical cancer has emerged [2,3]. This procedure maps lymphatic drainage from the primary tumor by injecting a tracer in the cervix. With this tracer, the surgeon is able to identify the first lymph node to receive efferent lymphatic drainage: the SLN. The disease status of SLNs is thought to reflect the disease status in other, non-SLNs [4].

Pathologists use an ultrastaging protocol to reliably assess the disease status of SLNs. This protocol consists of step sectioning at multiple levels and immunohistochemical staining with pancytokeratin antibodies, in addition to hematoxylin and eosin (H&E) staining. Ultrastaging increases the probability of finding low volume disease, defined as either micrometastasis (greatest diameter between 0.2 and 2 mm) or isolated tumor cells (<0.2 mm in greatest diameter), which are easily missed with standard sectioning of (S)LNs [5,6]. Evidence on the impact of low volume disease on survival of patients is limited and controversial, due to the low incidence of both low volume disease in SLNs and recurrence [3,7,8]. To date, clinical evidence indicates that the presence of isolated tumor cells (ITC) in SLNs is not prognostically associated with reduced survival [1,7,9,10]. Therefore, finding ITC does not necessarily impact therapeutic strategy whereas finding micrometastases does and usually adjuvant chemoradiation will be given [11].

Current guidelines advise routine use of immunohistochemistry (IHC) when ultrastaging SLNs from cervical cancer patients [11]. Immunohistochemistry facilitates detection of micrometastasis and ITC by demonstrating tumor specific proteins, even when these tumor cells are not seen on examination of multiple step sections [3,5,12]. However, the added clinical value of IHC – in terms of finding low volume disease that adjusts therapeutic strategy – remains uncertain, while its routine use is time-consuming and costly.

With this study, we aim to evaluate the added value of IHC in terms of detection of low volume disease in cervical cancer in relation to its costs and clinical impact. Clinical impact was defined as detecting low volume disease affecting therapeutic strategy.

2. Materials and methods

2.1. Patient selection

A retrospective observational cohort analysis of all cervical cancer patients who had undergone a robot-assisted SLN procedure between January 2008 and December 2020 was performed. Consecutive patients with a histopathologically proven primary invasive tumor of the cervix, staged as IA1-IB2 or IIA1 (for the retrospective nature of this study determined according to the 2009 FIGO staging guidelines [13]), and an indication for surgical treatment including a SLN procedure, were included in the analysis. Patients were excluded when treated with neoadjuvant chemotherapy. All procedures were part of standard clinical care, for which informed consent was routinely obtained. This study was approved by the institutional review board.

2.2. Sentinel lymph node procedure

A surgical team consisting of three gynecological oncologists performed all surgical procedures with the da Vinci Surgical System (Intuitive Surgical, Sunnyvale, CA, USA, type S until 2010, Si until 2018, and X or Xi since 2018).

SLNs were detected by a combined technique of a radioactive tracer technetium-99m nanocolloid (^{99m}Tc) and patent blue dye injected into the cervical stroma at four quadrants. An SLN was defined as the first lymph node(s) of each pelvic side to drain the lymphatic fluid of the primary tumor, identified intraoperatively with either a gamma counter (set to the 140 keV energy peak of ^{99m}Tc) or blue color, and preferably both. During surgery, SLNs were sent for frozen section examination. A complete pelvic lymph node dissection was performed after each SLN procedure. If the tumor status of SLNs was found to be negative at frozen section, radical uterine surgery was performed. If the tumor status of SLNs was found to be positive at frozen section, the intended radical uterine surgery was abandoned and chemoradiation was given instead, according to international guidelines [11]. In some cases with small tumors and on individualized basis, shared decision between surgeon and patient was made to omit additional pelvic lymph node dissection. Surgery was divided into two separate sessions in patients with fertility-sparing surgery and a >10% perceived risk of lymph node metastases; first, a pelvic lymph node dissection including SLN procedure without frozen section and, second, a radical vaginal trachelectomy in case of negative lymph nodes. Details on imaging protocols and surgical techniques have been previously described [14,15].

2.3. Pathological review

A pathologist specialized in gynecological cancer reviewed all SLNs. Lymph nodes were dissected and counted. Additional smaller nodes found by microscopy were added to the nodal count, distinguishing between the numbers of surgically and pathologically defined SLNs. Two or more smaller SLNs were sometimes paraffin-embedded in a single cassette, at discretion of the pathologist. In those cases, the tumor status of the cassette was reported as one outcome and not reported separately for each (small) SLN.

For intraoperative frozen section examination, the SLNs were halved along the long axis and examined at two levels 200 μm apart. On each level two consecutive slides were obtained. The first slide was stained with hematoxylin and eosin (H&E). The other slide was used for IHC if the H&E slide was negative. If the SLN was larger than 10 mm in diameter, it was sliced along the long axis and embedded into two or more cassettes. If the H&E stained slides obtained by frozen section examination appeared to be negative, the rest of the SLN tissue was processed according to the pathologic ultrastaging protocol (step sectioning with IHC). First, the SLNs were sectioned perpendicular to the long axis at 200 μm intervals. The number of levels was unlimited and whole SLNs were cut out until no tissue was left. Two consecutive slides were obtained on each level. The first slide on each level was stained with H&E. Routinely, the second slide (on the same level) was stained with IHC with pancytokeratin antibodies (AE1/AE3 for squamous cell carcinomas and CAM5.2 for adenocarcinomas). From July 2019 the institutional ultrastaging protocol in cervical cancer was changed. Routine IHC of frozen sections was discontinued and step sectioning of the SLNs and IHC was limited to a maximum of five levels. The pathologist (GJ) retrospectively re-evaluated the pathological slides of the cases with positive SLNs to confirm the reported tumor status of the slides stained with IHC and corresponding H&E stained slides.

Pelvic non-sentinel lymph nodes (non-SLNs) were processed by single section and examined with standard H&E staining.

2.4. Data collection

All variables were extracted from the institutional medical records and Dutch Pathology Registry (PALGA): age at diagnosis, body mass index (BMI, in kg/m^2), FIGO stage, type of procedure, tumor histology and size, presence of lymph vascular space invasion (LVSI), overall detection rate (defined as at least one SLN detected), bilateral detection rate (defined as at least one SLN detected in each pelvic side), anatomical location of SLNs, the pathology protocol (frozen section yes/no; ultrastaging yes/no), SLN count and tumor status, number of slides stained with IHC, and disease recurrence. Disease recurrence was defined as local and/or distant (outside the inner pelvis) presence of malignant tissue originating from the primary tumor, determined clinically, radiographically and/or histopathologically.

2.5. Statistical analysis

Primary outcome was the added value of IHC, defined as the prevalence of low volume disease, micrometastasis (0.2–2 mm in greatest diameter) or ITC (< 0.2 mm in greatest diameter) detected only with IHC. These results were compared to the prevalence of low volume disease after multiple step sectioning with H&E staining only. When two or more positive SLNs were found within a single patient, the patient was assigned to a group based on the largest SLN metastasis.

Additional outcomes of interest were the prevalence of macrometastasis (≥ 2 mm in greatest diameter), cost of processing SLNs with IHC, risk factors associated with low volume disease, and pathological value of frozen section examination.

The Statistical Package for the Social Sciences version 25.0.2 (SPSS; International Business Machines, Armonk, NY, USA) was used for statistical analysis. Continuous variables were compared using Student's *t*-test or, if distribution were non-parametric, Mann–Whitney U. Depending on normality, results are reported as mean ± standard deviation (SD) or as median supplied with range. Categorical data, reported as proportions, were compared between groups using Chi-square test or Fisher's exact test as appropriate. Paired categorical data were compared using McNemar test. With a univariate model using Chi-square, the association of low-volume metastasis with other risk factors was analyzed. Risk factors significant in univariate analysis were entered into a multivariate logistic regression model. Statistical tests were two-sided with significance set at $P < 0.05$, and with confidence intervals (CI) at the 95% level.

3. Results

3.1. Patient population

Clinical features of the patients who underwent SLN detection are summarized in Table 1. In total 234 patients were included with 89.7% staged as FIGO 1B1 and 67.9% diagnosed with squamous cell carcinoma of the cervix. In six patients no SLN was identified in either hemipelvis. In the remaining 228 patients at least one SLN was identified, leading to an overall detection rate of 97.4%. In 200 patients at least one SLN was identified on both pelvic sides, leading to a bilateral detection rate of 85.5%. In total 516 SLNs were harvested intraoperatively with either ^{99m}Tc, blue dye, or both: a median of two (range 1–5) SLNs per patient. The majority of SLNs (498, 96.5%) were localized in four main regions: near the common iliac artery, internal iliac artery, external iliac artery, and obturator vessels (**Fig. S1**). The remaining SLNs (18, 3.5%) were localized outside of the pelvic lymph node dissection field, including parametrium and presacral tissue.

3.2. Sentinel lymph node detection: pathological features of SLNs

Pathological features of the detected SLNs are summarized in Table 2. Upon pathological review, 639 SLNs (median of two per patient, range 1–9) were discovered in the 516 surgical SLN specimens, which were all reviewed by the pathologist. Hereof, 485 SLNs from 194 patients were reviewed with frozen section examination of which 379 SLNs (148 patients) were stained with IHC.

According to the ultrastaging protocol, 579 SLNs from 211 patients were routinely processed with IHC. Fifty-one SLNs were not processed with IHC, primarily because of evident tumor cells in frozen section examination or initial H&E staining.

Table 1
Baseline characteristics of cervical cancer cohort undergoing sentinel lymph node biopsy.

	Patients ($n = 234$)
Age (years), median (range)	39 (23–81)
BMI (kg/m^2), median (range)	23.7 (17.3–41.8)
Clinical FIGO stage (2009), n (%)	
IA1	9 (3.8)
IA2	8 (3.4)
IB1	210 (89.7)
IIA	7 (3.0)
Histology, n (%)	
Squamous cell carcinoma	159 (67.9)
Adenocarcinoma	60 (25.6)
Other (adenosquamous, clear cell, villoglandular)	15 (6.4)
Grade, n (%)	
I	48 (20.5)
II	112 (47.9)
III	69 (29.5)
Not reported	5 (2.1)
Lymph vascular space invasion, n (%)	106 (45.3)
Additional pelvic lymph node dissection performed, n (%)	219 (93.6)
Number of SLNs detected intraoperatively, median (range)	2 (1–5)
Node-positive patients at primary treatment	33 (14.1)
Adjuvant treatment received, n (%)	57 (24.4)
Radiotherapy	26 (11.1)
Chemoradiation	31 (13.2)

Percentages may not total 100 because of rounding. BMI, body mass index. FIGO, International Federation of Gynecology and Obstetrics; SLN, sentinel lymph node.

3.3. Sentinel lymph node detection: pathological outcomes per patient

Frozen section identified metastasis in 14 patients (excluding ITC). The false-negative rate of frozen section examination (excluding ITC) was 33.3% (7/21) per patient (see **Table S1**).

After final pathology of the SLNs, 50 SLNs from 37 patients contained metastases: macrometastasis in 17 patients (26 SLNs), micrometastasis in nine patients (12 SLNs), and ITC in 11 patients (12 SLNs). Of 17 patients with macrometastasis, three patients were diagnosed with micrometastasis or ITC in another SLN. The flowchart in Fig. 1 summarizes all cases with positive SLNs.

A complete pelvic lymph node dissection was performed in 219 (93.2%) patients. After final pathology of all lymph nodes (both the SLNs and non-SLNs), 33 patients were diagnosed with positive lymph nodes who required adjuvant therapy (i.e. without ITC). Of these, 26 patients were detected with macrometastasis or micrometastasis in at least one of the SLNs (Fig. 1). Of the remaining seven patients, two had metastatic lymph nodes in the parametrial tissue removed during radical hysterectomy and were not considered as having false negative SLNs. Four patients had either unilateral SLN mapping or mapping failure. In case of bilateral SLN mapping ($n = 185$ of patients receiving

Table 2
Pathological features of sentinel lymph node procedure in cervical cancer patients.

	Patients ($n = 234$)
Total number of SLNs analyzed	630
SLNs discovered by pathology, median (range)	2 (1–9)
Frozen section examination performed, n (%)	194 (82.9)
Frozen section with IHC	148 (63.2)
IHC ordered in final pathology, n (%)	211 (90.2)

SLN, sentinel lymph node; IHC, immunohistochemistry.

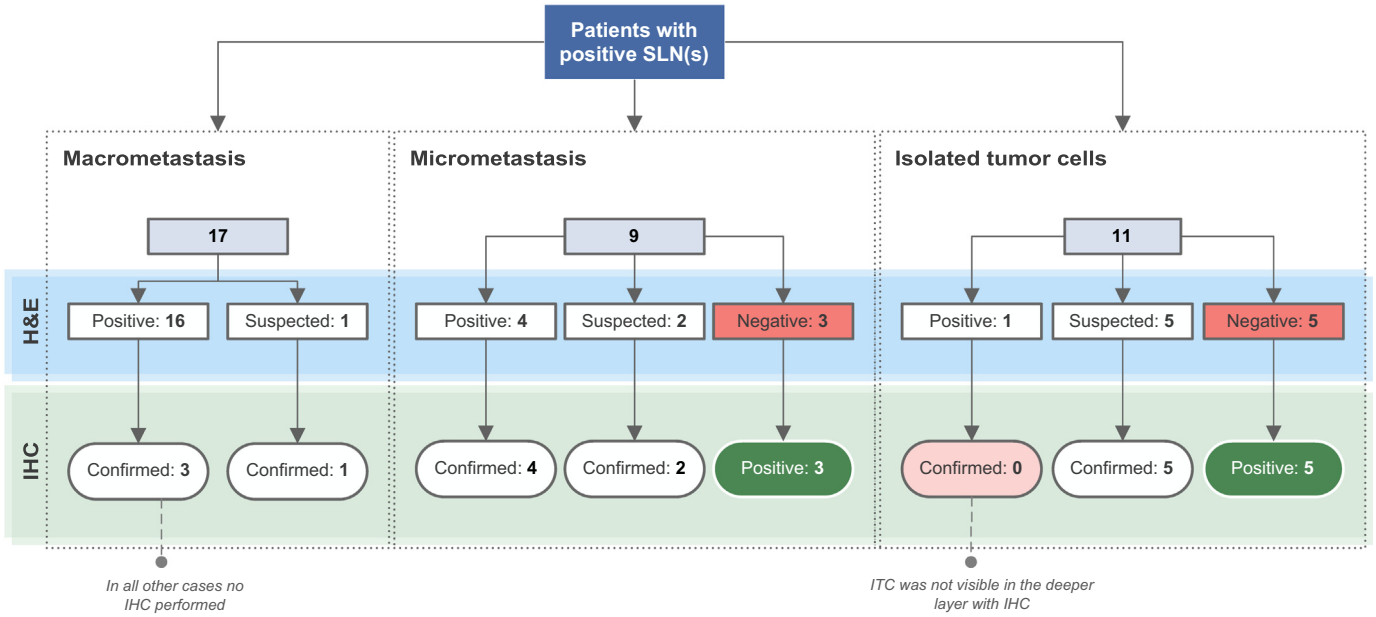


Fig. 1. Flow chart of positive sentinel lymph nodes in cervical cancer patients: cases identified after step sectioning with H&E (blue) versus cases identified only after IHC (green). In the group with macrometastases, IHC did not identify macrometastases that were not already identified with H&E. SLN, sentinel lymph node; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

pelvic lymph node dissection), the negative predictive value of the SLN procecure was 99.4% (158/159) with a false negative rate of 3.7% (1/27) excluding ITC (see **Table S2**).

3.4. Pathological value of immunohistochemistry

Among the 526 SLN samples (containing 579 SLNs) from 211 patients which were routinely stained with IHC, low volume disease was detected in 23 SLNs from 19 patients using IHC: nine patients with micrometastasis (13 SLNs) and 10 patients with ITC (10 SLNs). In eight of these 19 patients, low volume disease was not identified with H&E staining and detected only after IHC: three patients with micrometastasis (four SLNs) and five patients with ITC (five SLNs) (Fig. 1). The three patients who were identified with micrometastasis only after IHC, all received adjuvant therapy (chemoradiation). In these patients postoperative chemoradiation was only indicated by the outcome of the IHC. The routine use of IHC thereby affected the therapeutic strategy in 1.4% (95% CI 0.3%–4.3%) of the patients (3/211).

As summarized in Table 3, routine use of IHC significantly increased the rate of patients with low volume disease from 11 (5.3%) to 19 (9.1%) ($p = 0.04$).

Among the 11 patients with low volume disease in both H&E staining and IHC, eight were identified with atypical cells in H&E staining: IHC was needed to confirm low volume disease. In one patient ITC was only detected using H&E staining and not visible anymore in the sections stained with IHC.

Table 3
Low volume disease detected in cervical cancer sentinel lymph nodes with immunohistochemistry.

Patients ($n = 209$)[a]		
H&E ↓	IHC	
	No low volume disease[b]	Low volume disease
No low volume disease[a]	189 (90.4)	8 (3.8)
Low volume disease	1 (0.5)	11 (5.3)

H&E, hematoxylin and eosin; IHC, immunohistochemistry.
[a] Two patients, in whom IHC showed SLN metastasis, were excluded from this analysis because a macrometastasis was already found in the SLN on the contralateral side (identified without IHC).
[b] Either negative or macrometastasis.

3.5. Costs of immunohistochemistry

The integral costs to stain a single slide with IHC were on average €25 between 2008 and 2020, which included reagents, equipment maintenance and depreciation, and labor. Until July 2019, typically two slides per SLN were stained with IHC in frozen section examination. This added up to 914 slides (from 457 SLN tissue samples) with a total cost of €22,850 and a median of €150 (range €50–€450) per patient. In final pathology, an additional 2877 slides of 700 SLN tissue samples were stained with IHC from 2008 to 2020: a median of four slides (range 1–10) per SLN and a median of 12 slides (range 2–47) per patient. Staining these 2877 slides with IHC cost €71,925 with a median of €100 (range €25–€250) per SLN and a median of €300 (range €50–€1175) per patient. Overall, the costs of staining all slides (including frozen sections) with IHC between 2008 and 2020 thereby added up to €94,775 with a median of €125 (range €25–€300) per SLN and a median of €375 (range €100–€1625) per patient (see Fig. 2). Based on these findings, identifying one additional patient with low volume disease by routine IHC cost €11,846.88. Identifying one additional patient specifically with micrometastasis cost €31,591.67.

3.6. Risk stratification of low volume disease

Table 4 shows the results of the univariate analysis analyzing the correlation of low volume disease with other risk factors. The univariate analysis showed a significant correlation between presence of low volume disease and lymph vascular space invasion ($p < 0.01$). No other variables showed a significant correlation and multivariate regression analysis could therefore not be performed.

All eight patients with low volume disease – detected only with IHC - were diagnosed with lymph vascular space invasion (LVSI), in six patients LVSI was already found in prior biopsy or conisation. None of these patients showed atypical cells with H&E staining.

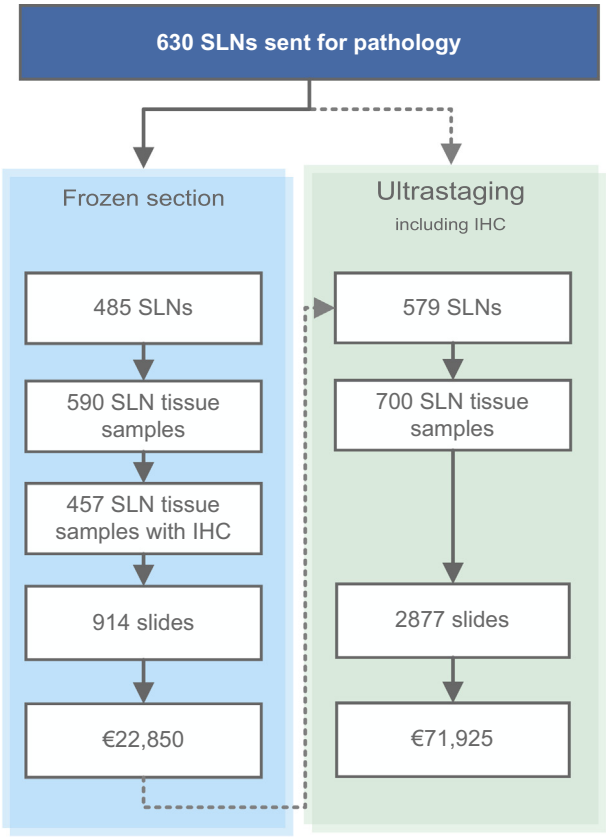


Fig. 2. Flow chart of the costs of immunohistochemistry use in cervical cancer sentinel lymph nodes: total costs of IHC performed during initial processing and frozen section (in blue) and total costs of IHC performed during ultrastaging (in green). SLN, sentinel lymph node; IHC, immunohistochemistry.

3.7. Oncological follow-up

In total 57 patients were postoperatively referred to adjuvant treatment (chemoradiation or radiotherapy) because of positive lymph nodes ($n = 29$), insufficient or positive resection margins ($n = 10$), parametrial invasion ($n = 5$), fulfilled Sedlis criteria (n = 5) [16], or a combination of these (n = 5). One patient was referred to chemoradiation as radical hysterectomy was considered unfeasible due to extensive fibrosis of the pelvis. As part of individualized treatment, one patient was referred to chemoradiation because of bilaterally detected SLNs with ITC in a stage IA2 adenocarcinoma of the cervix, previously treated with conisation. One patient was referred to chemoradiation as radical hysterectomy was abandoned because of a false positive frozen section of the SLN.

The five year disease free survival was 88.5%. Six patients with recurrent disease had lymph node metastases at primary treatment: five patients with macrometastasis and one patient with micrometastasis. Supplemental **Table S3** shows the location of recurrent disease in both node-negative and node-positive patients at primary treatment.

Table 4
Univariate analysis of risk factors for presence of low volume disease with immunohistochemistry in cervical cancer sentinel lymph nodes.

	Patients ($n = 211$)	Frequency with low volume disease	*P*
FIGO stage (2009)			0.38[a]
IA1/IA2	17	0.0%	
≥IB1	194	10.3%	
Histology			0.27
Squamous cell carcinoma	143	9.1%	
Adenocarcinoma	54	7.4%	
Other (e.g. adenosquamous, villoglandular)	14	21.4%	
Grade			0.62
I	46	10.9%	
II	98	7.1%	
III	62	11.3%	
Tumor size			0.46
< 2 cm	137	8.0%	
≥ 2 cm	74	12.2%	
Lymph vascular space invasion			<0.01
Negative	116	2.6%	
Positive	95	17.9%	

FIGO, International Federation of Gynecology and Obstetrics; [a]Fisher's exact test (>20% expected count <5).

4. Discussion

The present study concerned a cohort analysis of 234 cervical cancer patients who had undergone an SLN procedure in which the clinical value of IHC within the ultrastaging SLN protocol was evaluated. In 211 patients (90.2%) IHC was ordered. Using IHC, eight patients (3.8%) with low volume disease were identified in whom tumor cells were not identified upon step sectioning with H&E staining only: three patients with micrometastasis and five patients with ITC. Thereby, routine use of IHC significantly increased the rate of patients with low volume disease to 19 patients (from 5.3% to 9.1%). More importantly, IHC affected therapeutic strategy in three patients with micrometastasis, adding clinical value in 1.4% (3/211) of patients. Routine use of IHC however came with substantial costs: to identify one additional patient with a clinically relevant metastasis, approximately €31,592 had to be spend according to local staining costs.

Another important finding was that H&E stained slides from eight patients showed atypical cells suspicious for metastasis. In those cases, the pathologist needed IHC to confirm presence of small macrometastasis, micrometastasis or ITC (Fig. 1). In three patients IHC was performed to confirm clinically relevant metastasis (either macrometastasis or micrometastasis) suspected by H&E and thus confirmed the indication for adjuvant therapy (Fig. 1). When these cases are included, IHC on indication added clinical value in 2.8% (6/211) of patients.

The current guidelines for cervical cancer suggest that small macrometastases as well as micrometastases and isolated tumor cells can be identified in SLNs by using the ultrastaging protocol [11]. As outlined in a literature review by Cibula et al., ultrastaging is suggested to detect an additional 15% of patients with positive SLNs, considering macrometastasis, micrometastasis and ITC positive for metastasis [3]. A recent posthoc analysis of the SENTICOL I study reported that 8.1% of apparently node-negative patients were found to be node-positive only after ultrastaging, defined as step sectioning and IHC [17]. From this analysis it was not clear what type of metastasis was found, nor in how many patients. A comprehensive review of such ultrastaging protocols used in 127 studies showed that only 11 studies described the number of patients with SLN metastasis only detected after ultrastaging. In these 11 studies combined, 21% of the patients were detected with SLN metastasis only after ultrastaging (36/171 patients with SLN metastasis) [6].

Whereas the value of ultrastaging by step sectioning seems to be well established, less convincing evidence exists on the added value of IHC. In a small retrospective analysis of 48 early-stage cervical cancer patients, the researchers separately described detection by step sectioning and IHC. They reported that the rate of patients with SLN metastasis increased from 12 to 15 patients, of whom two patients were detected with step sectioning and one patient with IHC only (showing ITC) [5]. Salvo et al. reported 22% of node-positive patients (6/27) being converted to node-positive only after IHC, including ITC [18]. A recent observational cohort study of 92 cervical cancer patients reported that

six of 13 patients (46%) with positive SLNs (including ITC) were detected only after IHC [12] In both studies it is not clear what type of metastasis – i.e. clinically relevant or not – was additionally detected by IHC. In addition, the researchers did not describe the step sectioning intervals nor did they describe if a metastasis was already suspected in the corresponding H&E stained slides. In our IHC cohort of 211 patients, 33.3% (8/24) of node-positive patients were detected only after IHC, including ITC. One patient with ITC only was missed by IHC. Considering clinically relevant SLN metastasis – metastasis that adjust the adjuvant therapy (i.e. macrometastasis and micrometastasis) – 23.1% (3/13) of node-positive patients were detected only after IHC. If we include cases in which IHC was confirmatory for presence of metastasis (H&E slides showed atypical cells), 69.6% (16/23) of node-positive patients were identified only after IHC. In a study of 49 cervical cancer patients originating from 2004, Juretzka et al. assessed the added value of IHC in a different way; they reported that 8% of node-negative patients (4/49) were identified with lymph node micrometastasis only after IHC [19]. Choosing this approach, in our cohort 4.1% of node-negative patients (8/196) were identified only after IHC. This difference may be explained by the fact that Juretzka et al. retrospectively re-assessed all the pathology specimens of all pelvic lymph nodes with IHC. The four patients identified only after IHC, in retrospect, all received adjuvant therapy for other reasons (e.g. tumor >4 cm). Since this study was conducted before the SLN staging era, the researchers used a different ultrastaging protocol for pelvic lymph nodes than what is common nowadays [19].

To date, there have been few publications on the cost of the ultrastaging protocol, and specifically IHC, in cervical cancer. A study on the cost-effectiveness of the entire SLN procedure in cervical cancer reported that the SLN procedure including pathological ultrastaging was clinically superior and cost saving in comparison to traditional pelvic lymph node dissection [20]. The cost-effectiveness analysis was based on estimated costs of pathological ultrastaging at 580 Canadian dollars (≈€392) per patient and standard pathological processing at 375 Canadian dollars (≈€254) per patient. Recently, researchers studying the SLN procedure in ovarian cancer reported a median cost of ultrastaging of €96.8 to €124.5 per patient and €46.1 per SLN [21]. These estimated costs are lower than what we have reported (€275 to €375), mainly due to the low costs for each slide stained with IHC in this Spanish study (€3.5). Obviously, the total costs of pathology processing is heavily dependent on the number of submitted SLNs, the number of tissue blocks generated from these SLNs, and the number of levels cut from these blocks. Further, it is unclear whether the IHC costs described in the above studies concern the reagents costs only or integral costs including also equipment maintenance and depreciation and labor. Therefore, these international differences in costs of IHC should be interpreted with caution.

Pathological ultrastaging of SLNs in cervical cancer leads to an increased detection of low volume disease. The impact of low volume disease on the prognosis of cervical cancer patients remains controversial as the findings of recent studies are inconsistent, although most researchers agree that finding ITC in the SLNs does not affect survival [7–10]. Certain limitations can explain the inconsistencies in literature. The first limitation is the relatively low incidence of recurrence in combination with low incidence of low volume disease in the patients with cervical cancer [8]. Much larger cohorts are required to assess the prognostic value of low volume disease and the added value of additional treatment based on that. The second limitation is that the ultrastaging protocol has not been standardized yet. The intensity of pathology processing differs substantially internationally and even nationally [6]. Such differences inevitably affect the accuracy of detection of low volume disease and small macrometastasis by ultrastaging.

As shown in the present study, IHC facilitates detection of an additional group of patients with micrometastasis only, who are referred to adjuvant treatment. In the largest retrospective cohort study in cervical cancers patients to date, Cibula et al. reported a decreased overall survival in patients with micrometastasis, which was similar to patients with macrometastasis [1]. A recent study substantiated these findings and highlighted the importance of routine SLN ultrastaging since this detects 10% of patients with micrometastasis who would otherwise be missed [10]. In our cohort, three patients with micrometastasis would have been missed without IHC, with potential detrimental consequences for their survival.

False-negative SLNs were classified as cases in which positive non-SLN were detected on the same pelvic side as the histologically negative SLN, i.e. cases that would have been missed when the MSKCC algorithm is applied [22]. Subsequently a false negative rate of 6.3% was reported in case of at least unilateral SLN detection. The false-negative rate lowers to 3.1% if ITC is considered a relevant finding, comparable to the results of a recent analysis on the SENTICOL I data [17]. The relevance of detecting ITC was already confirmed by the study of Zaal et al., in which survival is significantly improved by pelvic lymph node dissection (>16 lymph nodes) when micrometastasis or ITC are found in the SLN [23].

The present study is limited by its retrospective single center design. All positive cases by IHC were re-evaluated by the pathologist in retrospect, who was not blinded for the outcome, in order to double check the reported outcome of H&E and IHC staining. In retrospect, when the outcome is known, the positive SLNs reported as being missed with H&E staining may show some atypical cells when thoroughly searching for an unlimited time. Nevertheless, in daily practice, this is not feasible as ultrastaging SLNs is already a time consuming process for the pathologist. Therefore, outcomes were based on the original pathology report. However, the ultrastaging protocol is not generalized yet and details of the protocol at our center may differ from others. In the same way the estimated costs of IHC may differ from other centers. The institutional ultrastaging protocol changed in July 2019, which mainly affected the number of IHC slides per SLN. We have no indications that the protocol change affected the pathological outcome of ultrastaging. To our knowledge, this is the first study to assess the added value of IHC in cervical cancer in relation to its costs.

Our findings regarding the costs of IHC raise the question if its routine use is a cost-effective strategy. Bethune et al. suggested that the shift towards selective use of IHC in breast cancer patients, instead of routine use, resulted in decreased ITC detection but at the same time led to significant cost savings [24]. In their cohort the rate of macrometastasis and micrometastasis remained equal. Our univariate risk model showed that LVSI had a significant correlation with presence of low volume disease and may be used to indicate IHC. This is consistent with previous studies [19], although other risk factors have been described. In an analysis of the SENTICOL I and II data, Guani et al. suggested that LVSI, FIGO stage, and depth of stromal invasion were correlated with low volume disease in univariate analysis, while LVSI and FIGO IB1 stage were significantly correlated in their multivariate analysis [7]. Colturato et al. retrospectively studied 83 node-negative patients between 2001 and 2007 and re-assessed all pelvic lymph nodes with IHC [25]. They suggested an algorithm for performing IHC on pelvic lymph nodes, based on FIGO stage (IB2 or II), insufficient lymphadenectomy (<12 nodes), cervical tumor >2 cm and stromal invasion >2/3. Now that the SLN procedure has emerged, ultrastaging of SLNs replaces the need for extensive pathological assessment (including IHC) of all pelvic lymph nodes and new algorithms for selective use of IHC, obtained from prospective studies, are needed.

Routine use of IHC on SLNs adds clinical value by increasing the detection of low volume disease in cervical cancer by nearly 4%, affecting therapeutic strategy-decisions in about 1% of patients. This added value comes at substantial costs. More selective use of IHC based on risk factors may therefore increase cost-effectiveness, while patient safety is maintained. Before this shift can be made, more research is warranted.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2022.02.011.

Author contribution

Conceptualization: IB, PvD, RZ. Methodology and investigation: IB, JH, GJ, PvD. Formal analysis: IB, JH. Validation: All authors. Writing – original draft: IB. Writing – review & editing: All authors. Supervision: CG, RZ.

Declaration of Competing Interest

RZ is proctor for robot-assisted surgery in gynecological oncology on behalf of Intuitive Surgical Inc. All other authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article. No funding was received.

References

[1] D. Cibula, N.R. Abu-Rustum, L. Dusek, M. Zikán, A. Zaal, L. Sevcik, et al., Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer, Gynecol. Oncol. 124 (3) (2012) 496–501, https://doi.org/10.1016/j.ygyno.2011.11.037.

[2] D. Cibula, N.R. Abu-Rustum, L. Dusek, J. Slama, M. Zikán, A. Zaal, et al., Bilateral ultrastaging of sentinel lymph node in cervical cancer: lowering the false-negative rate and improving the detection of micrometastasis, Gynecol. Oncol. 127 (3) (2012) 462–466, https://doi.org/10.1016/j.ygyno.2012.08.035.

[3] D. Cibula, W.G. McCluggage, Sentinel lymph node (SLN) concept in cervical cancer: current limitations and unanswered questions, Gynecol. Oncol. 152 (1) (2019) 202–207, https://doi.org/10.1016/j.ygyno.2018.10.007.

[4] L.R. Eiriksson, A. Covens, Sentinel lymph node mapping in cervical cancer: the future? BJOG. 119 (2) (2012) 129–133, https://doi.org/10.1111/j.1471-0528.2011.03134.x.

[5] E.D. Euscher, A. Malpica, E.N. Atkinson, C.F. Levenback, M. Frumovitz, M.T. Deavers, Ultrastaging improves detection of metastases in sentinel lymph nodes of uterine cervix squamous cell carcinoma, Am. J. Surg. Pathol. 32 (9) (2008) 1336–1343, https://doi.org/10.1097/PAS.0b013e31816ecfe4.

[6] P. Dundr, D. Cibula, K. Němejcová, I. Tichá, M. Bártů, R. Jakša, Pathologic protocols for sentinel lymph nodes ultrastaging in cervical cancer, Arch. Pathol. Lab. Med. (2019) https://doi.org/10.5858/arpa.2019-0249-RA.

[7] B. Guani, V. Balaya, L. Magaud, F. Lecuru, P. Mathevet, The clinical impact of low-volume lymph nodal metastases in early-stage cervical cancer: the senticol 1 and senticol 2 trials, Cancers (Basel) 12 (5) (2020) https://doi.org/10.3390/cancers12051061.

[8] M. Delomenie, H. Bonsang-Kitzis, A.S. Bats, C. Ngo, V. Balaya, H.T.N. Xuan, et al., The clinical implication of lymph nodes micrometastases and isolated tumor cells in patients with cervical cancer: a systematic review, Eur. J. Obstet. Gynecol. Reprod. Biol. 241 (2019) 71–76, https://doi.org/10.1016/j.ejogrb.2019.08.010.

[9] A. Nica, L.T. Gien, S.E. Ferguson, A. Covens, Does small volume metastatic lymph node disease affect long-term prognosis in early cervical cancer? Int. J. Gynecol. Cancer 30 (3) (2020) 285–290, https://doi.org/10.1136/ijgc-2019-000928.

[10] R. Kocian, J. Slama, D. Fischerova, A. Germanova, A. Burgetova, L. Dusek, et al., Micrometastases in sentinel lymph nodes represent a significant negative prognostic factor in early-stage cervical cancer: a single-institutional retrospective cohort study, Cancers (Basel) 12 (6) (2020) https://doi.org/10.3390/cancers12061438.

[11] D. Cibula, R. Pötter, F. Planchamp, E. Avall-Lundqvist, D. Fischerova, C. Haie Meder, et al., The European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology Guidelines for the Management of Patients with Cervical Cancer, Int. J. Gynecol. Cancer 28 (4) (2018) 641, https://doi.org/10.1097/IGC.0000000000001216.

[12] T.P. Diniz, C.C. Faloppa, H. Mantoan, B.T. Gonçalves, L.Y. Kumagai, A.N.O. Menezes, et al., Pathological factors associated with non-sentinel lymph node metastasis in early stage cervical cancer, J. Surg. Oncol. 123 (4) (2021) 1115–1120, https://doi.org/10.1002/jso.26341.

[13] S. Pecorelli, Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium, Int. J. Gynaecol. Obstet. 105 (2) (2009) 103–104, https://doi.org/10.1016/j.ijgo.2009.02.012.

[14] I.G.T. Baeten, J.P. Hoogendam, H.W.R. Schreuder, I.M. Jurgenliemk-Schulz, R.H.M. Verheijen, R.P. Zweemer, et al., The influence of learning curve of robot-assisted laparoscopy on oncological outcomes in early-stage cervical cancer: an observational cohort study, BJOG. 128 (3) (2021) 563–571, https://doi.org/10.1111/1471-0528.16399.

[15] J.P. Hoogendam, M.G. Hobbelink, W.B. Veldhuis, R.H. Verheijen, P.J. van Diest, R.P. Zweemer, Preoperative sentinel node mapping with (99m)Tc-nanocolloid SPECT-CT significantly reduces the intraoperative sentinel node retrieval time in robot assisted laparoscopic cervical cancer surgery, Gynecol. Oncol. 129 (2) (2013) 389–394, https://doi.org/10.1016/j.ygyno.2013.02.020.

[16] A. Sedlis, B.N. Bundy, M.Z. Rotman, S.S. Lentz, L.I. Muderspach, R.J. Zaino, A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: a Gynecologic Oncology Group Study, Gynecol. Oncol. 73 (2) (1999) 177–183, https://doi.org/10.1006/gyno.1999.5387.

[17] P. Mathevet, B. Guani, A. Ciobanu, E.M. Lamarche, F. Boutitie, V. Balaya, et al., Histopathologic validation of the sentinel node technique for early-stage cervical cancer patients, Ann. Surg. Oncol. 28 (7) (2021) 3629–3635, https://doi.org/10.1245/s10434-020-09328-2.

[18] G. Salvo, P.T. Ramirez, C.F. Levenback, M.F. Munsell, E.D. Euscher, P.T. Soliman, et al., Sensitivity and negative predictive value for sentinel lymph node biopsy in women with early-stage cervical cancer, Gynecol. Oncol. 145 (1) (2017) 96–101, https://doi.org/10.1016/j.ygyno.2017.02.005.

[19] M.M. Juretzka, K.C. Jensen, T.A. Longacre, N.N. Teng, A. Husain, Detection of pelvic lymph node micrometastasis in stage IA2–IB2 cervical cancer by immunohistochemical analysis, Gynecol. Oncol. 93 (1) (2004) 107–111, https://doi.org/10.1016/j.ygyno.2003.11.033.

[20] H. Brar, L. Hogen, A. Covens, Cost-effectiveness of sentinel node biopsy and pathological ultrastaging in patients with early-stage cervical cancer, Cancer. 123 (10) (2017) 1751–1759, https://doi.org/10.1002/cncr.30509.

[21] V. Lago, B. Montero, S. López, P. Padilla-Iserte, L. Matute, T. Marina, et al., Ultrastaging protocol in sentinel lymph node for apparent early stage ovarian cancer, Gynecol. Oncol. 161 (2) (2021) 408–413, https://doi.org/10.1016/j.ygyno.2021.03.001.

[22] B. Cormier, J.P. Diaz, K. Shih, R.M. Sampson, Y. Sonoda, K.J. Park, et al., Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer, Gynecol. Oncol. 122 (2) (2011) 275–280, https://doi.org/10.1016/j.ygyno.2011.04.023.

[23] A. Zaal, R.P. Zweemer, M. Zikán, L. Dusek, D. Querleu, F. Lécuru, et al., Pelvic lymphadenectomy improves survival in patients with cervical cancer with low-volume disease in the sentinel node: a retrospective multicenter cohort study, Int. J. Gynecol. Cancer 24 (2) (2014) 303–311, https://doi.org/10.1097/igc.0000000000000043.

[24] G.C. Bethune, M.A. Karkada, R. DeCoste, P.J. Barnes, D. Rayson, Isolated tumor cells in sentinel lymph nodes of primary invasive breast carcinoma: a cohort analysis, Clin. Breast Cancer 19 (4) (2019) 286–291, https://doi.org/10.1016/j.clbc.2019.03.002.

[25] L.F. Colturato, R.C. Signorini Filho, R.C.M. Fernandes, L.H. Gebrim, A.H. Oliani, Lymph node micrometastases in initial stage cervical cancer and tumoral recurrence, Int. J. Gynaecol. Obstet. 133 (1) (2016) 69–75, https://doi.org/10.1016/j.ijgo.2015.08.019.