Immunological aspects of conventional and new treatments for cervical cancer, an immunopharmacological approach

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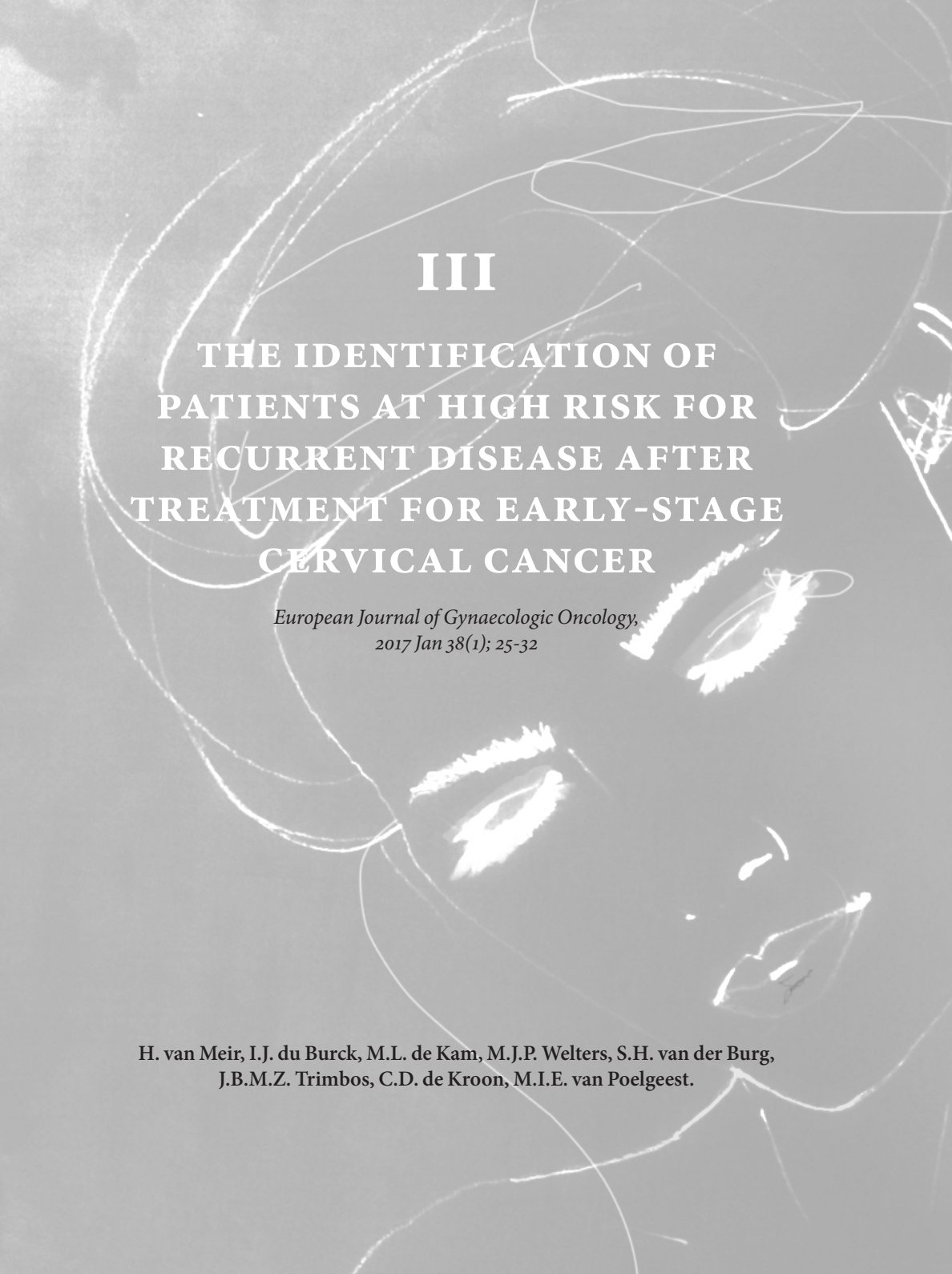

III

THE IDENTIFICATION OF PATIENTS AT HIGH RISK FOR RECURRENT DISEASE AFTER TREATMENT FOR EARLY-STAGE CERVICAL CANCER

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ABSTRACT

OBJECTIVE To investigate prognostic factors in patients with recurrent cervical cancer after treatment for early-stage disease in order to identify high-risk patients who might benefit from alternative treatment strategies.

STUDY DESIGN We retrospectively analyzed clinical and pathology data from 130 recurrent cervical cancer patients after surgical treatment for early-stage disease. Patients were compared with a recurrence-free control group matched for age, FIGO stage, and adjuvant treatment. Univariate and multivariate Cox regression analyses were performed to determine prognostic factors for recurrence and survival.

RESULTS Of 889 patients, 130 (14.6%) developed recurrent disease after primary treatment for early-stage cervical cancer. Local or loco-regional metastasis was observed in 45%, distant metastasis in 31%, and combined pelvic and distant metastasis in 24%. Median survival after recurrence was 12 months (range 1-107 months). Median 5-year survival was 96% in the control group and 29% in the recurrence group. Tumor size ≥ 40 mm and lymph node metastasis were independent unfavorable prognostic factors for overall and disease-free survival. The number of positive lymph nodes (≥ 1) and bilateral occurrence of pelvic lymph node metastasis were associated with adverse clinical outcome.

CONCLUSIONS Tumor size ≥ 40 mm and lymph node metastasis were independent unfavorable prognostic factors in surgically treated, early-stage cervical cancer patients. The combination of these factors was particularly associated with recurrence. Future trials should focus on the role of alternative adjuvant treatment strategies in patients at high risk of recurrent disease (e.g., by chemotherapy, immunotherapy or combinations thereof).

Introduction

Cervical cancer is the fourth most common cancer in women worldwide[1], and is staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system, based on clinical evaluation.[2] Most patients with cervical cancer present with early-stage disease (I-IIA), which generally has a good prognosis after primary treatment with either surgery or (chemo) radiation therapy.[3] In young patients, the surgical approach has advantages over radiotherapy because ovarian function is preserved and less sexual dysfunction occurs.[4,5] Radical hysterectomy with pelvic lymph node dissection results in excellent 5-year overall survival (OS) rates, ranging from 75% to 95%.[6]

Patients with lymph node metastasis, parametrial involvement, and tumor-positive surgical margins are treated with pelvic radiotherapy after surgery as this has shown to reduce the risk of recurrences with 47%.[7,8] More recently, adjuvant radiation in the context of unfavourable tumor factors has been suggested to be beneficial. These unfavourable factors include: tumor diameter ≥ 40 mm, tumor infiltration depth ≥ 15 mm, and the presence of lymphovascular space involvement (LVSI).[9-12] During last decade, adjuvant radiotherapy with concurrent chemotherapy was introduced, the addition of cisplatin improved progression-free survival and OS among women with high risk early-stage disease after radical hysterectomy and pelvic lymphadenectomy.[13,14]

Recurrent cervical cancer is associated with poor outcomes, with a reported 1-year survival rate of 15-20%[15], and median survival rates of 7-36 months after recurrence treatment.[16] For patients with metastatic disease, chemotherapy is the standard treatment, although it is neither curative nor associated with long-term disease control: response rates are between 20% and 35%, and median survival is only 8-13 months.[17,18] As chemotherapy has poor outcome and results in significant morbidity, alternative adjuvant treatment strategies in patients at risk for recurrent disease are crucial.We investigated tumor characteristics and clinicopathologic factors in patients who developed recurrence after primary surgery for early-stage cervical cancer. The aim of this study was to identify prognostic markers that can be used to stratify patients regarding the increasing risk of recurrence, and therefore those most in need of alternative therapies.

Materials and Methods

Between 1984 and 2009, 889 patients were surgically treated for FIGO stage I-IIA cervical cancer and underwent radical hysterectomy with pelvic lymphadenec-

tomy at the Leiden University Medical Center (LUMC). From 2001 on, the nerve-sparing Swift radical hysterectomy was performed.[19] Lymph nodes were divided into high nodes along the common iliac artery, superficial nodes along the external iliac artery and vein, and deep nodes from beneath the level of the external iliac vein in the obturator fossa. Histopathological characteristics were documented for each patient: tumor size, histological tumor type, parametrial involvement, tumor-positive lymph nodes, and surgical margins. Depth of invasion was measured in millimeters from the basement membrane of the surface epithelium. LVSI was considered positive when cancer cells were present within endothelium-lined spaces.

Indications for adjuvant radiotherapy included lymph node metastasis, parametrial involvement, and tumor-positive surgical margins. Since 1997, patients with tumor-negative lymph nodes but 2 or 3 unfavourable tumor parameters also received adjuvant radiotherapy. From the year 2000 on, patients with ≥ 2 tumor-positive lymph nodes, parametrial infiltration, or tumor-positive surgical margins were offered chemo-radiation therapy.

Follow-up by a gynecologic oncologist took place every 3 months for the first 2 years after surgery, every 6 months for the next 3 years, and annually thereafter. Patients receiving postoperative (chemo)radiation therapy were followed with 3 monthly appointments, alternately by a radiation oncologist and a gynecologic oncologist.

Recurrence of disease was defined as any new lesion diagnosed with physical examination, radiology, and histopathology. Local recurrence was defined as recurrent disease involving the vagina. Recurrence was loco-regional if it was located in the vagina as well as in the bladder, rectum, side wall of the pelvis, or inside the pelvis. Recurrence at other sites, including lungs, bones, supraclavicular lymph nodes, and various abdominal sites, was classified as distant. In our surgically treated cervical cancer group, 130 patients (14.6%) were diagnosed with recurrent disease. These patients were matched for age, FIGO stage and primary treatment with a control group without recurrent disease.

Statistical analysis was performed using SAS version 9.1.3. Fisher's exact test was used to analyze patient characteristics for categorical variables or factors. Continuous data was summarized by recurrence and compared with an unpaired *t*-test. The correlation between characteristics was analyzed using Spearman's correlation. Survival analysis was performed using Kaplan-Meier curves and Cox proportional hazards model. Independent prognostic factors were determined through multivariate analysis using the Cox proportional hazards model. The significant prognostic factors determined in the multivariate analysis, were used to stratify patients into risk groups. The frequency of lymph

node metastasis and location of recurrence were compared using Fisher's exact test. The statistical significance level was set at $p \leq 0.05$.

Results

Clinical and histological characteristics of patients with recurrent cervical cancer and the matched control subjects are outlined in Table 1. Notably, of 260 patients, only 38% had a documented FIGO stage IB1 or less; 38% had deep infiltrating tumors (≥ 15mm), 55% had LVSI, 15% had parametrial involvement, and 38% had lymph node metastasis.

The respective 5-year OS for the recurrence and control groups were 29% and 96% (figure 1A). In 67% of cases, recurrent disease occurred within 2 years after primary treatment, with a mean of 23.7 months (median 14; range 1-134). Survival after the diagnosis of recurrence was 28% after 24 months and 10.7% after 5 years, with a median survival of 12 months (95% CI 10-15; range,1-107). A disease free survival (DFS) of < 12 months was significantly associated with poor survival, compared with a DFS between 12 and 24 months (HR 0.55, $p = 0.0105$) and a DFS of > 24 months (HR 0.23, $p < 0.0001$) (Figure 1B).

Median survival was 58 months (95% CI 20-74) in cases of local recurrence, 24 months (95% CI 17-42) for loco-regional disease, and 39 months (95% CI 26-58) for patients with distant metastases (Figure 1C). Treatments for recurrent disease are listed in Table 2. Figure 1D depicts the OS for patients with recurrent disease, categorized by treatment modality.

TUMOR SIZE AND LYMPH NODE METASTASIS STRONGLY PREDICT SURVIVAL

Univariate analysis revealed that tumor infiltration depth ≥ 15 mm, tumor diameter ≥ 40 mm, and the presence of lymph node metastasis were significantly associated with impaired survival. On multivariate analysis, lymph node metastasis and tumor size ≥ 40 mm were significant predictors for OS ($p = 0.0046$ and $p = 0.0588$, respectively) and DFS ($p = 0.0015$ and $p = 0.0007$, respectively) (Table 3).

Patients with a tumor diameter ≥ 40 mm were at risk for the development of recurrent disease; a 62% incidence of recurrence was noted in this group, compared with 40% in the patient group with a tumor < 40 mm ($p = 0.0014$). Furthermore, mean time to recurrence was significantly shorter among patients with a tumor ≥ 40 mm than among patients with a tumor < 40 mm (12.3 months [95% CI 10.0-15.3] vs. 20.2 months [95% CI 15.9-25.7]; $p = 0.0027$).

Further analysis revealed that tumor size was associated with infiltration depth, LVSI, and lymph node metastasis. Tumor size correlated strongly with infiltration depth (Spearman correlation 0.61, $p < 0.0001$). Regarding LVSI, mean tumor infiltration depth was 14.8 mm (95% CI 13.5-16.1), compared with 10.9 mm (95% CI 9.4-12.3) in tumors without LVSI (unpaired *t*-test, $p < 0.0001$). Patients with a tumor ≥ 40 mm exhibited more frequent presence of lymph node metastasis than patients with a tumor < 40 mm ($p = 0.0295$).

Based on the presence or absence of these two prognostic factors, the 260 patients were stratified into the following risk groups: low (patients without risk factors), medium (patients with a tumor ≥ 40 mm *or* lymph node metastasis), and high (patients with a tumor ≥ 40 mm *and* lymph node metastasis). OS was significantly better in the low-risk group (hazard ratio 1.7, 95% CI 1.16-2.59) than in the medium and high-risk groups (hazard ratio 3.0, 95% CI 1.98-4.97) (Figures 2 and 3). Seventy-one percent of the 31 patients in the high-risk group that developed recurrent disease also had distant recurrence, which demonstrates that this group might experience impaired survival due to more distant tumor metastasis.

PROGNOSTIC IMPACT OF THE NUMBER AND SITE OF LYMPH NODE METASTASES

Sixty-two (48%) of 130 patients with recurrent cervical cancer had tumor-positive lymph nodes at surgery. For the recurrence and control groups, the 5-year survival rate was 70% for patients without tumor-positive lymph nodes, compared with 46% for patients with tumor-positive lymph node(s) (HR 2.17; 95% CI 1.53-3.09, $p < 0.0001$). Survival was significantly lower with an increasing number of tumor-positive lymph nodes ($p = 0.0001$) (Figures 4A, B). OS and DFS were especially poor in patients with ≥ 2 tumor-positive lymph nodes, with an OS of only 34% and a high risk of developing recurrent disease; 69% incidence of recurrence was noted in this group, compared with 48% in the patient group with 1 tumor-positive lymph node and 40% in patients without lymph node metastasis ($p < 0.0001$) (Figure 4B).

Of 98 patients with lymph node metastasis, 42 patients (43%) developed distant metastasis, and 21 patients (21%) had local or loco-regional recurrences. Bilateral occurrence of lymph node metastasis was associated with recurrent disease ($p = 0.034$). The number of lymph nodes removed during surgery and the extension of lymph node metastases to the common iliac or para-aortic nodes were not associated with the site of recurrence. Kaplan-Meier curves for OS and DFS for patients with positive lymph node metastases, by the site of

positive lymph nodes, are depicted in Figures 4C, D. Although not statistically significant, extension of lymph node metastases to the common iliac or para-aortic lymph nodes exhibited a trend toward impaired survival and higher risk of recurrent disease.

Comment

The aim of this study was to evaluate tumor characteristics of surgically treated early-stage cervical cancer patients in order to evaluate whether a subgroup at high risk of recurrent disease could be identified. The retrospective analysis was performed on a period of 25 years. The standard care has obviously been changed in this period of time with the introduction of Magnetic Resonance Imaging (MRI), the addition of FIGO stage IB1 and IB2, and the use of radiotherapy and subsequently chemotherapy.

After primary treatment for early-stage cervical cancer, recurrence occurred in 14.6% of the patients, indicating a good overall prognosis for the majority of patients. In this study, 65% of the patients who developed recurrent disease had undergone adjuvant (chemo)radiation therapy after radical surgery; therefore, recurrences occurred despite aggressive and combination primary treatment. This suggests that either the adjuvant treatment had nothing further to add after radical surgery, or it was not sufficient to provide any survival benefit.

Our study confirms results from other studies demonstrating that large tumor size is a prognostic factor in cervical cancer patients.[20,21] Since 1995, stage IB cervical cancer has been divided into subgroups based on clinical tumor size: stage IB1 indicates a tumor diameter < 40 mm, and stage IB2 a tumor diameter ≥ 40 mm (bulky tumor).[22] This sub-classification recognizes that tumors > 40 mm require different treatment approaches. With regard to our results, it states that appropriate selection of patients upfront for chemoradiation rather than surgery is crucial. Moreover, recent studies have shown that a morphologic characteristic, the Barrel Index (BI), the ratio of tumor width to tumor length, is also an independent prognostic factor for recurrence and survival in bulky cervical cancer.[23,24] This suggests that in addition to tumor diameter, tumor morphology might be helpful in identifying a subgroup of high-risk patients with a worse prognosis. Although we did not make this division in stage IB in barrel-shaped versus exophytic tumors in the present study, the division might also have been associated with clinical outcomes. As there are no clear guidelines regarding the best treatment approach for bulky cervical tumors, different treatment approaches are explored. Neo-adjuvant chemotherapy

followed by radical surgery offers the potential to reduce tumor volume, thereby facilitating primary surgery and positively effecting microscopic disease.[25] A meta-analysis of 6 randomized controlled trials[26] and the results of a phase III study by the Gynecologic Oncology Group (GOG)[27] demonstrated advantages in neo-adjuvant chemotherapy reducing the rate of lymph node metastasis and parametrial infiltration, thereby improving OS and DFS. In addition, there is promising evidence for enhanced activity of weekly platinum/taxane regimens to improve prognosis of patients with locally advanced cervical cancer.[28] EORTC trial 55994 is currently ongoing and aims to investigate whether neo-adjuvant chemotherapy followed by surgery confers a survival advantage compared with concomitant radiotherapy and chemotherapy in patients with stage IB2, IIA ≥ 40 mm, and IIB cervical cancer.

The present study demonstrated that the number of tumor-positive lymph nodes is a relevant prognostic factor for OS and DFS. Other studies have shown that the number of metastatic lymph nodes,[29-31] common iliac or para-aortic lymph node involvement,[31] and bilaterality of lymph node metastasis[32] are associated with clinical outcome. In general, patients with para-aortic lymph node metastasis are treated with extended-field radiotherapy, which is associated with significant morbidity.[33] Unfortunately, the heterogeneity in trial results and use of different treatment regimens result in a lack of consensus concerning treatment choice. Alternative treatment strategies have not been studied extensively. A small retrospective analysis showed that adjuvant chemotherapy in high-risk patients did not result in better outcomes compared with patients after adjuvant radiotherapy. However, a subgroup of patients with common iliac and > 2 lymph node metastases exhibited improved survival.[34] The choice for and type of adjuvant treatment in patients at high risk for recurrence should be examined further. The GOG-9926 study started in 2011 and examines the role of adjuvant paclitaxel and carboplatin in women with para-aortic lymph node metastasis after extended field radiation therapy.

Immunotherapy is as another alternative and promising treatment strategy for patients with cervical cancer. Because of the viral etiology and the expression of the viral oncoproteins E6 and E7, cervical cancer is regarded as highly immunogenic. Immunotherapy has been shown to lack clinical efficacy in end-stage patients with large tumor burden and immunosuppressive conditions,[35,36] but results from vaccination trials in patients with HPV16-induced pre-malignant vulvar lesions have shown that smaller lesions are more likely to regress in response to vaccine-induced HPV16-specific immunity.[37] Hence, activating the immune system by immunotherapy might be of value in patients with minimal residual disease. In our study, 72 of 130 patients with recurrent cervical cancer

developed distant metastases, suggesting that a substantial number of high-risk patients have residual micrometastases after primary treatment. Alternative systemic therapies that attempt to reduce the risk for recurrence are mandatory rather than a change of primary local treatment. Immunotherapy may well be an alternative adjuvant systemic approach in these patients, particularly because effective alternative therapies are identified for the clinical management of other malignancies. For example, immunotherapeutic options have emerged as a potential adjuvant treatment option in the context of high-risk surgically treated melanoma patients.[38] Future studies could introduce targeted therapies in an adjuvant setting for high-risk cervical cancer patients.

In summary, this study demonstrates that positive lymph node status and tumor size are prognostic factors associated with poor survival in patients surgically treated for early-stage cervical cancer. In particular, patients with both factors are at high risk for recurrent disease and might benefit from alternative adjuvant treatment strategies. New therapeutic approaches should be explored in these high-risk patients, such as (neo)adjuvant chemotherapy, immunotherapy, or combinations of these treatments.

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Table 1 Patient characteristics. Data are presented as n (%) unless otherwise specified. FIGO, International Federation of Gynecology and Obstetrics; EBRT, external beam radiation therapy; LVSI, lymphovascular space involvement.

Factors	Recurrence (n=130)	Control (n=130)	p
Median follow-up (range)	30.5 (5 - 203)	72.5 (12 - 287)	
AGE			
<40	45 (34.6%)	49 (37.7)	
40-70	75 (57.7%)	69 (53.1%)	0.78
>70	10 (7.7%)	12 (9.2%)	
FIGO STAGE			
IA	1 (0.8%)	1 (0.8%)	
IB	109 (84%)	109 (84%)	1
IB1	48 (44%)	49 (45%)	
IB2	25 (23%)	16 (15%)	
N/A or unknown*	36 (33%)	44 (40%)	
IIA	20 (15.4%)	20 (15.4%)	
PRIMARY TREATMENT			
Surgery	45 (34.6%)	45 (34.6%)	
Surgery + adjuvant EBRT	71 (54.6%)	71 (54.6%)	1
Surgery + adjuvant Chemo-EBRT	14 (10.7%)	14 (10.7%)	
HISTOLOGICAL TYPE			
Squamous cell carcinoma	91 (70%)	99 (76.1%)	
Adenosquamous carcinoma	9 (6.9%)	9 (6.9%)	
Adenocarcinoma	28 (21.5%)	21 (16.2%)	0.51
Undifferentiated	1 (0.8%)	0	
Unknown	1 (0.8%)	1 (0.8%)	
SITE OF RECURRENCE			
Local	29 (22%)		
Loco-regional	29 (22%)		
Distant	41 (32%)		
Loco-regional and distant	31 (24%)		
DEPTH OF INVASION			
<15 mm	65 (50%)	74 (56.9%)	0.6
≥15 mm	51 (39.2%)	49 (37.7%)	
Unknown	14 (10.8%)	7 (5.4%)	
TUMOR DIAMETER			
<40 mm	58 (44.6%)	86 (66.1%)	0.0014
≥40 mm	59 (45.4%)	36 (27.7%)	
Unknown	13 (10%)	8 (6.2%)	

LVSI			
Yes	78 (60%)	65 (50%)	0.06
No	41 (31.5%)	56 (43.1%)	
Unknown	11 (8.5%)	9 (6.9%)	
PARAMETRIAL EXTENSION			
Yes	24 (18.5%)	16 (12.3%)	0.2
No	104 (80%)	112 (86.2%)	
Unknown	2 (1.5%)	2 (1.5%)	
LYMPH NODE METASTASIS			
Yes	63 (48.5%)	35 (26.9%)	<0.001
No	67 (51.5%)	95 (73.1%)	
PATIENT STATUS			
Alive	13 (10%)	122 (94%)	<0.001
Dead	116 (89.2%)	8 (6%)	
Disease-free	7 (5.4%)	119 (91.5%)	
Stable disease	4 (3%)	0	
Progressive disease	2 (1.5%)	0	
Dead due to cervical cancer	116 (89.2%)	0	
Dead due to other causes	0	8 (6.2%)	
Lost to follow-up	1 (0.8%)	3 (2.3%)	

** There is no information on FIGO stage IB1 or IB2 cervical cancer (based on a tumor diameter of 40 mm) in patients treated and operated before 1995.*

Table 2 Treatments for recurrent disease.

Treatment	Patients, n=130
MONOTHERAPY	60 (46.2%)
SUR	6 (4.6%)
RT	24 (18.5%)
CH	28 (21.5%)
VAC	2 (1.6%)
COMBINATION THERAPY	54 (41.5%)
SUR + CH	4 (3.1%)
SUR + CH + RT	5 (3.8)
SUR + RT (+/- HT)	8 (6.2%)
SUR + VAC	1 (0.8%)
RT + HT	5 (3.8%)
RT + VAC	1 (0.8%)
CHRT (+/- HT)	21 (16.1%)
CH + HT	6 (4.6%)
CH + VAC	3 (2.3%)
SUPPORTIVE TREATMENT	16 (12.3%)

SUR = surgery; RT = radiotherapy; CH = chemotherapy; VAC = vaccination; HT = hyperthermia

Table 3 Regression analyses of predictors for overall and disease-free survival in cervical cancer patients after surgical treatment for early-stage disease.

Prognostic factors	Univariate analysis						Multivariate analysis					
	Overall Survival			Disease Free Survival			Overall Survival			Disease Free Survival		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
RECURRENT DISEASE												
yes vs no	26.3	12.82-53.97	< 0.0001									
AGE												
< 40 yrs vs 40-70 yrs	0.82	0.56-1.20	0.3	0.91	0.63-1.32	0.6						
> 70 yrs vs 40-70 yrs	0.85	0.44-1.64	0.6	0.86	0.44-1.66	0.6						
SMOKING												
yes vs no	1.07	0.73-1.58	0.7	0.99	0.68-1.44	0.9						
DEPTH OF INVASION												
<15 mm vs ≥ 15 mm	1.51	1.04-2.20	0.03	1.22	0.84-1.75	0.3	1.09	0.68-1.74	0.72	1.13	0.72-1.77	0.6
<10 mm vs ≥ 10mm	1.45	0.96-2.19	0.08	1.42	0.95-2.13	0.09						
TUMOR SIZE												
< 40mm vs ≥ 40 mm	1.63	1.12-2.37	0.01	1.96	1.36-2.82	0.0003	1.52	0.98-2.34	0.0588	2.12	1.38-3.27	0.0007
< 30 mm vs ≥ 30 mm	2.02	1.32-3.11	0.0013	2.16	1.42-3.29	0.0003	1.77	1.07-2.91	0.025	2.56	1.56-4.21	0.0002
LYMPH NODE METASTASIS												
yes vs no	2.17	1.53-3.09	< 0.0001	1.91	1.35-2.70	0.0002	2.20	1.28-3.75	0.004	2.46	1.41-4.29	0.0015
PARAMETRIAL EXTENSION												
yes vs no	1.5	0.96-2.34	0.08	1.36	0.87-2.11	0.18	1.12	0.63-2.01	0.7	1.06	0.59-1.9	0.85
LVSI												
yes vs no	1.42	0.96-2.10	0.08	1.36	0.93-1.98	0.11	1.12	0.69-1.82	0.63	1.17	0.73-1.86	0.5

HR, hazard ratio; CI, confidence interval; LVSI, lymphovascular space involvement.

Figure 1 (A) Five-year overall survival (OS) for surgically treated FIGO IA-IIA cervical cancer patients. (B) Five-year OS for patients with recurrent disease, categorized with respect to disease-free survival (DFS). DFS < 12 months vs DFS 12-24 months: HR 1.82, p = 0.0105. DFS < 12 months vs DFS > 24 months: HR 4.35, p < 0.0001. (C) Five-year OS of patients with recurrent disease, categorized with respect to site of recurrence (local, loco-regional, distant and combined loco-regional and distant). (D) Five-YEAR OS for patients with recurrent disease, categorized with respect to treatment modality. Types of monotherapy include chemotherapy, radiotherapy, surgery, or vaccination; combination therapy includes combinations of chemotherapy, radiotherapy, hyperthermia, surgery and/or vaccination; supportive therapy indicates no active treatment (palliative treatment only). Median OS was 17 months (range 1 – 91 months) for supportive therapy, 29 months (range 8 – 98 months) for monotherapy, and 51 months (range 28 – 102 months) for combination therapy. Combination therapy was associated with survival benefit vs monotherapy (p = 0.007) and supportive (palliative) therapy (p = 0.0017).

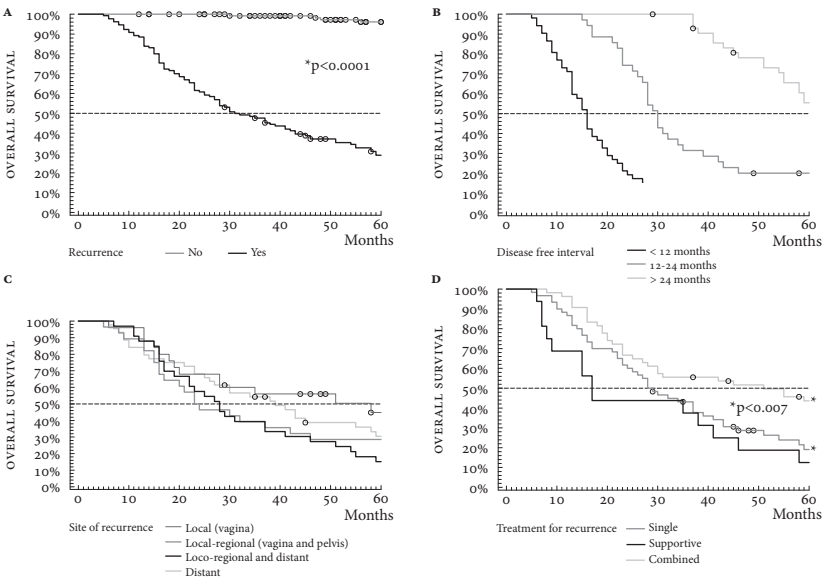

Figure 2 Overall survival based on prognostic factors. Low-risk: tumor size < 40 mm without lymph node metastasis; medium-risk: tumor size ≥ 40 mm or lymph node metastasis; high-risk: tumor size ≥ 40 mm and lymph node metastasis. Low-risk group vs medium-risk group: p = 0.01; low-risk vs high-risk group: p < 0 .0001.

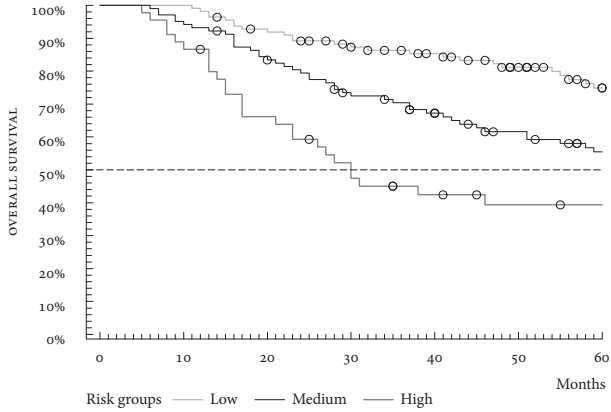

Figure 3 Disease-free survival based on prognostic factors. Low-risk: tumor size < 40 mm without lymph node metastasis; medium-risk: tumor size ≥ 40 mm or lymph node metastasis; high-risk: tumor size ≥ 40 mm and lymph node metastasis. Low-risk group vs medium-risk group: p = 0.007; low-risk vs high-risk group: p < 0.0001.

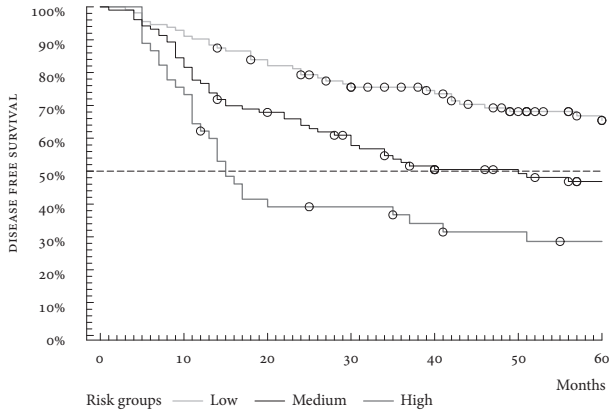

Figure 4 Overall survival (OS) and disease-free survival (DFS) for surgically treated patients with early-stage cervical cancer, categorized by number of tumor-positive lymph nodes (A, B) and by the site of tumor-positive lymph nodes (C, D). No lymph node metastasis, n = 162; 1 tumor-positive lymph node, n = 37; ≥ 2 tumor-positive lymph nodes, n = 61. (**A**) OS: None vs 1 tumor-positive lymph node: p = 0.056; None vs ≥ 2 tumor-positive lymph nodes: p < 0.0001. (**B**) DFS: None vs 1 tumor-positive lymph node: p = 0.26; None vs ≥ 2 tumor-positive lymph nodes: p < 0.0001. (**C**) OS: none vs common iliac or para-aortic tumor-positive lymph nodes: p < 0.0001; common iliac or para-aortic tumor-positive lymph nodes vs other tumor-positive pelvic nodes: p = 0.1. (**D**) DFS: none vs common iliac or para-aortic tumor-positive lymph nodes: p = 0.0002; common iliac or para-aortic tumor-positive lymph nodes vs other tumor-positive pelvic nodes: p = 0.1