



Detection of high-risk human papillomavirus DNA in tissue from primary cervical cancer tumor, pelvic lymph nodes and recurrent disease

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ABSTRACT

Objectives: The present study investigated Human Papillomavirus (HPV) DNA genotyping in primary tumor, pelvic lymph nodes (PLN) and recurrence in early-stage cervical cancer patients.

Methods: We conducted a hospital-based case-control study. From 2003 to 2015, 282 patients underwent surgery for cervical cancer in the Department of Gynecology, Aarhus University Hospital, Denmark. Twenty-nine recurrent cases were identified. HPV DNA genotyping was performed on formalin-fixed, paraffin-embedded tissue specimens from the primary tumor, PLN, and recurrent disease.

Results: In the primary tumor, HPV DNA was detectable in 18(72%) of 25 tissue specimens from recurrent cases and in 15(83%) of 18 controls. HPV DNA-positive PLN was significantly associated with recurrence, 83%(95%CI: 52–98%), compared to patients with HPV-negative PLN, 38%(95%CI: 18–62%)($p < 0.05$). HPV DNA genotyping was positive in eight of 12(67%) patients with recurrent disease. The genotype was identical in all three tissues types.

The positive predictive value for recurrence was the same for detection of HPV-DNA and metastases in the PLN, with reasonable sensitivity. The negative predictive value for recurrence, however, was best for HPV-DNA, 62%(95%CI: 38–98%).

Conclusions: In conclusion, our data suggest that the presence of HPV in pelvic lymph nodes is associated with an increased risk of recurrence.

1. Introduction

Human papillomavirus (HPV) is obligate for the development of cervical cancer [1–3]. Previous studies have reported the prevalence of high-risk HPV in both pelvic lymph nodes (PLN) and paraaortic lymph nodes (PAL) as well as in distant metastases; however, the impact of these findings in the clinical setting is not clear [3–12]. HPV-DNA in the cervical cancer metastases, including PLN and PAL, has been detected by different laboratory approaches, in different clinical settings, as well as by different measured outcomes [3–12], which makes comparisons and interpretation of the results difficult.

In early stage cervical cancer, the TNM classification is currently used as the preferred method to report the pathology findings. At this point, the most important prognostic finding is the presence of PLN metastases [13], which determines whether primary or adjuvant

chemo-radiation is given.

The demonstration of PLN metastases depends on several steps in the diagnostic process. Traditionally the surgical procedure performed includes complete pelvic lymphadenectomy. Recently, the sentinel node (SLN) procedure has been extensively evaluated. In the SLN procedure a tracer is injected into the uterine cervix to perform lymphatic mapping and SLN biopsy to spare the lymphatic drainage [14–17]. The subsequent pathologic examination of the lymph nodes is dependent of the surgical approach [14–16]. Traditionally, the complete lymph nodes are paraffin embedded, sectioned longitudinally and one slide originating from the central part of each node is examined [17]. In the SLN method, the protocol includes a more thorough investigation with tighter sections perpendicular to the long axis through the lymph node [16]. Apart from the high number of slides examined, the sectioning process leaves a substantial amount of waste material arriving from the

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areas between the examined slides. The consequence of more examined slides from the SLN's reduces the risk of missing micrometastases compared to the protocol after full PLN excision [14–19].

We undertook this study to evaluate HPV DNA detection in the surgical managed early-stage cervical cancer patients (FIGO stage IB) by examining tissue from primary tumor, PLN, and recurrent disease. Thus, we examined the prevalence of HPV DNA in tissue biopsies from patients with recurrent disease to determine whether there is congruence between the HPV genotypes detected in the primary tumor, PLN, and recurrent tumor tissue. Furthermore, we evaluated the potential of HPV DNA testing in PLN tissue as an alternative to microscopic examination for metastases, and the prognostic value of HPV DNA positivity in PLN.

2. Methods

2.1. Design

2.1.1. Participants and setting

We conducted a retrospective hospital-based case-control study. The study population comprised 282 women who had been surgically treated (i.e. radical hysterectomy and full PLN excision) for early-stage cervical cancer (i.e. FIGO stage IB) in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Denmark, in the period January 1, 2003 to December 31, 2015. The surgical procedure was performed in one step. Intra-operative observation of bulky nodes or obvious parametrial invasion induced termination of the surgical procedure.

The patients with International Classification of Disease 10 diagnostic code for cervical cancer and recurrence of cervical cancer were identified through a computerized list from the Danish Pathology Databank. Eligible patients had to have a histologically verified cervical cancer (i.e. squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma). Cases were eligible if the primary tumor was HPV-DNA positive and if they had been diagnosed with cervical cancer recurrence in the period from cervical cancer diagnosis until August 24, 2016, death, or emigration, which ever came first. Controls were randomly selected from the study population and had to have no record of cervical cancer recurrence in the period from cervical cancer diagnosis until August 24, 2016, death, or emigration, which ever came first. Controls, corresponding to the number of cases with an HPV-DNA positive primary tumor were matched to cases on age (+/-2 years), and an effort was made to closely relate the dates of surgery. Of note, only controls with an HPV-DNA positive primary tumor were eligible for final analysis (see Fig. 1 for elaboration). From the Danish Pathology Databank and medical records, we collected information on age at diagnosis, result of the pathological examination, FIGO stage, etc.

2.1.2. Data collection and management

Formalin-fixed, paraffin-embedded (FFPE) blocks containing tissue from the primary tumor, PLN, and recurrent tissue were collected at the Department of Pathology, Aarhus University Hospital, Denmark. As several FFPE blocks were available for each patient, the slides were reviewed and a representative slide with tumor tissue was chosen from the primary cervical tumor. In patients with metastatic lymph nodes, a representative slide was selected to ensure the presence of tumor cells in the tissue tested. For the patients with non-metastatic lymph nodes, the representative slide from the largest lymph node in each lymph node station was selected, a total of six, if available. Finally, in the recurrent cases, we selected the FFPE block containing the most tumor cells. Fine-needle aspirations from recurrent disease could not be used because the number of cells provided is insufficient for HPV testing.

The preparation of the blocks, DNA extraction, and HPV analysis were performed at the Department of Pathology, Aarhus University Hospital, Denmark. The FFPE tissue from the primary tumor, pelvic lymph node stations, and recurrent tumor tissue were collected from

the archive.

The “sandwich technique” was applied to enable histopathologic review of sections flanking the sections used for HPV genotyping. First, a 3- μ m section was cut, stained with hematoxylin and eosin (HE), and used for the primary microscopic examination and selection, as described above. For tissue from the primary tumor, the metastatic lymph nodes, and the recurrent disease, four 10- μ m sections were cut for the HPV genotyping after macrodissection. From patients with non-metastatic lymph nodes, two 10- μ m sections were cut from the largest lymph nodes from each of the available lymph node stations, up to six. Finally, a 3- μ m section was cut for HE staining to confirm the presence or absence of tumor tissue in the material selected for HPV testing. To avoid contamination, the microtome, tweezers, brush, and knife were cleaned with 1% sodium dodecyl sulfate and 99% ethanol before and after cutting every FFPE block.

DNA extraction was performed using the QIA Symphony DSP DNA mini kit (Qiagen, Venlo, the Netherlands) following the manufacturer's instructions. HPV detection and genotyping were performed using the INNO-LiPA[®] HPV Genotyping Extra II (LiPA) (Fujirebio Europe, Ghent, Belgium) according to the manufacturer's instructions. The INNO-LiPA[®] utilizes the SPF10 primer set, which amplifies a 65 base-pair region in the L1 open reading frame, followed by HPV genotyping by reverse hybridization. The assay allows the detection of 32 HPV genotypes, including all high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), potentially high-risk HPV genotypes (25, 53, 66, 70, 73, 82), and low-risk HPV genotypes (6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, 89). Laboratory personnel were blinded to the clinical data related to each sample. All microscopic examinations were performed by the first author (KF), and supervised by an experienced gynecological pathologist (EM).

2.2. Statistics

The data are described as binary data. Medians were presented with interquartile range (IQR). Proportions with exact 95% confidence intervals (95%CI) were calculated. A Fischer's exact test was used to assess the hypothesis of no difference in the proportions. Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity with exact 95%CI were calculated. Calculations were performed in Stata 13.1.

2.3. Ethics

This study was approved by the Central Denmark Region Committees on Health Research Ethics (reference number: 1-10-72-321-13, 3 December 2013), the Danish Data Protection Agency (reference number: 1-16-02-252-12, 19 September 2012), and The Danish Health Authority (reference number: 3-3013-203/1/, 25 January 2013). In accordance with Danish legislation informed consent was not necessary. The Danish Tissue Use Registry was searched to verify that the specimens were eligible for medical research.

3. Results

During 2003–2015, 282 patients underwent surgery due to FIGO stage IB cervical cancer in Aarhus University Hospital of which 29 (10%) were diagnosed with recurrence. A total of 33 patients were included; 18 controls with recurrence and HPV-DNA positive primary tumor were matched with 18 cases without recurrence, only 15 had HPV-DNA positive primary tumor (see Fig. 1 for elaboration). The overall median age was 47 years (IQR 22–72). The median interval from surgery to recurrence was 22 months (IQR 8–35). The distribution of HPV DNA strains detected in the primary tumor was HPV16 (17/51%), HPV18 (8/24%), HPV31 (3/9%), HPV39 (4/12%), and HPV45 (1/3%)(Table 1).

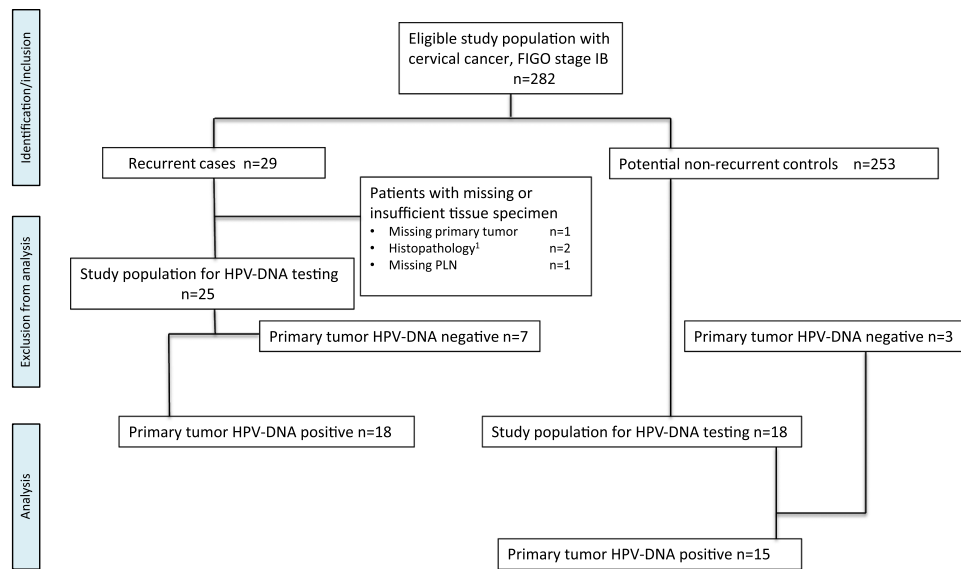


Fig. 1. Consolidated Standards of Reporting Trials diagram of the study population. FIGO indicates International Federation of Gynecology and Obstetrics. ¹Other than squamous cell carcinoma, adenosquamous carcinoma and adenocarcinoma.

Table 1

Clinical characteristics of 33 patients with early-stage cervical cancer (FIGO stage 1B) given primary radical surgical treatment in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark, from 2003 to 2015.

Characteristics	Recurrence (n = 18)	No recurrence (n = 15)
Age (years)		
< 45	5	6
≥ 45	13	9
Median age 47 years (IQR 22–72)		
Tumor size (largest diameter)	2 ND	
< 2 cm	2	6
2–4 cm	7	6
> 4 cm	7	3
Histopathology		
Squamous cell carcinoma	10	10
Adenocarcinoma/ Adenosquamous carcinoma	8	5
Parametrial invasion	2	0
Lymphovascular involvement	13	2
Pelvic lymph node metastases	5	1
Distribution of HPV genotypes		
HPV16	7	9
HPV18	6	3
HPV31	1	2
HPV39	3	1
HPV45	1	0
Adjuvant chemo-radiation	7	3

ND; not described.

3.1. HPV DNA congruence

Recurrence was detected by fine needle aspiration in six patients, and therefore no material was available for HPV DNA testing. In the remaining 12 patients, HPV DNA was detected in the recurrent tumor tissue from eight patients (67% (95% CI: 35–90%)) and in 100% (8/8) the HPV genotype was consistent in the primary tumor, PLN, and recurrent tissue. In 13% (2/15) of the non-recurrent cases, HPV DNA was detected in PLN with a 100% (2/2) congruent HPV genotype (Table 2).

3.2. HPV DNA and metastases in PLN

In the group with HPV DNA-negative PLN one (5% (95%CI:

Table 2

The prevalence of tumor tissue confirmed by microscopy and HPV DNA detection using the INNO-LiPA® HPV Genotyping Extra II (LiPA) (Fujirebio Europe, Ghent, Belgium) in primary tumor, in pelvic lymph nodes (PLN) and in tissue biopsy from recurrent disease from early-stage cervical cancer (FIGO stage 1B) patients treated in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark from 2003 to 2015.

Patients	Primary tumor Confirmed by microscopy / HPV detection	PLN Confirmed by microscopy / HPV detection	Recurrent tissue Confirmed by microscopy / HPV detection
Recurrence (n = 18)	+ / + (HPV18)	- / + (HPV18)	+ / NS
	+ / + (HPV16)	- / + (HPV16)	+ / + (HPV16)
	+ / + (HPV18)	+ / + (HPV18)	+ / + (HPV18)
	+ / + (HPV18)	- / -	+ / -
	+ / + (HPV39)	- / -	+ / NS
	+ / + (HPV18)	- / -	+ / NS
	+ / + (HPV16)	+ / + (HPV16)	+ / -
	+ / + (HPV18)	- / + (HPV18)	+ / -
	+ / + (HPV39)	- / -	+ / + (HPV39)
	+ / + (HPV16)	- / + (HPV16)	+ / + (HPV16)
	+ / + (HPV16)	+ / + (HPV16)	+ / NS
	+ / + (HPV16)	- / -	+ / NS
	+ / + (HPV18)	+ / -	+ / NS
	+ / + (HPV16)	- / -	+ / + (HPV16)
	+ / + (HPV31)	+ / + (HPV31)	+ / + (HPV31)
	+ / + (HPV39)	+ / + (HPV39)	+ / + (HPV39)
	+ / + (HPV45)	- / -	+ / -
	+ / + (HPV16)	- / + (HPV16)	+ / + (HPV16)
No recurrence (n = 15)	+ / + (HPV16)	- / + (HPV16)	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV18)	- / -	NR
	+ / + (HPV39)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV18)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV31)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV31)	- / -	NR
	+ / + (HPV16)	- / -	NR
	+ / + (HPV16)	+ / + (HPV16)	NR
	+ / + (HPV18)	- / -	NR

NS; fine needle aspiration, not sufficient material for HPV detection, NR; no recurrent disease.

Table 3

The prevalence of HPV DNA in pelvic lymph nodes (PLN) with and without microscopic metastatic PLN obtained at primary radical surgical treatment from 33 early-stage cervical cancer (FIGO stage IB) patients treated in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark, from 2003 to 2015.

PLN	Microscopic non-metastatic	Microscopic metastatic	Proportion	
	n	n	PP (95%CI)	
HPV DNA-negative	20	1	5% (1–24%)	21
HPV DNA-positive	7	5	42% (15–72%)	12
	27	6		33

Fischer’s Exact: $p < 0.05$.

1–24%) of 21 patients had PLN metastases compared to five (42% (95%CI: 15–72%)) of 12 patients in the HPV DNA-positive PLN group. There was a statistically significant difference between these proportions ($p < 0.05$) (Table 3).

3.3. HPV DNA positivity in PLN associated with recurrence

The significance of lymph node metastases and HPV DNA presence in PLN for recurrence is presented in Table 4. The presence of either metastases or HPV DNA-positivity in PLN is considered to give a higher risk of recurrence compared to non-metastatic and/or HPV DNA-negative PLN. The same proportion of patients had recurrence independent of the metastases or HPV DNA-positivity in PLN, five (83% (95%CI: 18–62%)) of six patients with microscopically confirmed PLN metastases and 10 (83% (95%CI: 52–98%)) of 12 patients with HPV DNA-positive PLN. In contrast the recurrence was lower, notably in the patients with HPV DNA-negative PLN 13 (38% (95%CI: 18–62%)) of 27 patients had recurrence, whereas eight (48% (95%CI: 29–68%)) of 21 patients with non-metastatic PLN had recurrence. The association between HPV DNA positivity and recurrence was statistically significant ($p < 0.05$) (Table 4).

3.4. Predictive value of HPV DNA in PLN for recurrence

The PPV for recurrence was the same with detection of either metastases or HPV DNA-positivity in PLN and the NPV was better for HPV DNA detection, 62% (95%CI: 38–82%), than for conventional microscopic detection of metastatic PLN, 51% (95%CI: 31–71%). The specificity is lower for HPV DNA: 87% (95%CI: 60–98%) compared to 93% (95%CI: 68–100%) for microscopic detection. The sensitivity for HPV DNA detection in PLN was 56% (95%CI: 31–79%) compared to 28% (95%CI: 10–54%) for detecting PLN metastases by conventional microscopic assessment (Table 5).

Table 4

The prevalence of recurrence with and without microscopically metastatic pelvic lymph nodes (PLN) and with and without HPV DNA in PLN obtained at primary radical surgical treatment from 33 early-stage cervical cancer patients (FIGO stage IB) treated in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark, from 2003 to 2015.

PLN		Recurrence	No recurrence	Proportion	n
		n	n	% (95%CI)	
PLN	Microscopic metastatic	5	1	83% (36–100%)	6
	HPV DNA positive	10	2	83% (52–98%)	12
PLN	Microscopic non-metastatic	13	14	48% (29–68%)	27
	HPV DNA negative	8	13	38% (18–62%)	21
		18/18	15/15		33/33

HPV/recurrence: Fischer’s Exact $p < 0.05$.

Microscopy/recurrence: Fischer’s Exact $p > 0.05$.

4. Discussion

In the present study, the primary aim was to examine the presence of HPV DNA in recurrent disease after treatment for early-stage cervical cancer (FIGO stage IB). A tissue biopsy, taken to confirm the recurrence, was available for examination in 12 of 18 (67%) patients with HPV DNA positive primary tumor and recurrence, and in eight of 12 (67%), HPV DNA was detected in the tissue samples. HPV DNA genotyping revealed 100% congruence in the HPV genotype detected in the primary tumor, PLN, and recurrent tumor tissue. In addition, the findings in this study suggest that both microscopic metastases and HPV DNA positivity in PLN have a high PPV for recurrence, whereas the NPV is weaker, but seems to be best for HPV DNA.

In 1993, kenberg et al. reported that HPV DNA is detectable in metastatic tissue. They examined frozen tissue with Southern blot hybridization from cervical cancer metastases in 17 patients with late-stage cervical cancer (FIGO stage IIA to IVB) and detected HPV DNA in 14 of 18 distant metastases [5]. In the present study, detection of HPV DNA in tissue biopsies from patients with the recurrent disease, after treatment for early-stage cervical cancer (FIGO stage IB), supports the previous findings. However, HPV DNA was not detected in 33% of biopsies of recurrent tissue. This may be due to method failure, or it could be interpreted as an expression of etiologic diversity. Nevertheless, the findings confirm that HPV DNA continues to be present in the tumor cells, probably as a driver of the cancerous process, and it is important for the maintenance of the disease.

In addition to the detection of HPV DNA in recurrent tissue, we evaluated the HPV genotype congruence in the primary cervical tumor, PLN, and recurrent tissue. In 67% of recurrent cases, HPV DNA was detected in the three tissue samples, with 100% congruence of the HPV genotype, also in the case of more rare HPV genotypes like HPV31 and HPV39. In 13% of the non-recurrent cases, HPV DNA was detected in the PLN. Moreover, detection of HPV in both PLN and tissue from recurrent disease gives rise to speculations about the significance of the individual immunological response for the outcome after treatment. HPV does not only initiate the process, but seems to stay present in the tumor tissue until recurrent disease. The present findings support the importance of the continuous elaboration of therapeutic vaccines or immunotherapeutic approaches, which will very likely refine the treatment of cervical cancer [20].

The most significant prognostic factor for the risk of recurrence after surgery for early-stage cervical cancer (FIGO stage IB) is detection of metastases in the PLN [13], and both the surgical and the pathological approach are important for the detection [14–19]. In the present study, the patients had complete PLN excision, i.e. bilateral removal of lymphatic tissue surrounding the common and external iliac artery and vein. The SLN procedure has been evaluated since the 1990s [15–17,21], and it is currently not a standard procedure for cervical cancer surgery at the majority of cancer centers in the world. In the Nordic Countries, the SLN procedure is mostly performed in clinical trials [22], and barriers for SLN procedure are the access to the

Table 5

The positive predictive value (PPV), the negative predictive value (NPV), the specificity and the sensitivity for recurrence after treatment for early-stage cervical cancer based on conventional microscopic assessment of pelvic lymph node (PLN) metastases (MIC) and HPV DNA detection (HPV) in PLN obtained at the primary radical surgical treatment from 33 early-stage cervical cancer patients treated in the Department of Obstetrics and Gynecology, Aarhus University Hospital, Aarhus, Denmark, from 2003 to 2015.

PLN				
	PPV	NPV	Specificity	Sensitivity
MIC	83% (95%CI: 36–100%)	52% (95%CI: 32–71%)	93% (95%CI: 68–100%)	28% (95%CI: 10–54%)
HPV	83% (95%CI: 52–98%)	62% (95%CI: 38–82%)	87% (95%CI: 60–98%)	56% (95%CI: 31–79%)

necessary technology, the availability of surgical skills, and the increased workload associated with the ultrastaging, both in the pathology laboratory and for the pathologist.

Prompted by the challenge of detecting PLN metastases, we were encouraged to consider the value of HPV testing as a surrogate marker for metastases. We expected that HPV DNA would be present in all patients with microscopic metastatic PLN, because several previous studies have confirmed the presence of HPV DNA in PLN. Lukaszuk et al. [6] included 116 cervical cancer patients (FIGO stage IB–IIB), and in 81 patients, HPV DNA was detected in frozen tissue from PLN with PCR for E6 and L1 after full PLN excision. In another study, the SLN procedure was performed in 59 cervical cancer patients (FIGO stage IA–IIB), and HPV was detected in 85% unilaterally and 40.7% bilaterally. HPV DNA was detected in 50% of 20 metastatic SLN and 5.6% of 90 non-metastatic SLN by E6-specific PCR [7]. Lee et al. [8] conducted a study in 57 cervical cancer patients (FIGO stage IB–IIA) detecting HPV DNA in 10 of 11 metastatic PLN by PCR. Dürst et al. [12] evaluated the HPV-mRNA as a molecular marker for tumor cells in non-metastatic SLN's and mRNA-HPV was detected in 52 (27%) from 189 cervical cancer patients (FIGO stage IA1–IIB).

In the present study, HPV DNA was detected in 10 (83%) of 12 patients with microscopic metastatic PLN. The missing HPV DNA detection in metastatic PLN is important and indicates that our results should be interpreted with caution if HPV DNA testing should be introduced as a surrogate marker for lymph node metastasis. Remarkably, HPV DNA was detected in the lymph nodes from 26% with non-metastatic lymph nodes. This can be caused by HPV shedding from lysing cells from the primary tumor or non-detected isolated tumor cells, i.e. solitary tumor cells or conglomerate of tumor cells up to ≤ 0.2 mm in the unexamined parts of the lymph nodes. Another explanation for these findings could be contamination, however, we find this less likely as we applied a strict contamination protocol during the process and all negative controls showed no signs of contamination.

Previous studies have detected HPV DNA in PLN [6–8]. Lukaszuk et al. [6] conclude that the presence of HPV DNA in lymph nodes increases the risk of recurrence. In the study by Lee et al. [8] the findings indicate that a low-risk of recurrence was related to HPV DNA-negative, non-metastatic SLN after 3 years of follow-up. This is supported by Dürst et al. [12] when they conclude that the absence of HPV-mRNA in non-metastatic PLN's significantly increase the recurrence-free survival. Careful interpretation of the present findings indicates that the estimates of the PPV of HPV DNA detection in PLN and the detection of microscopic metastatic PLN for recurrence are valuable; both methods have specificity close to 90%. However, the negative predictive value for recurrence is lower in both methods: HPV DNA 62% (95%CI: 38–82%) and microscopy 52% (95%CI: 32–71%). The sensitivity for HPV DNA detection is 56% (95%CI: 31–79%) and is 28% (95%CI: 10–54%) for conventional microscopic assessment of PLN metastases. Although this is in favor of HPV, it is still not near 90% as would often be the preferred minimum for a diagnostic test. The results are not comparable as detection of metastases to the PLN implies postoperative adjuvant chemo-radiation with a very high success rate. Therefore, it

will not immediately be attractive to initiate a change in the clinical setting and substitute microscopy with HPV DNA detection to decide the need for adjuvant oncologic treatment, closer surveillance, or discharge.

The study was performed meticulously in accordance with the study protocol; nevertheless, the use of older FFPE is very likely a limitation. There is a risk of DNA degradation, and especially for the HPV testing of the recurrent tissue there was only a small amount of tissue available from the core biopsies. The performance of studies at a single institution is often considered as a weakness, but the findings in the present study generates a perspective to be tested and verified in a larger study. Furthermore, study limitation is inherent in the retrospective design, and the study is challenged by the use of lymph nodes from full PLN excision compared to previous studies focusing on SLN procedure.

Another argument toward the value of the present study is that we have confined the study to lymph node metastases and HPV DNA detection. This is a simplification because the treatment planning for cervical cancer is complex [23,24]. We have not addressed other histopathologic high-risk criteria like tumor size, lymphovascular space invasion, invasion depth, etc. Furthermore, we have not considered next-generation sequencing technology. So far, the most significant finding in recent studies was the detection of increased levels of VEGF mRNA in cervical cancer cells, which has prompted clinical studies dealing with the addition of anti-angiogenic agents to standardize oncologic treatment in patients with metastatic, recurrent, or persistent disease [25].

In the present study, a very interesting finding is the congruence of HPV DNA in the primary tumor, PLN and the tissue biopsy from recurrent disease that highlights the significance of HPV in cervical cancer pathogenesis. The perspective of this study is that we continue the work with HPV DNA testing in PLN. We are planning a prospective study with HPV DNA testing in SLN, i.e. histopathologic review by ultrastaging and HPV DNA testing of the waste material from the sectioning process. We are challenged by the relative large amount of tissue in the waste material, and we have parallel investigation to develop a manageable method. We expect that using new FFPE tissue from SLN instead of older FFPE blocks from PLN will upgrade the result. The aim is to increase the NPV and the sensitivity, so the test can be incorporated into the clinical setting

5. Conclusion

We conclude that there is a perspective in introducing HPV DNA testing in early stage cervical cancer patients (FIGO stage IB) with non-metastatic lymph nodes. These patients are considered low-risk of recurrence and the HPV-DNA testing may have the potential to guide the surveillance, i.e. low-risk, HPV DNA positive patients will need a closer surveillance than the low-risk, HPV DNA negative patients.

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Conflicts of interest

The authors have no conflict of interest to report.

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