Practical aspects of cervical cancer

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The soul would have no rainbow if the eyes had no tears
- Native American saying

Aan mijn familie

Contents

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Chapter 1

General introduction

General introduction

There is no agreement about the optimal treatment of low stage cervical cancer (Fédération Internationale de Gynécologie Obstétrique (FIGO) stage 1a-2a). Surgery is technically feasible for cancer in these stages without clinically evaluable metastases. The choice of the optimal treatment is based on disease free and overall survival results and the short and long term complications. Especially in the higher range of these FIGO stages, the choice between radiotherapy and surgery becomes debatable.

Staging

The FIGO staging system is based on clinical evaluation. In addition to the physical examination, only few additional techniques (ultrasound of the kidneys and ureters, and chest film) may be used. Advanced imaging can be used to plan treatment, but may not alter the clinical staging. This decision was made by the FIGO because not all diagnostic tools and treatment modalities are generally available, e.g. not in low-resource settings, and clinical stage is used to compare treatment results world wide.(1-3) Advanced imaging is however gaining more and more interest.(1;4;5) The clinical parameters that are involved in the FIGO staging system are tumour diameter, infiltration depth, local extension to the vagina, parametria, or pelvic side wall, and in more advanced stages to the bladder or rectum. All these factors relate to patterns of tumour spread, making the FIGO stages highly dependent on local tumour extension parameters. Especially for low stage cervical cancer, prognosis is influenced mainly by growth characteristics of the primary tumour.

Treatment choice

Primary treatment choice for cervical cancer is mainly based on FIGO stage. Treatment modalities have been studied in clinical trials in different populations. The surgical treatment of stage 1a disease is well established. More recently, it has been advocated to consider less radical approaches of the parametrial tissues when the risk of parametrial spread only, is limited.(6) For larger early stage cervical tumours (1b-2a), both primary surgery and primary radiotherapy have been proposed as the best suited treatment options. Survival is similar with both treatments. Nevertheless, in most centres surgery is preferred for tumours with a diameter ≤4 cm.(1;4;7) Larger tumours (>4 cm) have been shown to have a worse prognosis after both primary surgery and radiotherapy compared to smaller diameter tumours and the debate about the best primary treatment modality is ongoing, in which neoadjuvant chemotherapy is also considered as a treatment option.(1;4;8-16) Apart from survival differences, surgery and radiotherapy have detrimental effects on women with cervical cancer. After radiotherapy, radiation effects on surrounding tissues are common, including vaginal stenosis (brachy therapy), and chronic radiation damage of the bladder and small or

large bowel. Furthermore, in case of recurrent loco-regional disease the sequence of surgery first, radiotherapy second generates less morbidity than the other way around: radiotherapy primarily and surgery for tumour recurrence. Surgery has the advance of ovarian function preservation in pre-menopausal women, while ovarian function is usually lost after radiotherapy. In addition, surgery preserves vaginal function better, and enables the study of pathological findings in order to select patients that might benefit from adjuvant treatment.

Surgical treatment for larger early stage tumours consists of a radical hysterectomy with pelvic lymphadenectomy.(1;4) Conventional surgical procedures have been shown to cause damage to the pelvic autonomic nerves. The pelvic autonomic nerve system consists of the inferior hypogastric plexus and the splanchnic nerves, and innervates the bowel, bladder, and vagina. Damage to these nerves is reported to lead to impaired bladder function (e.g. urgency, difficulties in bladder emptying), defecation problems (constipation, urgency, straining) and sexual dysfunction (diminished lubrication, dissatisfaction with sexual life). These iatrogenic damages can have a substantial negative impact of the quality of life (QOL) of the, often young, cervical cancer patients after surgery.(17-25)

Adjuvant treatment planning

The clinically evaluated parameters that are used for FIGO staging and primary treatment planning are also assessed per- and post-operatively in case of surgical treatment. Additional prognostic factors that are not taken into account in the FIGO staging system are positive (pelvic) lymph nodes, vaso-invasion, histological type, invasion depth relative to the cervical thickness, tumour free resection margins, and distant metastases other than visible on ultrasound or chest film.(1) Adjuvant treatment planning thus depends, like primary treatment planning, mainly on patterns of local growth and spread of the cervical tumour. A better knowledge of these aspects of the biological behaviour of cervical tumours could be of value to optimise both primary and adjuvant treatment planning.

Advances in technology and knowledge

Part of the prognostic factors, e.g. local extension, lymph node metastases, invasion depth relative to the cervical thickness, and distant metastases, are evaluable by several imaging techniques. CT-scan, MRI, PET-CT, or PET-MRI might be able to improve the pre-operative evaluation of the prognostic factors. The use of modern imaging techniques increases worldwide in places where these modalities are available. The findings are used for treatment planning, although both concordance with findings on pathological examination, and the implications on prognosis based on pre- instead of intra- and post-operative findings are not clear yet.(1;26;27) Likewise, other recent

advances in technical possibilities like immuno-histology and DNA-profiling may offer possibilities for more accurate (pre-operative) evaluation of tumour characteristics and clinical behaviour than the physical examination performed by the Gynaecologist. Opportunities and limitations of these modalities have to be explored and scientifically evaluated. The thorough evaluation of the clinical relevance of new findings with respect to treatment planning and prognosis is essential.

New findings and insights with respect to local growth and spread of cervical cancer have always moved the field of staging and treatment. Recent findings have led to a different understanding of the best pre-, per-, and post-operative care of cervical cancer patients.

In 1995, a separate FIGO stage class was assigned to stage 1b cervical tumours >4 cm (1b2; bulky tumours), which had proven to have a worse prognosis than smaller 1b tumours (≤4 cm; 1b1).(28) This sub classification reflects the view that tumour diameter is probably the most important factor in cancer prognosis. However, in 2004, two different growth patterns of cervical cancer stage 1b2 were found to have a different survival after primary surgical treatment. Barrel shaped tumours had a significantly worse prognosis as compared to exophytic growing tumours, which had a prognosis similar to low stage cervical cancer.(29) This finding might point to a more specific subgroup of cervical cancer patients (those with exophytic growing tumours) that would benefit from primary surgical treatment instead of radiotherapy. Notably, it is not always easy to identify the growth pattern of a tumour during the conventional pre-operative work-up.

Tumour morphology might be a reflection of certain characteristics that are important for tumour growth and spread. Well known unfavourable prognostic factors for survival after surgery for cervical cancer are tumour diameter, tumour in the parametria, positive pelvic lymph nodes, vaso-invasion, infiltration depth, and possibly histological type. Progression of normal cells into cancer cells is accompanied by changes in DNA. Specific genetic changes might be early predictors of clinical behaviour of cervical tumours. The recognition of tumours with favourable or unfavourable characteristics in an early stage, by identifying genetic changes associated with one or more prognostic factors, would be very helpful for individualising cervical cancer treatment. Recently, genetic profiling has taken a flight. With the help of new techniques, single nucleotide polymorphisms (SNPs) are detectable in formalin-fixed, paraffin-embedded (FFPE) tumour tissue, instead of only fresh or frozen tissue.(30-32) This enables the study of large numbers of tumours that are stored in the pathological archives.

Traditionally, the classification of the radical hysterectomy for cervical cancer in terms of radicality is based on the extent of the procedure (33-35), even in the area of more advanced technology.(36;37) The distance between the tumour and the surgical

dissection plane is looked upon as important, making the radical hysterectomy in essence a wide excision. Different surgical techniques taking notice of anatomical structures in the surgical area have been described. First developed in Japan, nerve sparing surgery has gained more and more acceptance for reasons of recovery, late complications, and quality of life (QOL).(18;20;23;38-43) A modification of the Wertheim operation has been routinely applied to radical hysterectomy for cervical cancer in the Leiden University Medical Center (LUMC) since 2001. This nerve sparing operation developed in the LUMC was described in detail previously.(17;43) Beneficial effects of the nerve sparing operation technique, as compared to non nerve sparing procedures, have been reported on sexual functioning, bladder function, and bowel function.(42;44;45) However, in spite of promising results, compromised radicality in the area of the parametrium and decrease in survival cannot be ruled out.(39;43;46-53)

In 2005, an interesting theory concerning local tumour growth has been postulated by Höckel et al. Embryological anatomy of the female pelvis is combined with a hypothesis about tumour spread. A topographically defined anatomical area, derived from common embryonic precursor tissue was described, and named the Müllerian ‘morphogenetic unit’ (MGU). The structures surrounding and surrounded by the MGU are derived from different embryological precursor tissue than the MGU itself, which is looked upon as very important for cancer spread and treatment according to Höckel. Höckel et al. postulated that, until late in disease progression, local spread of carcinoma of the uterine cervix is guided by positional information presented by the mesenchyme of the organ or tissue from which the tumour originates; the MGU for cervical tumour spread. Therefore, leaving part of the MGU (harbouring occult tumour cells) in situ during surgery enhances the risk of recurrences according to this theory. Nerves are not derived from the Müllerian ducts and can therefore be left in situ safely, without compromising radicality. The ‘total mesometrial resection’ (TMMR), developed by Höckel et al. on the basis of their findings and theory, assures the complete removal of the MGU. Höckel et al. propose to refrain from post-operative radiotherapy if the MGU is totally excised, regardless of resection margins or positive lymph nodes, providing that an extended staged lymphadenectomy is performed. The theory and treatment are supported by very good results in terms of recurrences and survival.(54-67) Considering those results and the possible advantageous consequences with respect to QOL, the proceedings of Höckel et al. are very interesting for the treatment of low stage cervical cancer patients. Although the theoretical argumentation of the TMMR is strong and the results with respect to survival are convincing, the translation of theory and embryological or cadaver-based findings into surgical practice can be perilous. The scientific evidence for the existence of the MGU as a clearly bordered area and the confinement of tumour spread to the MGU for an extended phase in malignant progression should be strengthened.

Recently, additional changes in the FIGO classification have been made. The new classification was introduced during the study period. The old and new FIGO stages are shown in Table 1.

Table 1 FIGO classification (deduced from Pecorelli 2009 (68))

"Old"	**> 2009**	
Stage 1		Confined to the cervix
1a1	idem	Can be diagnosed only by microscopy, invasion depth ≤ 3 mm and linear extension ≤ 7 mm
1a2	idem	Can be diagnosed only by microscopy, invasion depth > 3 mm and ≤ 5 mm and linear extension ≤ 7 mm
1b1	idem	Tumour clinically visible, or pre-clinical greater than 1a2, ≤ 4 cm
1b2	idem	Clinically visible, > 4 cm
Stage 2		Tumour extension beyond the uterus, but not to the pelvic side wall or the lower third of the vagina
2a		No parametrial involvement
	2a1	No parametrial involvement, ≤ 4 cm
	2a2	No parametrial involvement, > 4 cm
2b	idem	Parametrial involvement
Stage 3		Tumour extended to the pelvic side wall, lower third of the vagina, or presence of hydronephrosis or non-functioning kidney
3a	idem	Extension to the lower third of the vagina, but not the pelvic side wall
3b	idem	Extension to the pelvic side wall, or hydronephrosis, or non-functioning kidney
Stage 4		Tumour extension beyond the true pelvis, or involvement of bladder- or rectal mucosa
4a	idem	Spread of the growth to adjacent organs
4b	idem	Spread to distant organs

In the constant search for advances in diagnosis and treatment, one should be careful not to prematurely relate new and exciting findings to clinical behaviour or treatment response. Promising results from clinical or laboratory studies, often with a relatively short follow-up period, might encourage clinicians to adjust treatment protocols. Besides proceeding with new techniques, evaluating the current practice remains very important. Results of long-term follow-up in larger patient groups have to be used to reflect upon the chosen clinical practice and might enable the recognition of subgroups of patients that would benefit from different treatment strategies.

This thesis combines studies on practical aspects of cervical cancer. Practical aspects are those features that interfere with the daily clinical treatment policy of patients with this disease. Practical aspects are comprehensive, and involve surgical considerations, and considerations of tumour behaviour and spread, prognosis and treatment. The aim of the studies was to contribute to improving treatment strategies with an optimal balance between cancer cure and quality of life, tailored to the individual patient.

Outline of the thesis

Surgical aspects of cervical cancer are described in chapter 2, 3, and 4, and partly in chapter 5. Since 2001 the nerve-sparing technique has been routinely applied to radical hysterectomies for cervical cancer in the LUMC. To evaluate the danger of possibly compromised radicality in the area of the parametrium, recurrences and survival were monitored. In chapter 2 local recurrence rate, feasibility, and safety are compared between non-nerve-sparing and nerve-sparing radical hysterectomy in cervical cancer patients stage 1a-2a.

Inspired by the theory of the MGU and the TMMR, a modification of the Leiden nerve-sparing radical hysterectomy was developed in the LUMC in 2006. Chapter 3 describes this procedure, which is called the ‘Swift’ operation.

Sometimes small practical manners or renewals are not made publicly. This is regrettable because other surgeons and patients might benefit from such improvements. Also, discussion about possible favourable and unfavourable side effects is impossible without publications. In chapter 4 the use of distilled water in the achievement of local haemostasis during oncologic surgery is described, and the possible impact on the peritoneum and on tumour cells is addressed.

Chapter 5, 6, 7, 8 and 9 describe studies and findings on tumour behaviour and spread. The clinical findings and treatment results of Höckel et al. have not yet been confirmed by other research groups. In chapter 5 a closer look is taken at the MGU in vivo, to describe the possible difficulties in the translation of the theory of the MGU into clinical practice.

The local spread of cervical cancer in the parametria has been described. But the mechanism of spread has not been determined in detail. In chapter 6 patterns of parametrial tumour involvement in radical hysterectomy specimens of cervical cancer patients are described, with emphasis on continuous and discontinuous growth and the effect on metastasising potential.

A special manifestation of a recurrence that is generally considered as local is the scar recurrence. A tumour metastasis in the surgical scar after surgery for cervical cancer is probably rare, but its incidence is unknown. Chapter 7 presents incidence and characteristics of abdominal scar recurrences in patients who underwent primary surgical treatment for cervical cancer in the LUMC.

Because patients with exophytic growing bulky cervical cancer have a different response on surgical treatment as compared to barrel shaped tumours, the differentiation between those two morphologic types in the pre-operative patient work-

up is important. In chapter 8 MRI-derived characteristics that might further specify the relation between morphology and differences in survival, and support the clinical discrimination are presented.

Improvement of the pre-operative assessment of the established clinical parameters that are important for treatment choice would help the individualisation of cervical cancer treatment. Chapter 9 describes a whole genome single nucleotide polymorphism (SNP) analysis of the clinical prognostic factors barrel shaped or exophytic growth pattern, tumour diameter, vaso-invasion, infiltration depth, tumour in the parametria, positive pelvic lymph nodes, and histological type in bulky cervical cancer.

Chapter 10 comprises a summary of this thesis and a general discussion on the findings.

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Chapter 2

Nerve-sparing radical hysterectomy: local recurrence rate, feasibility, and safety in cervical cancer patients stage 1a-2a

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Abstract

To clarify the debate about the possible threat of sparing the pelvic autonomic nerves in radical hysterectomy for cervical cancer to radicality, comparative studies of nerve-sparing and conventional surgery are necessary. The aim of his study was to analyze and compare local recurrence rate, feasibility, and safety of nerve-sparing and non-nerve-sparing radical hysterectomy.

In a cohort study with 2 years of follow-up, 246 patients with cervical cancer of stages 1a to 2a were analyzed: 124 in the non-nerve-sparing group (1994-1999) and 122 in the group where nerve-sparing was the intention-to-treat (2001-2005). Local recurrence rate, local recurrence-free survival, feasibility, and safety were analyzed and compared.

The clinical characteristics of the treatment groups were comparable. Sparing the nerves unilaterally or bilaterally was possible in 80% of cases of the nerve-sparing group. Local recurrence rates in the non-nerve-sparing (4.9%) and nerve-sparing (8.3%) group were not significantly different. Mean local recurrence-free survival within 2 years were 22.7 and 22.0 months, respectively. Univariate and multivariate regression analyses showed that nerve-sparing treatment was not a significant prognostic factor for local recurrence. With respect to perioperative and postoperative parameters, operating time and blood loss were less in the nerve-sparing group and mortality was equal (1 patient); the postoperative course of the nerve-sparing group was similar to the state-of-the-art of conventional radical hysterectomy.

On the basis of the results of our study, we consider the nerve-sparing technique for cervical cancer stages 1a to 2a feasible and safe.

Introduction

Conventional radical hysterectomy causes damage to the pelvic autonomic nerves, which is believed to lead to impaired bladder function, defecation problems, and sexual dysfunction.(1-9) First developed in Japan, a nerve-sparing modification of the Wertheim operation has been routinely applied to radical hysterectomy for cervical cancer in the Leiden University Medical Center (LUMC) since approximately 2001.

Beneficial effects of the nerve-sparing operation technique, compared with non-nerve-sparing procedures, have been reported on sexual functioning, bladder function, and bowel function.(10-12) With respect to disease-free and overall survival (OS) after nerve-sparing surgery for cervical cancer, promising results have been reported.(13) However, concerns about radicality and safety remain. Preservation or removal of the most lateral and distal parts of the parametrium remains an issue of debate. Whereas some authors believe that parametrial lymph nodes distributed in these tissues may cause early tumour spread,(14-16) others found no evidence for this and do not advocate extensive parametrectomy.(17-21) Whether the modification of surgery with respect to the cardinal and sacrouterine ligament is a threat for radicality has, thus, remained a matter of discussion.(22-25)

In the present study, we precisely analyzed the results of nerve-sparing hysterectomy for cervical cancer in the LUMC (2001-2005) and compared these with the results of the period before the introduction of the nerve-sparing modification (1994-1999). Emphasis was on local recurrence, feasibility, and safety.

Patients and methods

The study was conducted with 2 LUMC cohorts: January 1994 to January 1999 and January 2001 to January 2005. All patients with cervical cancer stages 1a to 2a who were scheduled for a radical hysterectomy with curative intention as the primary treatment were included in the analyses. Both groups were followed prospectively, and the follow-up data were collected at regular intervals. The objective of the study was formulated after the introduction of the nerve-sparing technique.

Patients were preoperatively evaluated by the standard staging procedure, which included complete physical and gynaecologic examination, routine blood and urine analysis, chest radiography, and ultrasound to exclude ureteral dilatation. Cystoscopy, rectoscopy, magnetic resonance imaging, or computed tomography was performed only on indication. The stage of the disease was determined by using the FIGO staging system.

From 1994 to 1999, radical surgery consisted of non-nerve-sparing radical abdominal hysterectomy, combined with a pelvic lymphadenectomy. The surgical technique has been described elsewhere.(26) In 2001-2005, all patients were planned to undergo a nerve-sparing radical hysterectomy and pelvic lymphadenectomy. This nerve-sparing operation developed in the LUMC was described in detail previously.(1,24) Only in case nerve sparing was considered impossible during operation, the procedure was completed unilaterally or bilaterally as a conventional radical hysterectomy. In both time cohorts, the operation was converted to a less radical "te Linde" procedure in case of advanced tumour spread outside the cervix necessitating postoperative radiation.

Pathology assessment comprising histological type, infiltration depth, infiltration of parametrium and resection margins, vaso-invasion, tumour diameter, number of pelvic lymph nodes, and lymph node metastases was routinely performed. Patients received adjuvant radiotherapy in case of one tumour-positive lymph node or more and in case of parametrial involvement or non-radical surgical resection margins (<5 mm). Additional criteria from 1997 were presence of at least 2 of 3 of the following prognostic unfavourable factors: vaso-invasion, tumour diameter greater than 4 cm, and invasion depth greater than 15 mm. In individual cases of severe tumour extension, platinum-based chemoradiation was offered.

Since 1984, detailed records of all patients with invasive cervical cancer who were treated in the LUMC have been prospectively stored in a database containing more than 250 parameters per patient. The outcome measures were extracted from this database.

The primary outcome measures were recurrence rate and disease free survival with respect to local recurrence-free survival (LFS) within 2 years after surgery. Local recurrences were defined as vagina top recurrences or deep pelvic recurrences (loco regional recurrences) not considered as lymph node metastases. Recurrences were diagnosed during regular follow-up visits and confirmed on computed tomographic and/or magnetic resonance imaging scans. Whenever possible, histological or cytological confirmation was obtained. A follow-up period of 2 years was used because most central recurrences occur within the first year after primary treatment, with approximately 80% occurring within 2 years.(27-31)

Feasibility and safety of the nerve-sparing technique were assessed by investigating the frequency of successful nerve-sparing procedures, reasons of failure, blood loss during surgery, operating time, complications during surgery, postoperative hospital stay, resumption of bladder function, and postoperative complications during the hospital stay (short-term complications).

Statistical Analysis

Statistical analysis was performed with the SPSS 14.0 package. With respect to the comparability of the groups, the [chi]^2 test was used to test the association between discrete or categorical variables in the univariate analysis, and the t-test was used to compare means. The survival functions were estimated by the Kaplan-Meier method and were compared by Cox proportional hazards regression model. We chose a significance level of 95%. Univariate analysis was used to assess the effect of surgical and tumour parameters that could affect the local recurrence rate. We used binary logistic regression to measure the effect of age, surgical procedure, lymph node status, parametrial involvement, resection margins, tumour diameter, infiltration depth, vaso-invasion, adjuvant therapy, and (intention-to-treat (itt)) nerve-sparing treatment group on recurrence rate. In a multivariate analysis, Cox proportional hazards regression model was used to correct the effect of the nerve-sparing treatment on local recurrence rate. All data were analysed by itt.

Results

Between January 1994 to January 1999 and January 2001 to January 2005, 246 women were newly diagnosed with cervical carcinoma stages 1a to 2a and scheduled for a radical hysterectomy with curative intention.

General, preoperative, clinical characteristics of the 246 patients, divided into the 2 treatment groups consisting of the period 1994-1999 (124 patients) and the period 2001-2005 (122 patients), are summarized in Table 1. The last treatment group consisted of all patients who were planned for a nerve-sparing procedure, regardless whether sparing the nerves could be successfully established or not (itt). Comparison of the 2 groups showed no clinically relevant differences between the clinical parameters. In addition, no significant differences in FIGO stage were observed after division in advanced (Ib2 or worse) and not advanced stage.

Characteristics concerning the feasibility and safety of the 2 surgical modalities are presented in Table 2. With respect to the surgical procedure, the operating time in the period 2001-2005 was shorter than that in 1994-1999 (difference in means, -0.26 hours; 95% confidence interval [CI], -0.41 to -0.10), and blood loss during surgery was less (difference in means, -279.3; 95% CI, -504.39 to -54.24). The mean blood loss was 1115 ml in the first group and 840 ml in the last group. Median blood loss was 875 versus 700 ml. The mean blood loss in the first group differed considerably from the median (1115 vs. 875 ml) mainly because of one excess of 11000 ml. In the latest period, more lymph nodes were dissected during lymphadenectomy (mean difference, 4.74; 95% CI, 2.91-6.58).

Table 1 Clinical characteristics of 124 patients who had a non-nerve-sparing radical hysterectomy, and 122 patients who were scheduled for a nerve-sparing procedure

Group	Non-nerve sparing	Itt nerve-sparing	
			P-value
Time period	1/1/1994 – 1/1/1999	1/1/2001 – 1/1/2005	
N	124	122	
Age mean (min-max)	46.5 yr (25-81)	46.2 yr (23-80)	0.858
FIGO stage			0.314
1a	2.40%	2.50%	
1b1	67.70%	77.70%	
1b2	16.90%	9.90%	
2a	12.90%	9.90%	
Histological type (pre operative)			0.174
Squamous	60.80%	68.90%	
Adeno	37.60%	29.50%	
Other	1.60%	1.60%	

N=number; Itt=intention-to-treat

The nerve-sparing procedure was successfully completed bilaterally in 67% of the 122 patients in 2001-2005. In another 13%, it was possible to spare the nerves on one side. Table 3 shows the reasons for failure of nerve sparing. The main reasons were deductible to tissue (e.g., adhesions, fragility or firmness, scarification) and patient features that made the procedure technically impossible. In 10% of cases, the nerve-sparing procedure was abandoned for radicality reasons.

Table 2 Surgical and tumour characteristics

Group	Non-nerve sparing	Itt nerve-sparing		
			Mean difference	**95% CI**
Duration mean (min-max)	3.2 h (2.0-5.5)	2.9 h (1.0-4.5)	0.26	0.10 to 0.41
Blood loss mean (min-max)	1115 ml (175-11000)	840ml (125-4500)	279.31	54.24 to 504.39
Pelvic nodes removed median (min-max)	17 (5-33)	22 (6-45)	4.7	2.9 to 6.6
			Odds ratio *	**95% CI**
Conversion to Te Linde	8.80%	5.70%	0.694	0.26 to 1.89
Success rate of nerve-sparing			xxx	xxx
Both sides	x	67.20%		
One side	x	13.10%		
Para aortal lymphadenectomy	5.70%	1.60%	3.713	0.756 to 18.244
			P-value	**d.f.**
Histological type (post operative)			0.174	6
Normal	15.60%	17.50%		
CIN	5.70%	1.70%		
Squamous	50.00%	58.30%		
Adeno	27.10%	20.90%		
Other	1.60%	1.70%		
			Odds ratio *	**95% CI**
Lymph node metastases	16.90%	26.20%	1.744	0.939 to 3.238
Parametrial involvement	7.30%	7.40%	1.018	0.390 to 2.657
Resection plane < 5mm free	12.10%	17.20%	1.511	0.739 to 3.091
Infiltration depth > 15 mm	16.00%	25.40%	1.786	0.915 to 3.485
Tumour diameter > 40 mm	18.30%	24.80%	1.469	0.790 to 2.729
Vaso invasion	34.90%	37.70%	1.129	0.651 to 1.958
			P-value	**d.f.**
Adjuvant Therapy			0.031	2
Radio therapy	32.30%	39.30%		
Chemo radiation	0.80%	5.70%		
None	66.90%	54.90%		

Table 3 Reasons for (uni- or bilateral) failure of nerve sparing procedure, as stated in the surgical report

	N	**Percentage**
Tissue	4	10.0
Lack of space	1	2.5
Radicality	4	10.0
Identification	7	17.5
Bleeding	4	10.0
Adiposity	4	10.0
Conversion to Te Linde	5	12.5
Unknown	11	27.5
Total	**40**	**100.0**

Surgical and tumour parameters, measured by pathological investigation, which could affect the local recurrence rate, are also depicted in Table 2. In the first group, 33% of patients received adjuvant therapy (32% radiotherapy and 1% chemoradiation), whereas in the second group, 45% of patients underwent adjuvant treatment (39% radiotherapy and 6% chemoradiation). This difference between the groups was statistically significant (d.f.=2, p=0.031). The other differences were not significant. The

percentages of patients with a postoperative histological result of "Cervical Intraepithelial Neoplasia" (CIN) or "normal" reflected the cases where no residual tumour was found in the radical hysterectomy specimen, after previous loop electrosurgical excision procedure or conisation with an apparent removal of the malignant tissue.

Complete 2 years' follow-up was obtained from 122 patients in the first group and 120 patients in the second group. In both groups, 1 patient died postoperatively during the hospital stay, and both groups had 1 patient lost to follow-up after 10.2 and 13.3 months, respectively.

Table 4 Recurrences within 24 months after radical hysterectomy

Group	**Non-nerve sparing**	**Itt nerve-sparing**		
			Odds ratio *	**95% CI**
Local recurrence **	4.9%	8.3%	1.758	0.618 to 4.998
Locations of all recurrences				
Local	4.9%	7.5%		
Loco regional	0.0%	0.8%		
Regional	3.3%	6.7%		
Distant	2.5%	5.0%		
Any recurrence	10.7%	20.0%	2.096	1.012 to 4.343
			Mean difference	**95% CI**
LFS*** (all patients)				
Mean	22.7 mth	22.0 mth	0.303	-3.022 to 3.629
Median	24.0 mth	24.0 mth		
LFS*** (local recurrences)				
Mean	11.9 mth	11.6 mth	0.293	-6.074 to 5.487
Median	10.8 mth	11.8 mth		
Time to recurrence mean	11.7 mth	13.3 mth	1.7	-2.2 to 5.7

* Odds Ratio for intention-to-treat (nerve-sparing / non-nerve-sparing)
** Local and loco regional recurrences
*** LFS = Local recurrence free survival
Itt=intention-to-treat; CI=Confidence interval; d.f.=degrees of freedom

Percentage and locations of recurrence are shown in Table 4. Total local plus loco regional recurrence rates were 4.9% for 1994-1999 and 8.3% for 2001-2005 and did not differ significantly between the groups. Mean disease-free survival with respect to LFS were 22.7 and 22.0 months, respectively, for the 2 groups as whole. The mean LFS for patients who had a local recurrence were 11.6 and 11.9 months, respectively. The Kaplan-Meier curve of the LFS is shown in Figure 1. The curves did not differ significantly (p=0.273). Univariate logistic regression indicated lymph node status, conversion to te Linde modification, vaso-invasion, and age as prognostic factors for the development of a local recurrence. Of these, only lymph node status remained as an independent prognostic factor for local recurrence in the multivariate logistic regression (hazard ratio (HR), 3.4; 95% CI, 1.078-10.974). Nerve-sparing treatment group was not

a statistically significant prognostic factor for local recurrence in the univariate analysis or in the multivariate analysis.

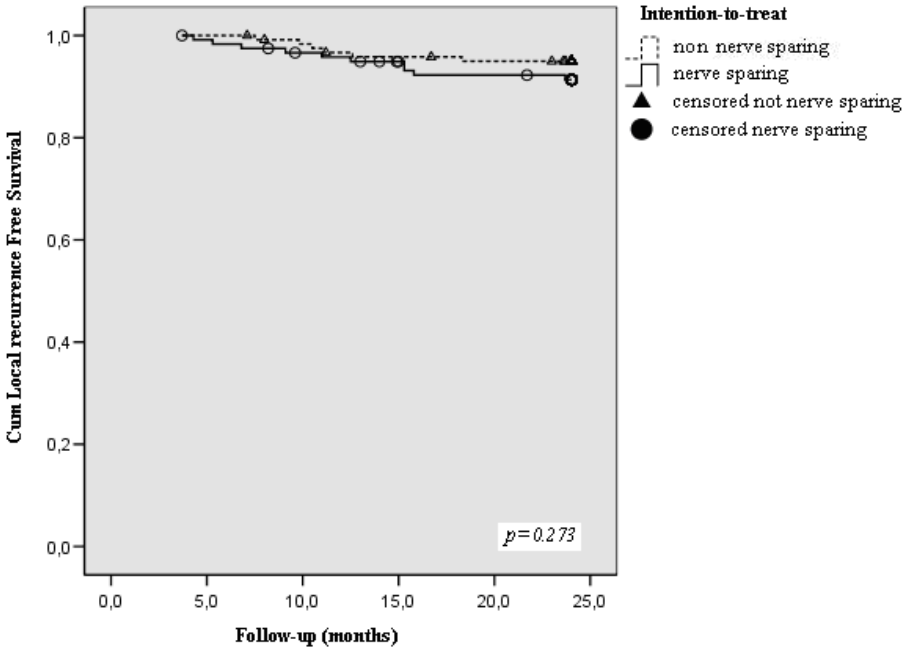

Figure 1 Survival functions (LFS)

Table 5 shows the effect of nerve-sparing treatment group on local recurrence rate after subsequent addition of known prognostic disadvantageous parameters to the multivariate Cox proportional hazards regression model. A HR of 2.171 remains with a 95% CI of 0.609 to 7.738, being not statistically significant.

Table 5 Effect of (itt) nerve-sparing (ns) treatment group on local recurrence after successively added different parameters in a Cox regression model

Factor	HR ns treatment group*	95% CI	P-value
ns treatment group (rude)	1.748	0.635 to 4.809	0.280
+ Conversion to te Linde	1.937	0.700 to 5.362	0.203
+ Lymph node status	1.59	0.567 to 4.458	0.378
+ Resection margins positive	1.619	0.574 to 4.566	0.363
+ Parametria positive	1.626	0.573 to 4.616	0.361
+ Tumour diameter > 40 mm	1.657	0.579 to 4.740	0.347
+ Infiltration depth > 15 mm	1.51	0.481 to 4.739	0.480
+ Vaso invasion	1.937	0.563 to 6.663	0.294
+ Adjuvant therapy	2.068	0.592 to 7.223	0.255
+ Age	2.171	0.609 to 7.738	0.232

* HR ns treatment group= Hazard ratio of intention-to-treat nerve-sparing / non-nerve-sparing for local recurrence rate

The column 'HR ns treatment group' shows the hazard ratios of (itt) nerve-sparing treatment group after successive addition of the subsequent factors which are depicted in the column ‘Factor’.

Table 6 describes parameters concerning the postoperative course and complications of the 122 patients in the (itt) nerve-sparing group. With respect to the postoperative period, the median hospital stay of 7 days after the operation shows that the maximum of 32 is an exception. The median period of 5 days until the removal of the catheter, mostly suprapubic, reflects the high percentage (82.5%) of patients who had spontaneous micturition directly after removal of the catheter. The only death in this group was the result of a septic shock after a bowel perforation with fatal course within 2 days despite extensive treatment. Of the other short-term complications, only one, a missed ureteral injury, required re-laparotomy.

Table 6 Post operative course and complications of 122 patients who had a radical hysterectomy with lymphadenectomy (itt nerve-sparing) for cervical cancer stage 1a-2b in the period 2001-2005

	N	Percentage
Ureter lesions during surgery		1.7%
Post operative hospital stay median (min-max)	7.0 days (5-32)	
Catheter type		
Supra pubic		86.1%
CAD		9.8%
Intra ureteral		0.8%
Unknown		4.2%
Time to removal of catheter median (min-max)	5.0 days (3-29)	
Spontaneous micturition after catheter removal		82.2%
Post operative complications		
Mortality		0.8%
Wound infection		0.8%
Pelvic infection		0.8%
Urinary tract infection		7.5%
Respiratory tract infection		2.5%
Fever		6.7%
Deep venous thrombosis		0.0%
Pulmonary embolism		0.8%
Bowel obstruction		0.8%
Post operative haemorrhage		0.8%
Hb at discharge median (min-max)	6.6 mmol/L (5.2-8.3)	

itt = intention-to-treat; CAD = catheter à demeure (indwelling catheter); Hb = haemoglobin

Although the total number of recurrences (local, loco regional, regional, and distant) was not among the outcome measures of the study, the data were present and we included these in Table 4. Because of the unexpected finding of 20.0% recurrences in the nerve-sparing group compared with 10.7% in the non-nerve-sparing group, we performed post hoc univariate and multivariate analyses. The multivariate analysis included the same factors as shown in Table 5. Although the odds ratio for the nerve-sparing group was 2.096 with a 95% CI of 1.012 to 4.343, the HR for nerve sparing with respect to the total number of recurrences showed to be 2.111 with a 95% CI of 0.929 to 4.798 in the multivariate analysis, being not statistically significant. We also analyzed the 5-year OS. The Kaplan-Meier curves are shown in Figure 2. There was no statistically significant difference between the curves (p=0.417).

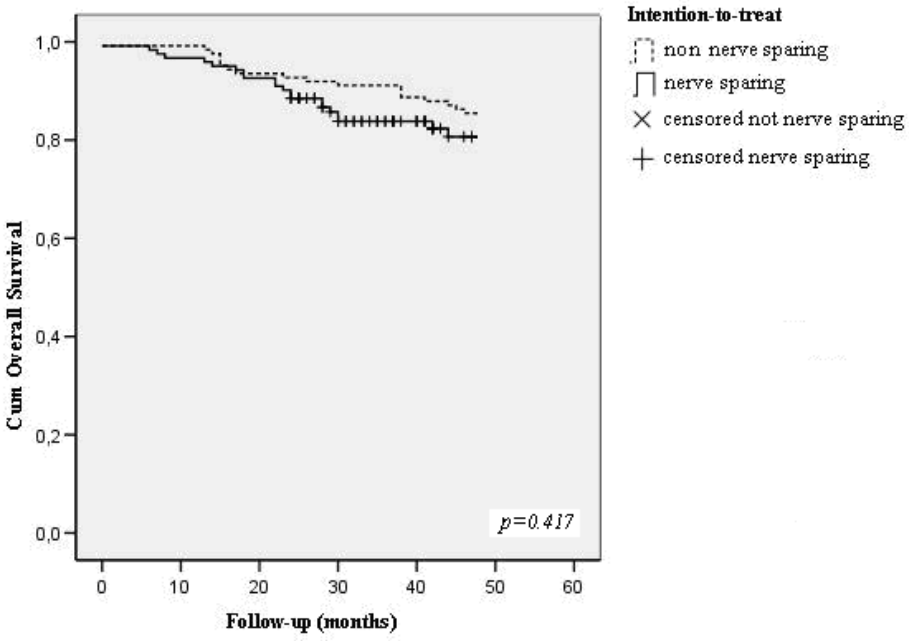

Figure 2 Five years Overall Survival (OS)

Discussion

This paper presents the results of nerve-sparing hysterectomy for cervical cancer stages IA to IIA in the LUMC (2001-2005) and compares the itt nerve-sparing group with a patient group before the introduction of the nerve-sparing technique (1994-1999). All patients scheduled for radical hysterectomy were included in the analyses, and the consecutive character of the study groups is a strong feature of this study. On the basis

of the results of this study, we consider the nerve-sparing technique for cervical cancer stages 1a to 2a feasible and safe.

Analysis of our study results showed no statistically significant change in local recurrence rate and LFS after radical hysterectomy between the periods before and after introduction of nerve-sparing surgery for cervical cancer stages 1a to 2a. This finding of unaffected local and loco-regional recurrence rate is emphasized by the comparison of the 2-year LFS (Fig. 1) and the 5-year OS (Fig. 2) of the 2 groups. Although OS was not a primary end point of our analysis, the survival curves support the conclusion that nerve-sparing is safe from an oncologic point of view.

The characteristics of the treatment groups were comparable. With respect to prognostically unfavourable factors, the 2 groups showed no significant differences. Despite this statistical consideration, the percentage of tumour-positive lymph nodes and infiltration depth of greater than 15 mm tended to be higher in the nerve-sparing group of 2001-2005. The 95% CIs showed an evident tendency toward the unfavourable side for the nerve-sparing group, which is reflected by the different proportions of adjuvant therapy, and should be considered as of clinical importance. On the whole, the latest group tended to have worse characteristics.

The HR of the 2001-2005 group for local recurrences decreased considerably after correction for the only independent prognostic factor for local recurrence rate, positive lymph node(s). After subsequent addition of the different major prognostic parameters, the changes in HR were as expected. Although the best guess for the HR remained 2.171, the difference between the groups is not statistically significant and gives no immediate cause to believe that the local recurrence rate has changed after introduction of nerve-sparing surgery.

Our local recurrence rates and LFS within 2 years were consistent within the range of known data from the literature, for both treatment groups.(27-31) This defined our study group as comparable to other patient populations and, therefore, permissible to extrapolate.

An unexpected finding of a post hoc analysis was the difference in total number of recurrences (local, loco-regional, regional, and distant) between the 2 treatment groups. We could not explain this finding as a result of the nerve-sparing modification of the surgical technique. The HR of the nerve-sparing group for recurrence of 2.111 was not statistically significant, but the 95% CI was 0.929 to 4.798. The finding might be explained by a coexisting tendency of operating patients who had more prognostically unfavourable characteristics in the most recent period.

In the group where nerve sparing was intended, the procedure could be successfully completed unilaterally or bilaterally in most cases. Operating time decreased after the introduction of nerve sparing, as did the blood loss. This was most likely the result of a learning curve and could possibly reflect a shift toward not closing the peritoneum after the procedure, which took place during the studied period.(26)

In 2003, a study presenting the state-of-the-art of radical hysterectomy in gynaecological oncology centres was published with data from a randomized multicenter clinical trial examining the clinical significance of surgical drains after radical hysterectomy (European Organization for Research and Treatment of Cancer), representing 12 gynaecological oncology departments from across Europe.(32) Comparing the perioperative and postoperative results and complications mortality, secondary bleeding, wound, pelvic, and respiratory tract infection, and bowel obstruction, the results in the nerve-sparing group of our study showed to be similar to the state of the art. In our (itt) nerve-sparing group, there were less postoperative urinary tract infections and thrombosis but slightly more lesions of the ureter during surgery. The last difference is most likely the effect of the inclusion of small lesions, which could be repaired during the procedure, in our analysis. The mean postoperative hospital stay was 6 days shorter in our study group (7 vs. 13 days), and the mean duration of the surgical procedure was 60 minutes shorter than in the European collaborative series.(32)

Our results concur with recently published new insights in embryologically derived anatomy of the female pelvis and mechanisms of tumour spread.(33-36) In view of these publications, the deep lateral parametrium does not exist in the form as it is looked upon conventionally. Adjusting the radical hysterectomy based on these new principles should be considered, with nerve sparing by dissection of a complete sacrouterine ligamentous sheet instead of the separation between the ligamentous and nervous part being a natural consequence of the modification.

Our study is, to the best of our knowledge, the only prospective study comparing the results of nerve-sparing and non-nerve-sparing radical hysterectomy for cervical cancer. Analyzing all data by itt warranted the best possible comparison of the treatment groups. A small group of patients where nerve sparing failed on one side and an even smaller group where nerve sparing was not possible at all were analyzed as part of the nerve-sparing group. Also, we could not exclude an effect of the ongoing time on operation results. Although the comparability of the 2 groups gives no reason to believe these effects were major, comparing the 2 treatment modalities in a randomized controlled trial could be considered to give a definite answer to the question whether nerve sparing is a threat to radicality. In view of ethical deliberations of those favouring and not favouring nerve sparing, this could probably be best accomplished in a

multicenter setting, yet in that way, there will always be a bias for different surgeons between the 2 groups.

Although the results of our study are promising, longer follow-up is recommended. In the meantime, our findings indicate that nerve-sparing radical hysterectomy is a safe and feasible procedure for cervical cancer patients of stages 1a to 2a. With respect to the deep lateral parametrium, our study gives no indication to fear an increase of early tumour spread by sparing the pelvic autonomic nerves. Given the long-term morbidity after iatrogenic damage to the pelvic autonomic nerves, and the possibility to improve quality of life after the operation, we feel that a nerve-sparing approach should be an integral part of all radical hysterectomies in this group of patients.

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Chapter 3

The Swift operation: a modification of the Leiden nerve-sparing radical hysterectomy

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Abstract

In 2002, our group introduced an operation to avoid damage to the pelvic autonomic nerves during radical hysterectomy that proved to be feasible, effective, and safe. During the last five years, we have adapted our surgical technique to make this procedure easier and safer in terms of radicality. We report on the changes in the surgical approach and the results in the first 15 consecutive patients. The Swift operation is more radical in the area of the uterosacral ligaments than the original operation, and it dissects the hypogastric nerve free under direct vision. In the area of the parametria, it is more radical in the deep lateral part. The vascular parametrial tissue is dissected and separated ventrally from the ureters. From October 2006 to February 2007, 15 consecutive patients with cervical cancer stage IA2 to IB2 underwent the Swift operation. The extra operating time amounted to 20 min, which was similar to the original operation, and with no extra blood loss. The suprapubic catheter was removed after a median of five days. Up until now (February 2008), no recurrences have been seen in these patients. It was concluded that the Swift procedure is easy to perform and that it offers advantages over the original operation in terms of safety and radicality.

Introduction

In 2001, we proposed a surgical technique to avoid damage to the autonomic nerves in the pelvis during radical hysterectomy.(1) The rationale for preserving these nerves during surgery is to avoid the well-known sequelae of nerve damage, such as impaired bladder function (2), rectal motility disorders and sexual dysfunction (3–5). Our nerve-sparing surgical technique was based on extensive cadaver studies (6) and the experience of Japanese surgeons (7;8), and an approach was chosen to make the technique applicable for Western patients with a higher mean body mass index (BMI) and different disposition of fat in the pelvis as compared to Asian women. Our proposal was not an entirely new form of radical hysterectomy but, rather, the description of individual nerve-sparing steps that could be incorporated into any particular type of radical hysterectomy. The technique was, therefore, easy to follow and adopt.(9;10) We have shown that the nerve-sparing technique is feasible and that nerve damage can actually be avoided.(11) Furthermore, the 5-year survival rate before and after the introduction of the nerve-sparing technique in our department did not change. Preliminary data on the objective sexual function following nerve-sparing surgery indicated that blood flow response following sexual arousal is not different between patients who underwent nerve-sparing radical hysterectomy on one hand and healthy controls on the other.

The winds of change do also blow in surgery and, over the years, we have considered adaptations to our technique to make it easier and safer in terms of radicality. In this paper, we report on these modifications and we demonstrate the feasibility of the modified approach in the first 15 consecutive patients.

Patients and methods

From October 2006 to February 2007, 15 consecutive cervical cancer patients who underwent radical hysterectomy were included in a feasibility study. An adaptation of the original nerve-sparing operating technique (1) was performed, which is described in detail below. Clinical characteristics of the patients, the tumours and the perioperative course were recorded. Patients were admitted to the hospital for around a week; discharge was dependent on their condition and home situation. All patients received a suprapubic bladder catheter at the end of the operation. On the fifth postoperative day, the bladder function was tested and urinary retention measured. When there was spontaneous micturition and the urinary retention was less than 100 ml, the catheter was removed under antibiotic prophylaxis.

Surgical technique

The successive steps of the adapted nerve-sparing operation are summarised in Table 1. To obtain a better insight into the neuroanatomical structure of the pelvic autonomic nerves, we refer to a detailed review on this subject.(12)

The operation results in a tissue specimen containing the uterus with parametrium and paracervical tissue and two distinct tissue flaps on both sides: a horizontal mesometrial flap containing the uterine vessels and the surrounding tissue and a dorsal tissue flap containing the uterosacral ligament, rectal pillar, peritoneum and subperitoneal connective tissue. The horizontal vascular mesometrium contains the uterine artery and veins, lymphatic vessels, small lymph nodes, uterovaginal branches of the autonomic pelvic plexus, loose connective tissue and, usually, small calibre fatty tissue. The dorsal tissue flap, the ligamentous mesometrium, is a three-dimensional structure of dense connective tissue running in a dorsal and inferior direction and is horseshoe-shaped in the transverse plane, which encases the rectum. The hypogastric nerve runs laterally to it. The tissue specimen resembles the silhouette of a Swift bird (Figure 1) and, therefore, the operation is called the Swift operation.

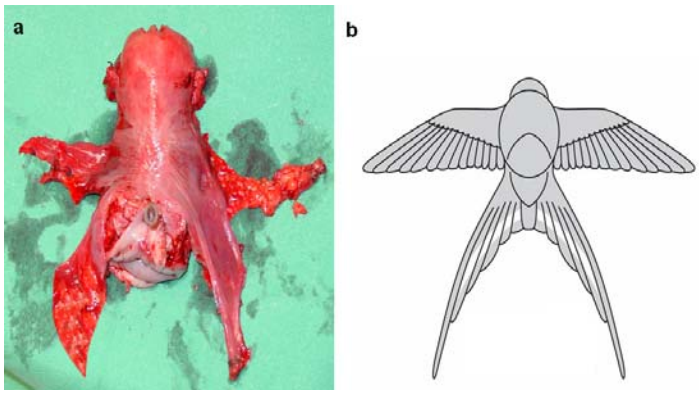

Figure 1 Comparison of the freed tissue specimen (a) with a Swift bird (b)

Table 1 Swift operation in steps

- Transect the round ligaments and open the peritoneum of the broad ligament way up to the common iliac arteries
- Identify and dissect the ureters from as cranially as possible up to the entrance of the ureteral canal
- Lymphadenectomy along the external iliac vessels, the common iliac artery, the hypogastric artery, from the obturator fossa and the presacral area, clearing all of the lymph node-bearing fatty tissue. Also, the area lateral to and underneath the superior vesical arteries (‘lateral parametrium’) is cleared from lymphatic tissue
- Develop the parietal spaces (see (14) for a more extensive description) by blunt dissection of the loose connective tissue lateral to the superior vesical arteries up to the bifurcation of the iliac vessels
- Identify the origin of the uterine vessels from the pelvic side wall and dissect along the distal border of the vessels to separate the uterine vessels (‘mesometrium’) from the underlying bladder mesentery. The bladder mesentery consists of the inferior vesical artery and veins, lymphatic vessels, fatty tissue and the distal part of the inferior hypogastric nerve plexus, with branches to the ureter, bladder, vagina and clitoris
- Transect the uterine vessels at their origin and dissect this tissue (containing blood vessels, lymph vessels, fatty tissue and loose connective tissue) down along the pelvic side wall, then turn upwards in a medial direction, over the ureter to its medial side, using haemoclips or LigaSure sealing (Figure 3)
- Develop the presacral space by blunt dissection between the rectum medially and the ureters laterally
- Put the peritoneum flap attached to the rectum under tension in a clamp and identify the hypogastric nerve system attached to the subperitoneal and dense connective tissue, grasp it in a Babcock clamp and dissect it laterally. The hypogastric nerve can be identified best at the entrance of the ureteral tunnel. At this level, the autonomous nerves run parallel to and closely dorsally to the ureter
- Open the pouch of Douglas peritoneum and develop the prerectal space
- Cut the peritoneal flap that has been separated from the hypogastric nerve halfway round the circumference of the rectum and continue the dissection to include all of the uterosacral ligaments close to where the ureters enter the ureteral canal
- Open the bladder peritoneum, cut the supravaginal septum and dissect the bladder down from the cervix and the vagina
- Open the rest of the ureteral canal (anterior leaf of the vesicouterine ligament) using Oberholt clamp or Uchida’s ureter spoon
- Remove the ureter from the posterior leaf of the vesicouterine ligament by dissecting the loose connective tissue embedding the ureter. The ureters are now completely freed up to their entrance into the bladder
- Place clamps or LigaSure on the thin sheath of dense connective tissue along the cervix and vagina. This tissue has been called the medial part of the posterior leaf of the vesicouterine ligament, paracolpium or vesicovaginal ligament. It is almost continuous to the distal attachment of the uterosacral ligaments
- Place Burke clamps on the vagina and cut the vagina below these clamps
- Remove the uterus with the mesometrium wings and the long-tailed uterosacral ligaments
- Close the vaginal cuff with interrupted resorbable sutures
- Rinse the operating field with sterile water to detect persistent venous bleeding and provide for adequate haemostasis
- Close the abdomen

Compared to the original nerve-sparing operation, there are three differences.
First, in the Swift operation, the uterosacral ligament is not divided any more into a medial and lateral part. The hypogastric nerve is dissected free from the uterosacral ligaments and the surrounding tissue, permitting a far more radical resection of the entire uterosacral ligaments and rectal pillars (Figure 2 right). In this way, the hypogastric nerve is approached from the lateral side as opposed to our previous technique, which approached it medially.

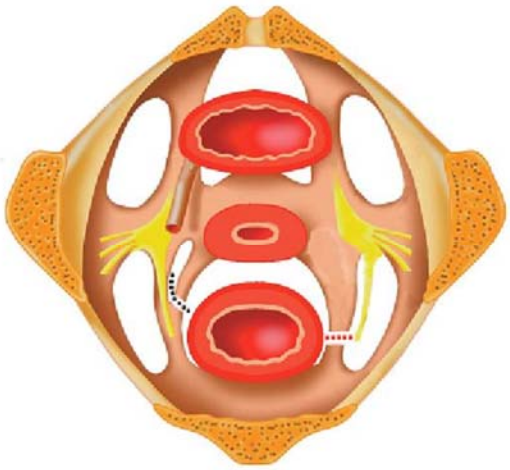

Figure 2 Dissection of the uterosacral ligament in the Swift operation (right)

Second, the parametrial resection has a different direction of the dissection plane. In the original operation, it was like the bow of a ship running from a ventro-lateral to a dorso-medial direction. In the Swift operation, the dissection plane follows the shape of the tip of an Ionic column of an ancient Greek temple (Figure 3). The mesometrial tissue is dissected free from the ventral side of the ureter and stays attached to the uterus as a horizontal tissue flap resembling the wings of a Swift.

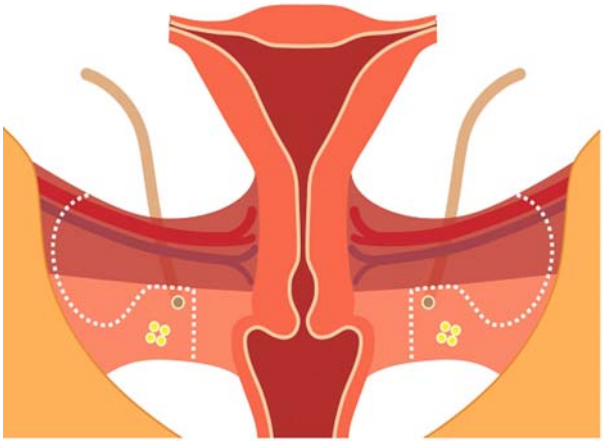

Figure 3 Dissection plane of the Swift operation

Third, the resection of the lateral part of the posterior leaf of the vesicouterine ligament, as described previously (1), is only performed when it is necessary to obtain radically free surgical margins in this area (R0 status).

Results

The results of the feasibility study are shown in Table 2. In all patients, the Swift operation could be performed. The amount of blood loss, operating time and admission time were within the normal ranges for this kind of surgery.(1;13) The suprapubic catheter was removed between 4 and 10 days postoperatively (mean 6 days, median 5 days). In all cases, there were radically (> 5 mm) free margins (R0 status) and in three patients, lymph node metastases were found in a total of six nodes: dorso-medially to the external iliac vein (two nodes); between the external iliac artery and vein (one node); laterally to the common iliac artery (one node) and in the medial part of the obturator fossa (two nodes). The extra operating time due to the Swift procedure amounted to 20 min, which means that the Swift procedure takes just as long as our former nerve-sparing technique.

Table 2 Characteristics of 15 patients who underwent the Swift operation

Consec. number	Age	Stage	Tumour diameter at surgery	Cell type	Operating time (min)	Blood loss (m)	Hospitalisa-tion (days)	Removal of SP catheter (days)	Resection status (margins)	Remarks
1	33	Ib1	2.9	Adeno	210	450	6	5	R 0	
2	42	Ib1	3.5	Squamous	180	750	7	5	R 0	
3	61	Ib2	5	Squamous	180	1200	8	7	R 0	1/30 nodes +
4	45	Ib1	3	Squamous	180	400	8	10	R 0	
5	45	Ib1	2	Squamous	240	800	8	6	R 0	
6	50	Ib1	3	Adeno	240	350	8	5	R 0	
7	29	Ib2	4.5	Squamous	195	630	8	8	R 0	3/33 nodes +
8	30	Ib1	2.5	Squamous	180	700	7	5	R 0	
9	36	Ib1	3	Squamous	180	700	9	7	R 0	
10	53	Ia2	1.5	Squamous	240	800	7	5	R 0	
11	57	Ib1	3.9	Squamous	180	800	10	9	R 0	
12	35	Ib2	4.5	Adeno	210	400	8	7	R 0	
13	46	Ib1	3	Squamous	210	380	7	5	R 0	
14	52	Ib1	2.5	Squamous	180	400	8	5	R 0	
15	33	Ib2	6	Adeno	150	500	6	4	R 0	2/29 nodes +

SP = suprapubic catheter

Discussion

This clinical study shows that the Swift procedure was easy to perform and feasible in all 15 consecutive cases. The postoperative restoration of bladder function was illustrated by spontaneous micturition with a urinary retention of less than 100 ml after a median of five postoperative days. Due to the short follow-up in this study, no data regarding the long-term effects on the bladder function can be provided.

The adaptation of the original operation

The adaptation of the original Leiden operation of nerve-sparing radical hysterectomy deserves some explanation. The first difference concerns the sparing of the hypogastric nerve fibres in the vicinity of the uterosacral ligaments. These fibres originate from the superior hypogastric plexus that is located ventrally from the promontory and run attached to the lateral side of the peritoneum that covers the rectum. This peritoneal sheath is normally opened to identify the ureter and obtain access to the presacral area. More distally, the hypogastric nerve is embedded in loose connective tissue that is part of the most lateral section of the uterosacral ligament. By stretching the peritoneal leaf lateral to the rectum, the hypogastric nerve can easily be identified on its lateral side and dissected free from all adjacent tissue. This dissection can be continued to the point where the nerves run dorsally from the ureter in front of the beginning of the ureteral tunnel. Here, the nerve fibres fuse with the parasympathetic fibres from the sacral roots S2–S4 to form the inferior hypogastric plexus. By dissecting the hypogastric nerve free, a very radical resection of rectal pillars, uterosacral ligaments and peritoneal and subperitoneal tissue can be achieved, without any fear of damaging the nerves. We perform this step with the use of LigaSure®. Dissecting the hypogastric nerve free in this way is actually easier than the splitting of the uterosacral ligament, as described previously (1), because the nerve is in sight during the whole procedure. Also, in obese patients, the identification of the nerve in this area is no problem. An additional advantage of the Swift approach is that it is no longer necessary to open the peritoneum covering the uterosacral ligaments. This was mandatory in the original operation to permit the splitting of the uterosacral ligament into a medial and a lateral part, but it often caused extra blood loss due to persistent oozing from the peritoneal edges in that area.

The second difference regards the dissection plane during the parametrial resection. In the Swift operation, the resection starts at the origin of the uterine vessels at the pelvic side wall. Then, it runs in a dorsal direction, bends medially and slightly ventrally (like the shape of an Ionic column) and follows a course just ventrally from the ureter. Medially from the ureter, it runs dorsally again (Fig. 3). In this manner, the entire vascular part of the parametrium (“mesometrium”) remains attached to the lateral side

of the uterus that is removed as a tissue specimen. The nerve-bearing tissue is avoided more effectively with the new technique.

By staying ventrally to the ureter, the hypogastric plexus can never be damaged, as it is always located dorsally from the ureter. In this sense, the Swift procedure is safer for avoiding the nerves than the previously described “bow-of-a-ship” dissection.

It can be stated that the Swift procedure is safer in terms of nerve-sparing compared to the original operation both at the uterosacral and the parametrial areas. At the same time, it is as radical in the parametrial resection and more radical in the uterosacral part.

The deep lateral parametrium

In the Swift operation, as in the previously proposed nerve-sparing radical hysterectomy, the deep lateral part of the parametrium is not removed. Is this a problem from a radicality point of view? We think not. We have seen no significant difference in the survival or local recurrence rate since we started with nerve-sparing surgery in 2001/2002. Some concern about the importance of removing the deep lateral portion of the parametrium has been expressed by the authors of the so-called “giant section” studies, in which the whole of the parametrium from the uterus to the pelvic side wall was embedded for pathology assessment. In these studies also, lymph nodes in the deep lateral part of the parametrium were found. In the giant-section studies, parametrial resection is performed before the pelvic lymphadenectomy, making it impossible to distinguish these nodes as “deep lateral parametrial” or “deep medial obturator.” We have found that, after an extensive and deep lymphadenectomy in which the lymph-bearing tissue dorsally from the superior vesical arteries is also removed, apart from the autonomic nerves, hardly any tissue remains in the area that is called the deep lateral parametrium. The only tissue that can be found is directed in a more distal course towards the bladder and is to be regarded as bladder mesentery. Furthermore, in the giant section technique, it is not possible to distinguish between parametrium and uterosacral ligamentous tissue.

Another argument against the obsession to remove the deep lateral part of the parametrium is the rate of lymph node metastases in this area. Winter et al. (14) found lymph node involvement in the deep lateral parametrium in 2.2% of FIGO stage Ib1 cervical cancer patients. Burghardt et al. (15) demonstrated, in stage 1b1–2b patients with negative pelvic lymph nodes, the likelihood of tumour involvement of the deep lateral parametrium to be only 0.9–1.3%.

Total mesometrial resection

The Swift operation is inspired by and resembles the total mesometrial resection (TMMR) described by Höckel et al.(16) This operation is based on the hypothesis that the local spread of cervical cancer cells is not a random process, but is guided by positional information within a defined permissive area. This area is the adult state of the embryological field through which the uterine cervix has developed. The theory behind TMMR indicates that this area, called the morphogenetic unit (MGU), should be completely removed during radical hysterectomy, but that it is unnecessary to remove tissue outside the borders of the MGU. The MGU consists of the vascular parametrium ventrally from the ureter and the uterosacral ligament/rectal pillar complex as far dorsally as half way round the circumference of the rectum. Therewith, the structure of the MGU closely resembles the shape of the tissue specimen after a Swift operation.

In the TMMR theory, the autonomic pelvic nerves, the posterior leaf of the vesicouterine ligament and the deep lateral part of the parametrium are outside the MGU and, as such, represent no risk for local tumour spread. The theory of Höckel et al. is very interesting from an oncologic point of view, because it suggests an orchestration of the way cancer cells spread in the vicinity of the primary tumour. The total mesorectal excision (TME) is based on the same principle and has caused a revolution in the treatment results of rectal cancer. Höckel's group recently published the results of 126 TMMR operations (17), and the results are spectacular, as well as better than the current state-of-the-art results in the surgical treatment of cervical cancer (13).

The TMMR and the Swift operations have reached a similar end point, albeit via different routes. The main difference between the two operations is a far more extensive lymphadenectomy in TMMR. This is done with a therapeutic intention to dispense with adjuvant radiation in all cases. In the Swift operation, the adaptations described in this paper were derived by the search for an optimal combination of sparing the autonomic pelvic nerves on the one hand and the radical removal of cervical cancer on the other. TMMR is based on a new and promising theory on the positional spread of cervical cancer cells. Sparing the autonomic nerves in the TMMR operation is not a goal in itself, but a logical consequence, because the nerves are not part of the MGU.

Whatever approach is followed in the surgical treatment of cervical cancer, avoiding damage to the autonomic pelvic nerves should be an important element for the kind of radical hysterectomy that is performed. This paper, which describes the Swift nerve-sparing radical hysterectomy, provides guidelines for such an approach. It has been developed over more than eight years of extensive study of the course and function of the pelvic autonomic nerves and the steps of pelvic surgery. It is feasible, and even easy to perform, is rational from an oncologic point of view and it concurs with a recently launched theory of the local tumour spread of cervical cancer.

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Chapter 4

The use of distilled water in the achievement of local haemostasis during surgery

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Abstract

Distilled water is used worldwide to check on haemostasis at the end of pelvic oncologic operations. Nevertheless, reports about this method are lacking. The aim of this study was to explain the method and to discuss possible side effects. After the addition of distilled water to the surgically exposed pelvis, rapid lysis of erythrocytes results in a transparent fluid in which a small source of bleeding is easily recognizable. A possible side effect of the lavage might be contribution to the formation of peritoneal adhesions by confusing the abdominal defence system. Systemic side effects are not to be expected. Although tumour cells might suffer from hypotonic distilled water lavage, the current use of distilled water at the end of surgery is probably not effective to lyse tumour cells. Our findings support the on-going use of distilled water lavage to achieve haemostasis after extensive pelvic surgery.

Introduction

After extensive pelvic surgery, e.g. oncologic operations or surgery for endometriosis, the surgeon can be confronted with a raw, oozing area in the pelvis. Stopping small venous bleeders to achieve adequate haemostasis is often a difficult task in these areas. Distilled water lavage makes the detection of the sources of bleeding much easier. While lavage with NaCl 0.9% to clean the operation field after surgery is widely accepted and often mentioned in literature, strangely enough we haven't seen any publication on the use of distilled water (aquadest). The aim of this report is to explain the use of distilled water for the purpose of achieving haemostasis during surgery. Besides informing about its usefulness and ease, we want to provide background information about the mechanism that makes distilled water applicable for this purpose. Apart from the beneficial effects of distilled water, it is imaginable that the addition of a hypotonic fluid as such to the pelvis might not be without any side effects. We briefly discuss these elements.

Mechanism

Clinical application

Before closing the abdomen, 500 cc of distilled water is poured into the abdominal cavity. In contrast to NaCl 0.9%, which gives a blurred view through an opaque fluid, distilled water remains clearly transparent and ensures a bright sight in the small pelvis. As a consequence, small venous bleeding is easily recognizable and the bleeding site can be accurately traced. When sources of bleeding have thus been discovered, the distilled water is suctioned out of the pelvis and the bleedings are stopped in the appropriate way. In general, two sequential lavages are used. Figure 1 shows two examples.

Physiology

Within a certain range of external solute concentrations, erythrocytes behave as an osmometer: their volume is inversely related to the solute concentration in a medium. The erythrocyte shrinks in hypertonic solutions and swells in hypotonic solutions. When an erythrocyte has swollen to about 1.4 times its original volume, it begins to lyse (burst). At this volume the properties of the cell membrane abruptly change, haemoglobin leaks out of the cell and the membrane becomes transiently permeable to most molecules.(1)

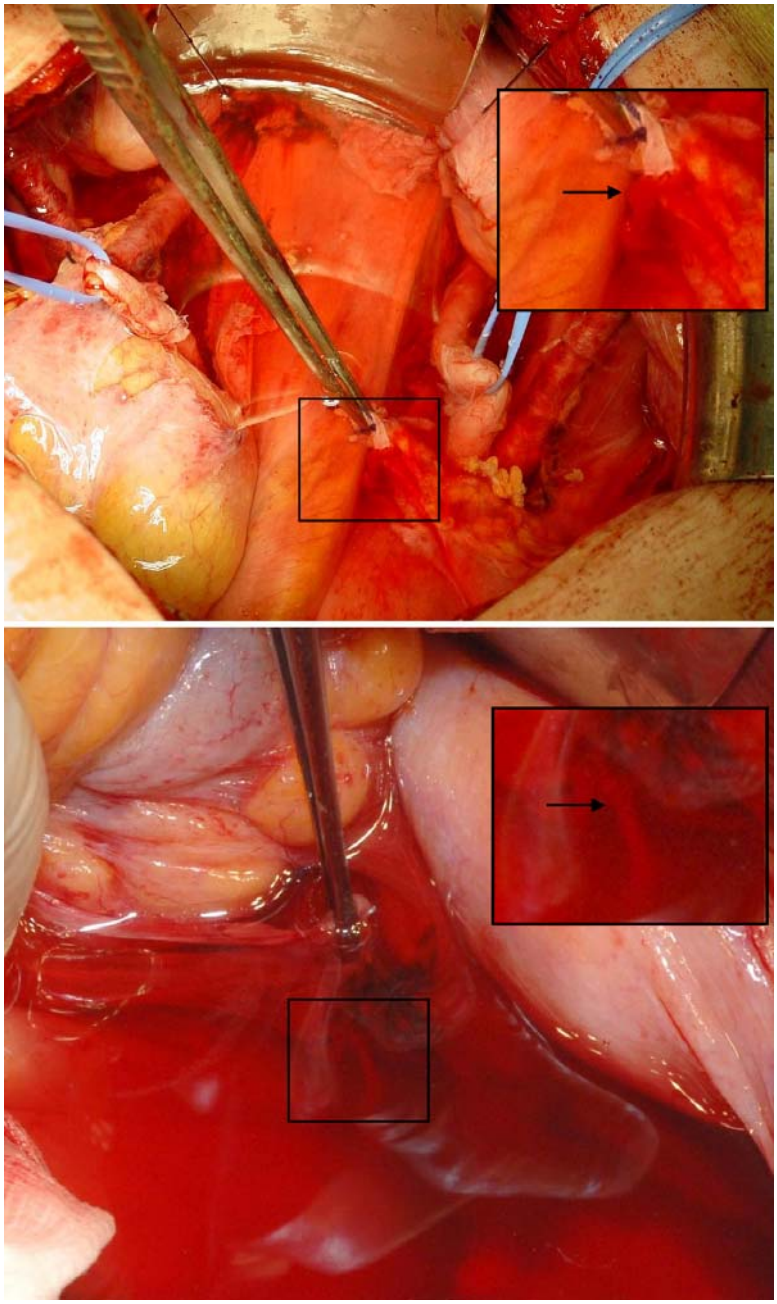

Figure 1 Two examples of the use of distilled water to localize small venous bleeding sites. The water is clearly transparent. The arrows point to the emerging stream of blood.

NaCl is isotonic to the red blood cell at a concentration of 154 mM. This corresponds with NaCl 0.9%. The red blood cell has its normal volume in isotonic NaCl. Erythrocytes remain intact in NaCl 0.9%, resulting in an opaque suspension. Distilled water on the other hand is hypotonic to red blood cells. The red blood cell will therefore swell and haemoglobin, containing the haem that gives the red colour to erythrocytes, leaks from the cell resulting in a transparent red-pink-coloured solution. Apparently, erythrocytes in clear fluid colour the fluid red and opaque, whereas haemoglobin in clear fluid leaves the fluid transparent.

Laboratory experiment

To further explore the appearance of a traceable blood stream when using distilled water lavage, we performed an in vitro experiment. Ten microlitres of human erythrocytes was injected in tubes containing 990 µl NaCl 0.9% or distilled water (concentration 1:100). A traceable stream dropping to the bottom was visible in both tubes. After stirring the tubes both solutions got turbid, but for the distilled water solution this only lasted a moment. Approximately 5 s after the stirring, the distilled water solution turned bright red-pink, clearly transparent. The NaCl solution stayed opaque red-pink. After 30 s, a drop of fluid of both solutions was examined under the microscope. In the NaCl solution, normal erythrocytes were visible, whereas no normal erythrocytes were visible in the distilled water solution. Subsequently, another 10 µl of erythrocytes was added to both solutions. The traceable stream was visible again in the red-pink distilled water solution, whereas a stream could not be seen in the turbine NaCl solution. Some time after stirring, the distilled water solution turned bright red-pink again, while the NaCl solution got more opaque. Successively, volumes of 10 µl erythrocytes were added to the tubes. When continuing the process, it took longer before the distilled water solution turned bright red-pink after stirring and the colour of the solution got darker. At a concentration of 16:115 (160 µl erythrocytes in 990 µl H_2O), the distilled water solution was no longer transparent and a blood stream was not visible anymore.

This experiment mimics the clinical situation and shows that a traceable blood stream does appear in both distilled water and NaCl 0.9%, but is only visible in a transparent fluid. In contrast to NaCl, distilled water retains its transparency after the addition of blood as a result of the rapid lysis of erythrocytes. The difference in transparency can be compared with the difference between fruit juice (NaCl) and lemonade (distilled water). Erythrocytes added to the solution cause the osmolarity of the solution to rise, and the difference in hydrostatic pressure to decrease, resulting in a prolonged time of cell lysis. Probably because more intact or swollen erythrocytes will be present in the distilled water solution after continuous mixing with blood or other intra-abdominal fluid,

the solution becomes less transparent. This sometimes necessitates more than one lavage cycle.

Possible side effects

Peritoneal damage

Peritoneal mesothelial cells play a primary role in the abdominal defence system. They can express specific surface markers that enable them to promote the margination and migration of neutrophil granulocytes and produce important molecules such as pro-inflammatory cytokines, nitric oxide, growth molecules, tissue plasminogen activator and plasminogen activator inhibitor (2). Peritoneal mast cells have been shown to have a direct effect on peritoneal adhesion formation. Mast cell mediators induce fibroblast proliferation and mast cell hyperplasia has been shown to be present in human peritoneal adhesions.(3)

It is not known by which mechanism peritoneal mesothelial cells behave when exposed to different concentrations of lavage solutions, but they are damaged by solutions that are far from physiological, such as peritoneal dialysis fluids. Even solutions that are more physiological have been shown to cause changes in synthesis of specific molecules.(4–6) In vitro studies showed that peritoneal lavage with normal saline causes up-regulation of pro-inflammatory cytokines, and that per-operative lavage solutions influence the peritoneal defence mechanisms. Both antiseptic lavage solutions and physiologic salt caused cell death and decreased integrity in the mesothelial monolayer. (7;8)

Polubinska et al. performed an in vitro study of mesothelial monolayers exposed to NaCl 0.9% and different peritoneal dialysis fluids, including hypertonic solutions with low glucose degradation products concentration. Although the hypertonic fluid caused damage to the cell membrane, the viability of the cells was not reduced and fybrinolytic activity was preserved. In contrast, cells exposed to NaCl 0.9% showed changes that could promote the formation of peritoneal adhesion formation. In a rat study of this group, hypertonic lavage solution was reported to suppress intra-peritoneal inflammation and mesothelial hyperplasia as compared to normal saline. The authors advise the use of hypertonic solutions with low glucose degradation products concentration for both peritoneal dialysis and rinsing of the peritoneal cavity per-operatively.(6;9) However, hypotonic solutions were not studied and the effects reported in this study could probably be attributed to the glucose dehydration product concentration rather than to osmolarity. Adhesion formation was not studied and the study groups were small. Kanakura et al. studied numbers and types of mast cells in the peritoneal cavity of mice after injection of distilled water or saline. Although the intra-

peritoneal injection of saline did not significantly affect the number of peritoneal mast cells, the intra-peritoneal injection of distilled water eradicated small and medium (differentiated) mast cell colony-forming cells, while the number of large mast cell colonies increased.(10)

The effects of lavage fluid volume and incubation time on the above-described mechanisms are not known, neither is their role in vivo.

Based on the existing literature, we assume that peritoneal lavage with any solution will result in disturbance of the homeostasis in the pelvis. Lavage with distilled water might alter the abdominal defence system by up-regulation of pro-inflammatory mediators. This might lead to the formation of adhesions. The extent of this effect of distilled water in proportion to other media is not clear. The same mechanisms might damage the lining of the per-operatively exposed organs and tissues, but we have no means to accurately quantify this effect in vivo.

Systemic effects

One can speculate on a systemic effect of distilled water pelvic lavage. The absorption of distilled water used as an irrigation fluid in endoscopic procedures has been reported to disturb the circulatory system and lead to clinical symptoms known as the transurethral resection (TURP) syndrome. Acute changes in intravascular volume and plasma solute concentrations might contribute to hypotension, and even acute renal failure has been reported.(11) Hypotonic distilled water directly entering the circulation through opened veins or absorbed more slowly, can cause haemolysis. Intra-vesicular pressure above 30 mmHg and increasing resection time have been proposed to increase the absorption of fluid.(12;13) Averagely, 15 l of irrigation fluid is used during a 60-min-lasting TURP procedure.(14;15) Still, severe changes in blood values after TURP with distilled water were rarely reported and it has been shown to be a safe irrigating fluid for TURP.(15)

Since in the procedure we describe only small volumes of distilled water are used for a short time at the end of the surgical procedure and without pressure, we have no reason to suspect systemic side effects as a result of the application of distilled water in the pelvis at the end of the surgical procedure.

Lysis of tumour cells

Besides possible negative side effects, distilled water lavage to attain haemostasis might also have an unintentional beneficial effect.

Although there is no consensus about the optimal lavage method, e.g. incubation time to achieve effective lysis and the potential of sequential lavages, several studies showed a harmful effect of distilled water on tumour cells. Human tumour cell lines seem to be sensitive to osmotic shock in vitro. Fechner et al. found significant bladder tumour cell dead after 10 min of exposure to distilled water lavage.(16) Mercill et al. observed a decrease in DNA synthesis in different tumour cell lines after exposure to distilled water for 1 to 10 min. However, the remaining cells did not lose their replication capacity.(17) In a study of Huguet et al., colorectal cancer cell lines were incubated with distilled water and no surviving cells were found beyond 12 min incubation. Water introduced into the peritoneal cavity in vivo was contaminated by intra-peritoneal secretions, compromising the osmotic lysis effect. However, this contamination was reduced by sequential lavages: after three lavages a final resultant osmolarity of 10 mM was attained. A solution of 10 mM was able to produce 100% cell lysis of colorectal cancer cells in vitro after 32 min of incubation.(18) Lin et al. found that the application of 10 l of distilled water divided into at least five cycles retained for 3 min resulted in a significant better survival time after curative liver resection in patients with spontaneous rupture of hepatocellular carcinoma.(19) Although it is assumed that tumour cell injury is caused by osmotic shock, the exact mechanism of potential cell injury by distilled water is not known. Selzner et al. assessed the effects of 1, 3 and 5 min of hypotonic exposure on cell viability in three different human colon cancer cell lines. All three cell lines challenged with distilled water exhibited a dramatic decrease in viability in a time-dependent manner, but only exposure of >15 min to distilled water was associated with significant increases in cell lysis. According to Selzner N et al. cell death is related to temporary cell swelling that triggers activation of apoptosis. Essential receptors for this apoptosis pathway were not detected in normal cells (human fibroblasts) after a challenge with either distilled water or isotonic media.(20)

Based on these reports we assume that the lavage method that we use to achieve haemostasis is not adequate for complete tumour cell lysis. The incubation time is short and numbers of lavages are relatively small. Nevertheless, free tumour cells in the pelvis might to some extent suffer from hypotonic distilled water lavage.

Conclusion

Distilled water is a helpful tool to achieve haemostasis per-operatively. Erythrocytes burst in the hypotonic distilled water, ensuring a transparent solution in which a blood stream can be easily traced to its origin. After suctioning the water out of the pelvis, bleedings can be stopped.

The impact of the method on the total amount of blood loss or operating time cannot easily be estimated since other per-operative factors outweigh the effect of distilled

water in that respect, but distilled water is certainly relevant for surgeons during a difficult step of the surgical procedure.

A possible negative side effect of this method is damage to the peritoneal mesothelial cells. Distilled water lavage might confuse the abdominal defence system and might contribute in this way to the formation of peritoneal adhesions. We could not assess the possible harmful effect of distilled water lavage on surgically exposed surfaces. The described method is not suspected to cause systemic side effects during or shortly after surgical treatment. Distilled water lavage might induce tumour cells lysis, but the currently used method is probably not sufficient to achieve this beneficial effect.

In the absence of evident indications on possible negative side effects of distilled water lavage as described above, we consider it sufficiently safe to apply this useful method in surgical practice.

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Chapter 5

Visualising the Morphogenetic Unit

S.A.H.M. van den Tillaart

Introduction

The mechanisms of local spread of cervical cancer are for the greater part unknown. Our knowledge consists primarily of observations of clinical behaviour and studies of laboratory markers that seem to be related to certain clinical characteristics. At present, none of these markers has been proven reliable enough to be used in the daily clinical practice. The clinical consequences of tumour extension to the surrounding tissues, invasion of vessels, pelvic lymph nodes and distant metastases, have been mapped. Their relation to survival and treatment response has been studied in large patient groups.(1) And thus, treatment is based on observations. But clinical findings do not reveal anything about the cause of tumour behaviour. We see that a tumour grows in a certain direction, but we do not know why.

Local tumour spread restricted to an exclusive area

As shortly mentioned in the introduction of this thesis, recently Höckel et al. put forward the Morphogenetic Unit (MGU) as the predisposed area for tumour growth, in a theory based on embryological anatomical studies. The MGU is proposed as the exclusive area for local tumour spread in the first stages of disease progression. The mesenchyme of this anatomical area, derived from common embryonic precursor tissue, is said to present positional information that guides local spread of cervical tumours.(2)

Höckel, Fritsch et al. studied the development of the female genital tract out of the Müllerian tubes from foetus to adult and described the development of the MGU in embryos and foetuses (see Figure 1), and in female cadavers. The borders of the MGU are formed during embryonal development and remain so when the internal genitalia develop further.

Höckel et al. describe three distinct Müllerian Morphogenetic Units. The proximal unit consists of the bilateral fallopian tubes and their mesosalpinx. The intermediate unit is substituted of the uterine corpus and bilateral peritoneal mesometrium (broad ligament). The distal part is located subperitoneal and consists of the cervix, proximal two-thirds of the vagina, their neurovascular supply structures (the uterine artery and vein(s), utero-vaginal branches of the autonomic nerve system, lymphatic vessels, lymph nodes, and fibrofatty tissue), and a coat of condensed connective tissue including the dense connective tissue of the recto-uterine pouch which corresponds to the recto-uterine and utero-sacral ligaments.(2)

According to Höckel et al. local spread of tumour cells is not a random process. The spread of tumour within the MGU can be continuous with the primary tumour, but also result from occult tumour cells, that migrate from the primary tumour into the surrounding tissue.(2;3)

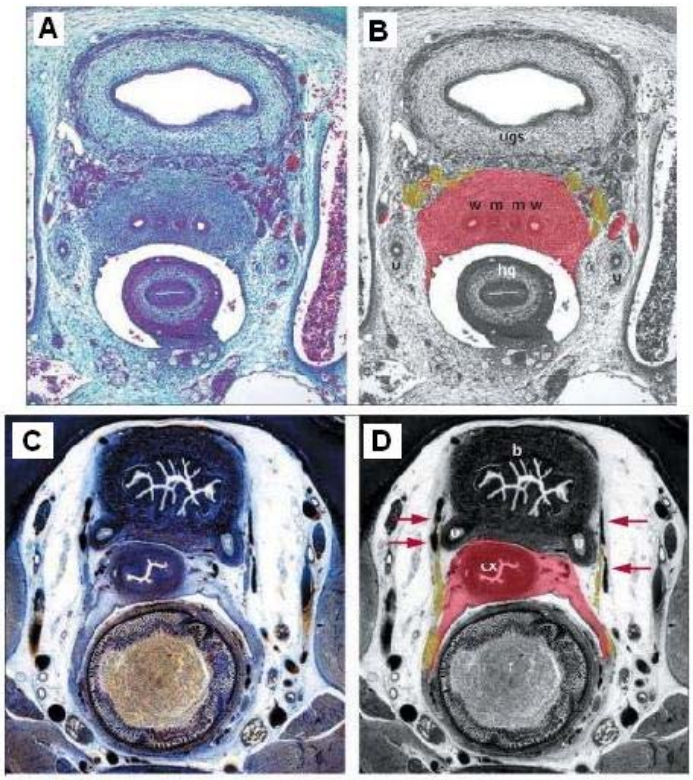


Figure 1 (A,B)Transverse sections of female embryo aged 8 weeks at the level where ureters are lateral to Müllerian aggregation. And (C,D) transverse sections of a female foetus aged 24 weeks at the level of ureters (u) entering the bladder. The Müllerian mesenchyme is present as horseshoe-shaped condensed connective tissue (B,D; red area). Autonomic nerves (B, D; yellow area) and blood vessels (B, D; red spots). Magnification: 20 x. w = Wolffian duct; hg = hindgut; r = rectum. Reprinted from:(2) with permission from Elsevier, Lancet Oncology.

Complete removal of the MGU

With the theory of occult tumour spread in mind, it is essential that the MGU is excised completely during the surgical treatment of low stage (FIGO 1b – 2a) cervical cancer to ensure the removal of all cancer cells. Only the most distal part of the MGU remains in situ to preserve the vaginal function. The tissues adjacent to the MGU, even if in close relation to the MGU must not be removed to minimize iatrogenic morbidity. The MGU is separable from the autonomic nerve plexus, rectum, bladder, bladder mesentery, and ureters. For this purpose, Höckel et al. developed the Total Mesometrial Resection

(TMMR). If TMMR is carried out successfully, the resection margins of the surgical specimen do not need to be radically free (> 5 mm) because the tissues outside the MGU are not at risk to harbour tumour cells. Neither is adjuvant radiotherapy necessary in case of positive lymph nodes, provided that systematic pelvic and (if lymph node metastases were found per-operatively) periaortic lymphadenectomy has been performed.(2-11) See Figure 2.

Compared to conventional (none-nerve-sparing, and to a smaller extent also nerve-sparing) radical hysterectomy, the TMMR is more extensive in certain directions to remove all tissue prone for tumour spread, but spares functional tissues in other parts.

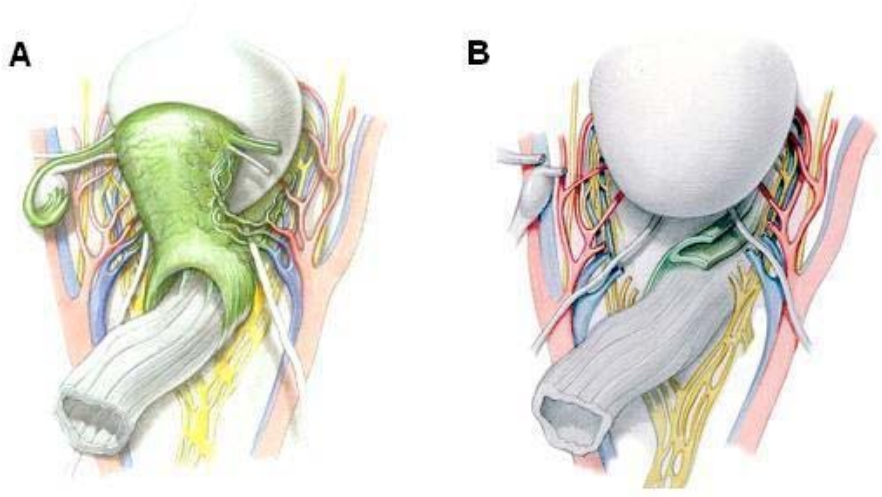


Figure 2 (A) Graphical representation of the MGU highlighted in green. Reprinted from: (11) with permission from Gynecologic Oncology. (B) Schematic representation of the surgical goal of the TMMR: postsurgical pelvic anatomic situs. Non-Müllerian paracervical tissue such as the inferior hypogastric plexus (star) and the bladder mesentery (arrow) remain in situ. Remainder of the MGU highlighted in green. Reprinted from: (12) with permission from Elsevier, Lancet Oncology.

Inspired by the theory of Höckel et al. and the TMMR, a modification of the Leiden nerve sparing radical hysterectomy (13) was developed in our centre. This technique was called the ‘Swift’ operation and is described in detail in chapter 3 of this thesis.(14) We do not refrain from adjuvant radiotherapy in case of positive pelvic lymph nodes because lymphadenectomy is performed less extensive and not with therapeutic intentions.

Höckel et al. described the MGU and its borders in their papers on the TMMR.(2;12) The proximal and intermediate parts of the MGU are well known anatomical structures that are easily recognizable and bounded. The distal part of the MGU is located subperitoneally and consists of the cervix, proximal vagina, and a vascular and ligamentous mesometrium (see Figure 3). These mesometria are more complex.

The vascular mesometrium contains the uterine artery and veins, lymphatic vessels, a few small lymph nodes, uterovaginal branches of the autonomic pelvic nerves, loose connective tissue, and usually small amounts of fatty tissue. It adheres to the bladder and the bladder mesentery antero-laterally. The ureters cross through the vascular mesometrium.

The ligamentous mesometrium corresponds to parts of the posterior broad ligaments, uterosacral ligaments, rectouterine and rectovaginal ligaments, and rectovaginal septum. It is a three-dimensional structure of dense connective tissue running in a dorsal and inferior direction. It is horseshoe-shaped in the transverse plane and runs up to halfway the circumference of the rectum on both sides, and follows the pelvic curvature sagittally. The fibro-fatty retroperitoneal and subperitoneal tissue of this part of the distal MGU is medially fused with the anterior and lateral mesorectum continuous with the endopelvic fascia overlying the coccygeus muscles, and laterally with the hypogastric nerve and the inferior hypogastric plexus.(2;12)

Ventrally the MGU has to be separated from the bladder mesentery. According to Höckel, the distal part of the MGU is covered by a continuous bordering lamella, which distinguishes it from the adjacent structures and enables its complete surgical resection. The tissue composition of the mesometrium differs from a true ligamentous structure, and due to the thin bordering lamella the mesometrium can be separated from the superior part of the bladder mesentery. This bordering lamella is reported to be visible between the different parts of the vesicouterine ligament in the female foetuses (7) and on H&E stained sections of TMMR operation specimens (12). There are venous anastomoses between the bladder mesentery and the vascular mesometrium that crosses the ureters at the anterolateral surface of the MGU.(2;12)

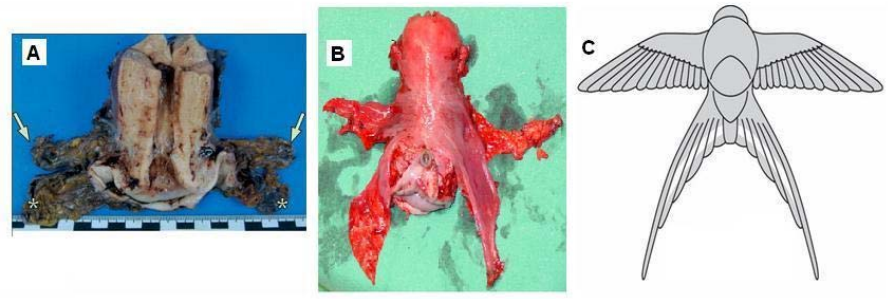


Figure 3 (A) TMMR specimen. Arrows indicate the vascular mesometrium, stars the ligamentous mesometrium. Reprinted from: (12) with permission from Elsevier, Lancet Oncology. (B) Operation specimen of the Swift procedure, resembling the shape of a (C) Swift (bird). The vascular and ligamentous mesometrium are pictured as the tail and wings of the Swift. Reprinted from: (14) with permission from Gynecological Surgery.

Enabling TMMR

Since according to Höckel the MGU has to be excised completely and unnecessary damage must be prevented, recognizing the borders of the MGU during surgery is essential. The oncologic surgeon has to operate meticulously according to the borders of the MGU. The first requirement for complete removal of the MGU is the clear visualisation of the MGU during surgery, or the presence of anatomical landmarks to serve as a beacon. Furthermore, if a surgical approach is justifiable as long as the tumour is confined to the MGU, pre-operative evaluation of the localisation of the primary tumour could select patients with FIGO stage IIB cervical tumours, who are eligible for surgery.

Visualising the MGU

The translation of theory and embryological findings into daily practice can be perilous. Höckel et al. provided embryological sections (e.g. Figure 1), graphics (e.g. Figure 2), per-operative pictures, and MR images (see Figure 4) to illustrate the extension of the MGU.

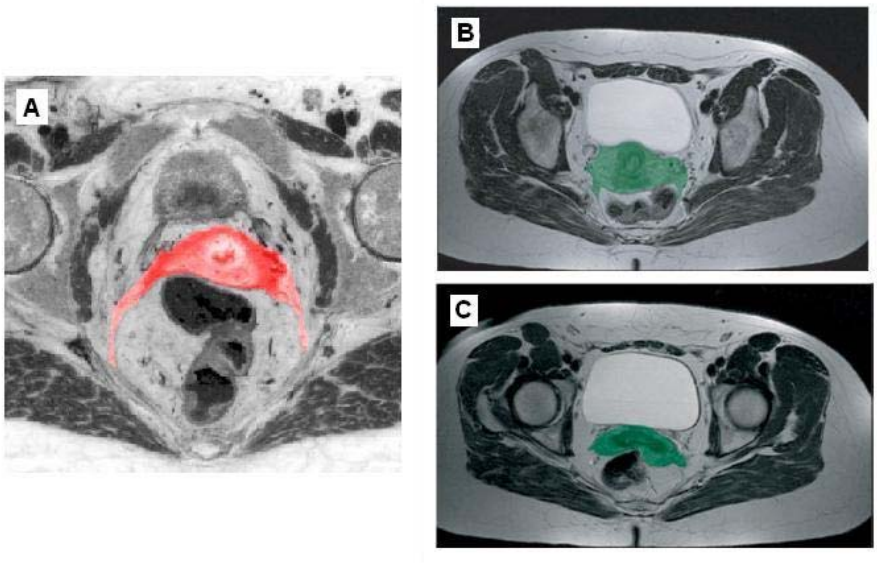


Figure 4 MRI scans with the Müllerian compartment highlighted in red (A) (reprinted from: (8) with permission from Der Pathologe) and green (B,C) (reprinted from: (12) with permission from Elsevier, Lancet Oncology); (A and B) transverse at the level of the cervix and (D) transverse at the level of the proximal vagina

Recently, Touboul et al. described an anatomical study of the parametrium by laparoscopy and cadaver dissection. Their findings were consistent with the anatomical model of Höckel et al. Touboul et al. also found specific signals on MR images of the parametrium, and found the connective tissue of the parametrium to extend in accordance with Höckel's description of the supra- and infraureteral parametrium (15). Whether the areas with the distinct signal on MRI could be sharply separated from the surrounding tissue, and had a clear border, was not described.

MRI is the visualization modality of choice for the cervical area (1) and thus for the MGU, and possibly to determine pre-operatively whether a cervical tumour is localised within the borders of the MGU. Cervical cancer patients might have a disturbed anatomy due to the tumour. Since we first wanted to see whether the MGU was visible at all, our study subjects were healthy pre-menopausal women. Our hypothesis was that the horse-shoe like shaped part of the MGU (tails of the Swift) that partly surrounds the rectum was likely to be visible on a high resolution MRI scan. Our imaging could provide further evidence for the theory of Höckel et al.

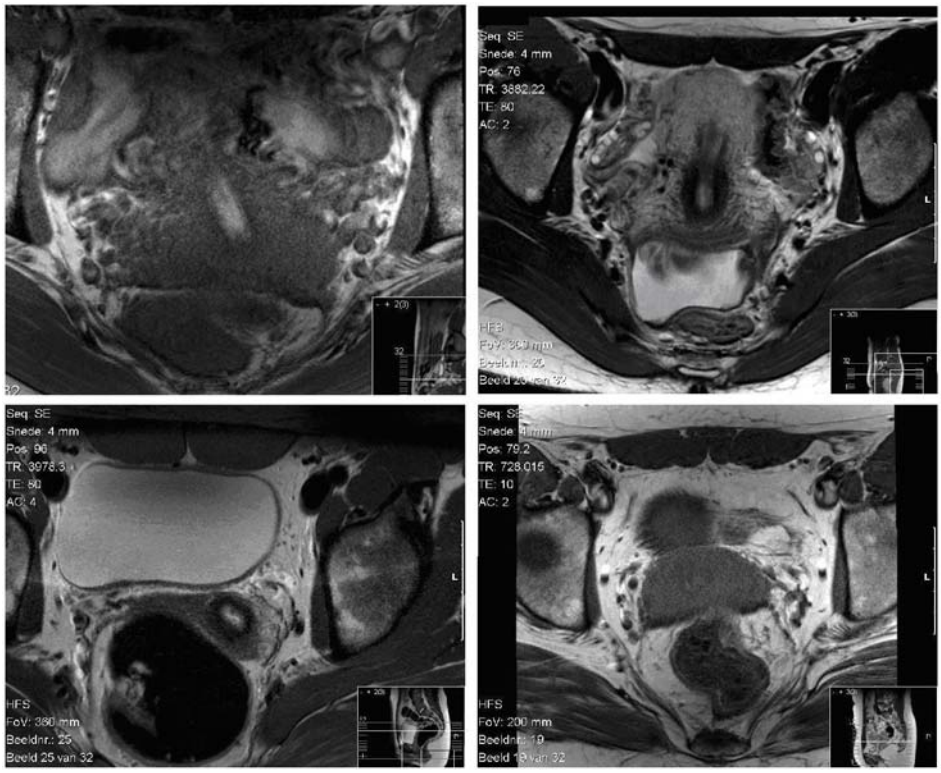
Findings

The MGU on MRI

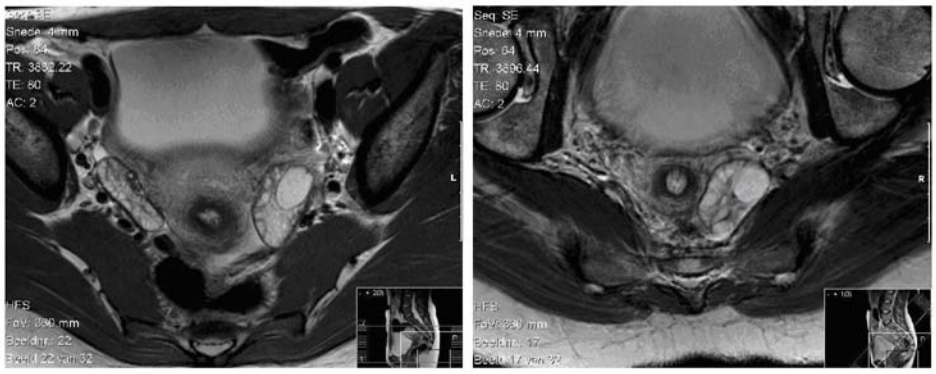
Fifteen healthy pre-menopausal female volunteers were scanned on a 3 Tesla MRI. (Permission was obtained from the Medical Ethical Committee of the LUMC and from all volunteers.) The 3 Tesla MRI has a good resolution and an acceptable level of disturbance. Subsequent T2 weighted images in transverse and oblique directions were viewed.

With the embryological images of Höckel and Fritsch in mind, we could distinguish a horseshoe-shape that was described as the shape of the MGU in the dorsal plane (ligamentous mesometrium, tails of the Swift). Such a shape, alike the shape of the MGU as indicated on Figure 4 (B) of Höckel was present in most women, on one or both sides. However, it was difficult to define borders of this area that might represent the MGU. The lining of the horse-shoe-shaped part that runs laterally from the cervix to half way the circumference of the rectum is hard to follow. It is not clear which of the different lines might represent the end, or a bordering lamella, of the MGU. And it was not clear whether these structures, if present, ran up to halfway the rectum. See Figure 5 (A). In women with an ovary in Douglas, a structure runs clearly from the cervix around the cavum Douglasi. Whether this structure would come to halfway the circumference of the rectum in a normal situation is not possible to say with this distorted anatomy. See Figure 5 (B). In most women it seemed that the structures that formed the medial and ventral part of the horseshoe-shape were vessels rather than ligaments or lamellae. See Figure 5 (C). But in other women, thin structures that might be ligaments were visible, although frequently more than one of these lines were present. See Figure 5 (D). In some images, the grey tint of the fat seemed to differ between the mesorectum and the area surrounding the cervix, but this fat pad usually continued towards the bladder and did not seem to be restricted to a cervical MGU. See Figure 5 (E). In some women, we did not see horseshoe-shaped tail-like structures running towards the mesorectum at all. The differences between the individuals were numerous.

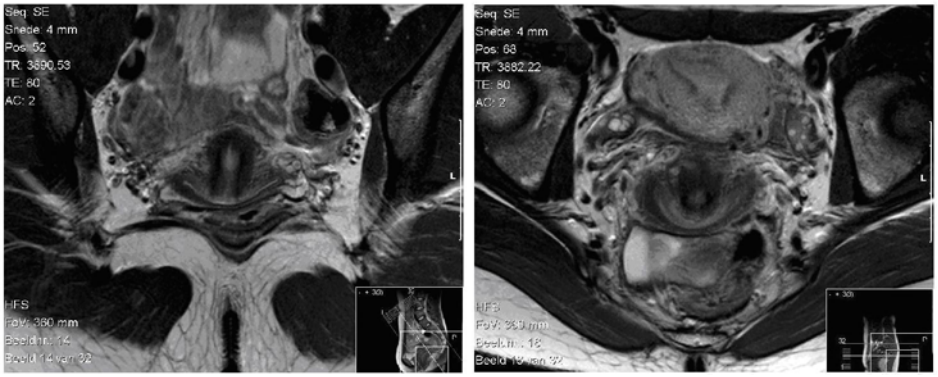
One of the reasons for these differences may be the differences in fat disposition among our volunteers who were, in general, thin. Another reason might be that the angle of the MR images was not always in the direction of the MGU. It would not be easy to aim this angle because the MGU is a real three-dimensional structure with various axis angles in all directions.



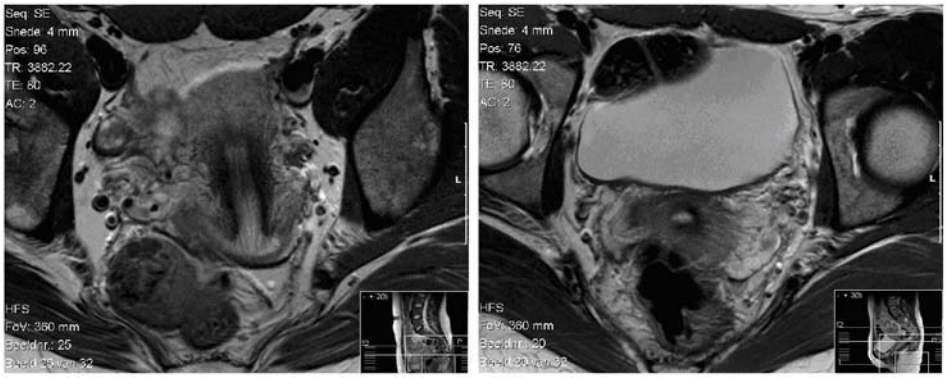
A Horseshoe-shaped structure running from the cervix towards the rectum



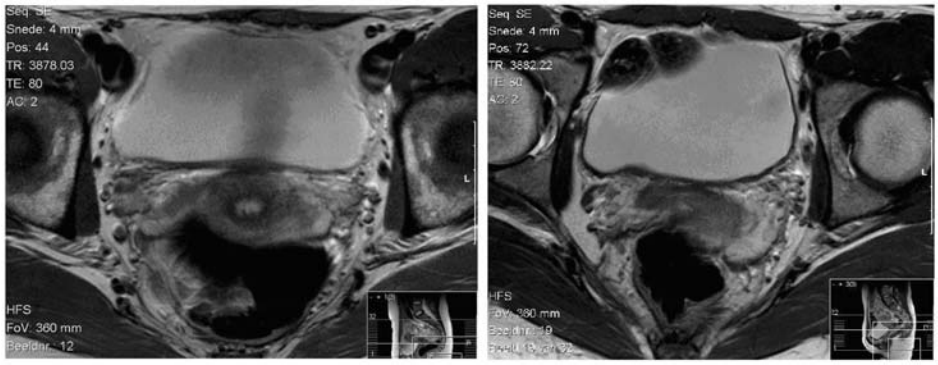
B Clearly ‘wing-like’ structures from cervix in a lateral direction surrounding the rectouterine pouch



C Mesometrium, or vessels?



D Which line is a bordering lamella?



E Fat pad near cervix has a different grey tint, but runs towards the bladder instead of confined to the cervical ‘MGU’

Figure 5 T2 weighted MR images of the pelvis (area of the MGU) of healthy female volunteers in transverse and oblique directions

The MGU during TMMR / Swift

Concerning the practical application of the theory of Höckel in surgical practice, we have developed the Swift operation.(14) During this procedure that resembles the TMMR we have assured the removal of the MGU as described by Höckel et al. The practice of defining the exact border of the morphogenetic units of the uterine cervix can sometimes be more complicated than the theory suggests. Below we will describe our experiences with the TMMR during the Swift operation.

During radical hysterectomy the vascular and ligamentous mesometrium are the most challenging parts of the distal MGU. Of those, the dorsal part is the easiest part. After localizing the hypogastric nerve fibres at the inner site of the peritoneal leaf adjacent to the rectum, it is always possible to start the dorsal resection halfway the circumference of the rectal tube. Taking this as a starting point it is easy to define the lines of resection close to the rectum medially and medial to the lateralized nerve fibres on the other side. Working stepwise in a ventro-distal direction the ligamentous mesometrium of the morphogenetic unit can easily be distinguished from adjacent tissue and resected radically.

The most lateral part of the resection is also clear. The point where the uterine vessels originate from the pelvic side wall is always clear, and one should realize that the uterine artery almost invariably branches from the superior vesical artery rather than from the hypogastric artery. This part of the specimen, the vascular mesometrium, has a clear dorsal border in the underlying ureter although the resection of the deep uterine vein is more dorsal (and lateral) to the course of the ureter.

The difficult part is defining the border between the distal part of the mesometrium and the bladder mesentery. Especially in obese patients these tissues often seem to fuse and there can be cumbersome bleeding from transversing veins that can obstruct the view of the bordering lamellae and hamper a clear assessment of the border between the mesometrium and the bladder mesentery. During surgery there are surprisingly few anatomical landmarks in this area to guide the surgeon and variation in radicality or completeness of the MGU resection is quite possible during this part of the operation.

In defining specific tissue specimens for surgical resection there are two ways of recognition. The first is to see the borders of the particular area. Removing a whole organ is the best example of this. Unless the uterus is grossly affected by outside disease, appreciating the exact borders of the organ is easy and therewith the exact removal of all uterine tissue, nothing more and nothing less. In TMMR, this is the case with the proximal and intermediate parts of the MGU. The second is the use of anatomical landmarks of adjacent areas. Talking about the complete removal of the

cervical MGU the-halfway-circumference-point of the rectum is such a landmark. The rest of the dorsal and lateral resection of the MGU is guided by clearly identifiable borders of this tissue. The absence of borders or anatomical landmarks in the transitional area between mesometrium and bladder mesentery can be a problem. Empirically it seems that this problem is most prominent in the very skinny or very obese patient, but in some patients these parts seem to ‘fall apart’ naturally indicating that a true bordering lamella does actually exist in that area.

Concluding

We were not able to provide absolute imaging evidence for the theory of the MGU, but we have found indications, as others did (Touboul et al.) of an anatomical support of Höckel’s hypothesis. It should be recognized that the absence of indisputable borders, linings and anatomical landmarks do not hamper adequate surgery. Every experienced surgeon knows about gross anatomical variations among individual patients. Therefore, the logical desire to operate along lines of clearly visible borders or landmarks should be no reason to abandon useful or necessary surgery. The theoretical argumentation of the TMMR is extensive and the results of Höckel et al. with respect to survival are convincing. Justification of the wide spread implementation of the TMMR and especially abandoning radiotherapy for patients with high risk histopathological features when the tumour is confined to the MGU might be premature based on existing evidence.(16-18) The best proof to support Höckel’s theory would be an improvement of recurrence and survival results in a randomised controlled trial, which takes the extensive lymph node dissection into account. However, it will take years to enable a reliable survival analysis. In the meantime, we will continue to perform the total excision of the MGU as good as possible since the theory is very much plausible, there are no opposite effects of this procedure, and nerve-sparing is an integral part of the operation.

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Chapter 6

Patterns of parametrial involvement in radical hysterectomy specimens of cervical cancer patients

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Abstract

A tumour in the parametria, either continuous with or separate from the primary malignancy, is an unfavourable prognostic factor in cervical cancer. The incidence of a parametrial tumour localized in blood or lymph vessels, or in tissue, and the relationship of these involvement patterns with pathologic characteristics and prognosis were investigated. Seventy-nine of 763 surgically treated cervical cancer patients (10%) had a tumour in the parametria in hysterectomy specimens. The available patient material was reviewed to discriminate between continuous and discontinuous parametrial tumour growth. The involvement pattern for discontinuous growth was specified on the basis of immunohistochemical staining with different specific markers. Fifty percent of the parametrial tumour involvement found postoperatively was caused by continuous extension of the primary process into the parametria. In the other 50%, the parametrial tumour was separate from the primary process. In this discontinuous group, we found a frequent presence of tumour in the lymph nodes and/or lymph vessels (together 79%) and even a rare appearance of tumour in the blood vessels (14%). A tumour was further found in unspecified vessels in 2 patients (5%), and as isolated foci in 6 patients (14%). Fourteen patients (33%) had more than 1 involvement pattern. Positive pelvic lymph nodes were more frequent in the discontinuous group. The involvement pattern was no independent predictor of overall survival. Parametrial blood vessel involvement was related to the development of distant metastases. The majority (79%) of parametrial involvement in the discontinuous group is caused by lymphatic metastases. Parametrial blood vessel involvement might be an independent predictor for the development of distant metastasis.

Introduction

Cervical tumours extend into the parametria in a proportion of patients. Parametrial involvement of radical hysterectomy specimens from cervical cancer patients has been shown to be an independent unfavourable prognostic factor for disease-free survival (DFS) and overall survival (OS), and necessitates adjuvant therapy.(1–4) During the pathologic examination, a tumour in the parametrium is found to be continuous with or separate from the primary process. The parametria are a part of the cervical plexus drainage area.(5;6) Besides the direct spread by continuous growth, the tumour may spread to the parametria as a result of lymphovascular space invasion (vaso-invasion). It has been postulated that tumour cells, individually or as part of a collective, might migrate toward certain parts of the parametria and that these “occult tumour cells” can form focal metastases.(7) Cervical cancer has a lymphatic metastasizing pattern. Haematogenous-spread metastases are seen infrequently.

The frequency of parametrial tumour in different types of vessels (blood or lymph vessels) or tissue, and whether these different involvement patterns have a different impact on prognosis, is unknown. Patients with a tumour in the lymph vessels might have a different postoperative course of disease than patients with a tumour in the blood vessels, and might need a different treatment approach.

Several reports mention the processing of giant sections, including the parametria, to examine involvement of the parametria in cervical cancer(1;8–12), often with emphasis on the parametrial lymph nodes.(1;7;9). These studies were carried out using haematoxylin and eosin (H&E) staining, and therefore were not able to differentiate lymph vessels from blood vessels.

In this study we specified and quantified the share of the different involvement patterns of tumour in the parametria on the basis of immunohistochemical staining techniques. Furthermore, we related the different involvement patterns and their clinical and pathologic characteristics to postoperative survival.

Materials and methods

Patients

Since 1984, all cervical cancer patients who were treated at the Department of Gynaecology at the Leiden University Medical Center were registered in a database and were prospectively followed. From this database, we extracted the information of all patients who had surgery as the primary treatment for cervical cancer (squamous cell carcinoma and adenocarcinoma) during the period 1984 to 2008. Clinical data and

pathology reports were reviewed. Patients who had a tumour in the parametria at pathologic examination were included in the study. The included patients did not receive neoadjuvant chemotherapy or radiotherapy.

Treatment

All the patients were evaluated preoperatively using the standard staging procedure, which included a complete physical and gynaecologic examination (conducted under anaesthesia if necessary), routine blood and urine analysis, chest radiography, and ultrasound to exclude ureteral dilatation. At our centre, the standard treatment for cervical cancer stage <=2a, and for stage 2b if technically feasible, was radical abdominal hysterectomy and pelvic lymphadenectomy.(13) The procedure was converted to a less radical procedure in case of an advanced tumour spread outside the cervix necessitating postoperative radiation. This procedure consisted of hysterectomy without radical removal of the whole parametrium in case of evident macroscopic parametrial involvement or lymph node involvement proven by frozen section investigation. In these cases, the proximal parametrium was excised and if the procedure was converted before lymphadenectomy, enlarged or macroscopically suspected lymph nodes were removed. The surgical specimens were examined by a pathologist. The parametria were fixed immediately in formalin and separately embedded in paraffin blocks. The blocks were stored in the archives at the Department of Pathology.

Methods

The original H&E-stained slides of the parametria were retrieved from the archive. These and the pathology reports of the patients were reviewed by a pathologist (G.J.F.) to distinguish whether the tumour growth in the parametrium was clearly continuous or discontinuous. In case of discontinuous involvement, 8 consecutive slides were cut from the original tissue blocks, and immunohistochemical staining was performed on freshly cut, 4-µm thick, buffered, formalin-fixed, paraffin-embedded tissue sections according to standard procedures. The slides were incubated overnight with mouse monoclonal antibodies as follows: (1) anti-cytokeratin AE1/AE3 [AE1-AE3, NeoMarkers Inc, 1:400, citrate antigen retrieval (AR)] to detect epithelial cells and determine their exact location in the tissue; (2) anti-calretinin (5A5, Novocastra, 1:160, citrate AR) to distinguish mesothelial cells from tumour; (3) anti-CD31 (JC70/A, Dako, 1:200, citrate AR), (4) anti-CD34 (Q-bend/10, NeoMarkers Inc, 1:200, citrate AR) to detect endothelial cells (vessel walls); (5) anti-D2-40 (D2-40, Signet, 1:80, citrate AR), which is a specific marker for lymphatic endothelium, to differentiate lymph vessels from blood vessels; and (6) anti-SM-actin (ASM-1, Progen, 1:800) to detect smooth muscle layers. In addition, H&E staining was performed.

If not all tumour-containing parametrial blocks were available for staining, classification of these cases was, as far as possible, based on the pathology reports and original H&E slides.

All the stained slides were viewed by a pathologist (G.J.F.). The tumour involvement in the parametrium was classified as either in lymph nodes, lymph vessels, blood vessels, unspecified vessels, or independent of other structures in the tissue (not further determined, apparently isolated “foci”). All the involvement patterns in each patient were recorded. Clinical and pathologic characteristics were extracted from the database.

Statistical analysis

Statistical analysis was carried out with the SPSS 16.0 package. The [chi]^2 test was used to test the association between discrete or categorical variables in the univariate analysis, and the t test to compare the mean values. We chose a significance level of 95%. Odds ratios (OR) were calculated by logistic regression analysis. Cox regression was used to analyse the effect of the different parameters on DFS and OS, expressed with hazard ratios (HR).

Results

Study group

During the period 1984 to 2008, 664 cervical cancer patients received primary treatment with a radical hysterectomy at our centre, and 99 patients underwent a less radical operation. In 79 of 763 patients (10%), a tumour was found in the parametria during surgery or at the pathologic investigation.

Involvement patterns

The general, surgical, and pathologic characteristics of the 79 patients with parametrial involvement are shown in Table 1. Tumour growth in the parametria was caused by direct extension of the primary tumour into the parametria (continuous growth) in 37 of the 79 patients (47%). The tumour cells in the parametria of the remaining 42 patients (53%) lay separately from the primary tumour (discontinuous growth). The distribution of continuous and discontinuous growth was similar in patients who had a radical hysterectomy or a less radical procedure.

In 3 cases not all tissue blocks that contained tumour-positive parametria were available for further processing. In 2 other cases, the newly cut slides did not contain

tumour cells. In only 1 of the 42 patients there were no blocks available at all. The available blocks of the other 41 cases were further processed and all were successfully stained. Two examples of the immunohistochemical staining are shown in Figures 1 and 2.

Table 1 Characteristics of the total group of 79 patients with tumour growth into the parametria; specified in 37 patients with continuous and 42 patients with discontinuous parametrial involvement

	Total group (N=79)		**Continuous (N=37)**		**Discontinuous (N=42)**				
	Mean	**Mn-max**	**Mean**	**Mn-max**	**Mean**	**Mn-max**	**P-value***		
Age	50	25-86	49	25-86	51	26-80	0.67		
Tumour diameter			44	20-80	47	10-110	0.5		
	N	**%**	**N**	**%**	**N**	**%**	**Pvalue***	**OR****	**95%-CI**
FIGO stage									
1a	1	1	0		1	2	**0.049**		
1b	53	67	21	57	32	76			
2a	21	27	15	41	6	14			
2b	4	5	1	3	3	7			
Surgical procedure									
Wertheim	49	62	23	62	26	62	0.981		
Less radical	30	38	14	38	16	38			
Positive lymph nodes	52	66	19	51	33	79	**0.011**	**3.5**	**1.3 - 9.2**
Resection margins									
> 5 mm free	40	51	19	51	21	50	0.91		
< 5 mm free	39	49	18	49	21	50			
Vaso-invasion									
No	12	15	7	19	5	12	0.329		
Yes	65	82	28	76	37	88			
Unknown	2	3	2	5	0				
Histological type									
Squamous	66	84	31	84	35	83	ns		
Adeno	10	13	4	11	6	14			
Not clear	3	4	2	5	1	2			
Tumour diameter									
< 40 mm	35	44	13	35	22	52	0.27		
> 40 mm	36	46	19	51	17	41			
unknown	8	10	5	14	3	7			
Parametrial infiltration									
Continuous gowth	37	47	n/a		n/a				
Not continuous	42	53	n/a		n/a				
Specific infiltration pattern									
In lymph node(s)	nfs		nfs		21	50			
In lymph vessel(s)	nfs		nfs		20	48			
In blood vessel(s)	nfs		nfs		6	14			
In vessel(s)	nfs		nfs		2	5			
Focus (foci)	nfs		nfs		6	14			
Unknown	nfs		nfs		6	14			

CI = confidence interval; n/a = not applicable; nfs = not further specified
* p-value of comparison of distribution of the parameters between the continuous and discontinuous group
** odds ratio of discontinuous versus continuous group

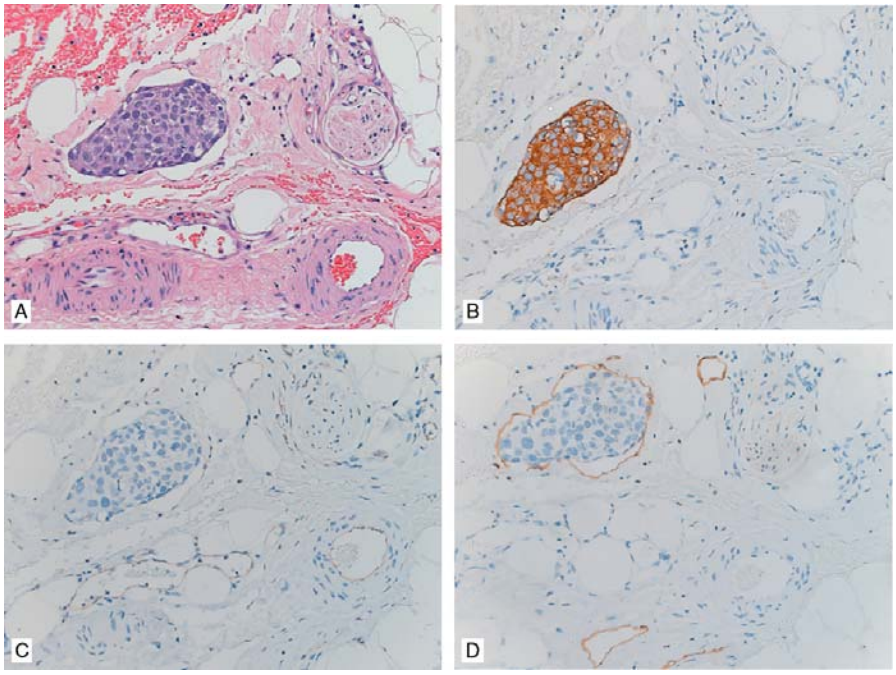

Figure 1 Examples of the staining of different structures in the specimens. The image represents cervical tumour cells in a lymph vessel surrounded by tissue containing blood vessels (magnification: 20 x). (A) Haematoxylin and eosin staining; (B) brown staining of anti-Keratin shows tumour cells; (C) brown staining of anti-CD-31 shows blood vessels; (D) brown staining of anti-D2-40 shows lymph vessels.

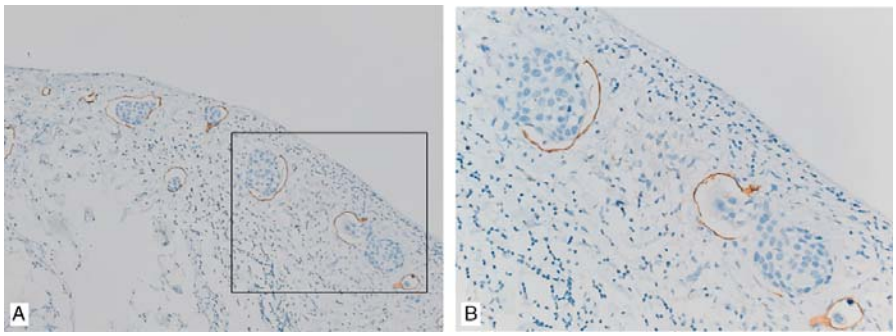

Figure 2 Example of a metastasis of a cervical tumour growing out of a parametrial lymph vessel into the surrounding tissue. (A) Lymph vessel walls are stained brown with anti-D2-40 (magnification: 10 x). The area indicated by the black rectangle is enlarged in (B) (magnification: 20 x).

Table 2 summarizes the overall results of the immunohistochemical staining of the discontinuous group, and Table 3 shows the specification of the simultaneous presence of different tumour manifestations. In 14 patients (33%), more than 1 pattern of tumour growth were present. A tumour was found in the parametrial lymph nodes in 21 patients (50%). For 11 of these (52%) patients, lymph nodes were the only detected mode of tumour spread to the parametria. A tumour in lymph vessels was found in 20 patients (48%). In 9 of these patients (45%), no tumour was found outside the lymph vessels. Blood vessels containing tumour cells were found in 6 patients (14%), and were the only manifestation of tumour in the parametria in 1 case (17%). In 2 patients (5%), the tumour seemed to be localized in a vessel, but the type of vessel could not be deduced from staining. Apparently isolated tumour foci were found in 6 patients (14%), of which 3 patients (50%) had no other tumour manifestation in the parametria. In 6 patients (14%), we were unable to deduce the involvement pattern of 1 or more tumour manifestations based on the slides. Lymph nodes or lymph vessels were most frequently involved. The majority of parametrial involvement in the discontinuous group was caused by lymphatic metastases: altogether, 33 of the 42 patients (79%) had a tumour in the lymph system of the parametria (lymph nodes or lymph vessels), and the parametrial tumour was found solely in the lymph system in 20 of the 42 patients (48%).

Table 2 Parametrial tumour spread in 42 patients with discontinuous growth of the primary tumour

	N	**Percentage**
Lymph node(s)	11	26.2
Lymph vessel(s)	9	21.4
Blood vessel(s)	1	2.4
Vessel(s)	1	2.4
Focus (foci)	3	7.1
Unknown	3	7.1
Lymph node(s) and Lymph vessel(s)	6	14.3
Lymph node(s) and Blood vessel(s)	1	2.4
Lymph node(s) and Unknown	1	2.4
Lymph vessel(s) and Blood vessel(s)	2	4.8
Lymph vessel(s) and Focus (foci)	1	2.4
Blood vessel(s) and Focus (foci)	1	2.4
Lymph node(s) and Lymph vessel(s) and vessel(s) and Unknown	1	2.4
Lymph node(s) and Lymph vessel(s) and Blood vessel(s) and Focus (foci) and Unknown	1	2.4
Total	**42**	**100**

Table 3 Specification of the simultaneous presence of different manifestations of tumour in the parametria of 42 patients without continuous growth of the primary tumour

		Lymph node(s) N=21	Lymph vessel(s) N=20	Blood vessel(s) N=6	Vessel(s) N=2	Focus (foci) N=6	Unknown N=6	No other manifestations N=28
Lymph node(s)	N	X	8	2	1	1	3	11
N=21	% within Lymph nodes	X	38%	10%	5%	5%	14%	52%
Lymph vessel(s)	N	8	X	3	1	2	2	9
N=20	% within Lymph vessels	40%	X	15%	5%	10%	10%	45%
Blood vessel(s)	N	2	3	X	0	2	1	1
N=6	% within Blood vessels	33%	50%	X		33%	17%	17%
Vessel(s)	N	1	1	0	X	0	1	1
N=2	% within Vessels	50%	50%		X		50%	50%
Focus (foci)	N	1	2	2	0	X	1	3
N=6	% within Focus	17%	33%	33%		X	17%	50%
Unknown	N	3	2	1	1	1	X	3
N=6	% within Unknown	50%	33%	17%	17%	17%	X	50%

Characteristics and survival of patients with continuous and discontinuous growth

General, surgical, and pathologic characteristics of the 37 patients with continuous and the 42 patients with discontinuous growth in the parametria are shown in Table 1. The comparison showed that the distribution of the FIGO stages was significantly different between the continuous and discontinuous groups (P=0.049). FIGO stage 2a was more frequent in the group with continuous growth (41% vs. 14%), whereas stages 1b and 2b were more frequent in the discontinuous group (76% vs. 57% and 7% vs. 3%, respectively). The mean tumour diameters were similar in the continuous (44 mm; range: 20–80) and discontinuous (47 mm; range: 10–110) groups (p=0.5).

Tumour-positive pelvic lymph nodes were present significantly more often in the discontinuous group [79% vs. 51%; OR: 3.5; 95% confidence interval (CI): 1.3–9.2]. Tumour diameter of more than 4 cm seemed to be more frequent in patients with continuous growth, but the difference was not significant (59% vs. 44%; p=0.270). The histological type was comparable in the continuous and discontinuous groups.

Follow-up data are shown in Table 4. Of the patients with available follow-up data, 60% of patients with discontinuous growth had a recurrence during follow-up compared with 43% of patients with continuous growth. This difference was not significant (p=0.269). The mean DFS was 27 months in the continuous group and 33 months in the discontinuous group, and the OS was 48 months in the continuous group and 46 months in the discontinuous group. Regression analyses for DFS of all patients with positive parametria (with the factors discontinuous or continuous growth, age, surgical procedure, resection margins, vaso-invasion of the primary tumour, tumour diameter of more than 4 cm or less than or equal to 4 cm, histological type, and pelvic lymph node status) showed that tumour diameter of more than 4 cm (HR: 2.8; 95% CI: 1.2–6.3) and resection margins that were not tumour free (HR: 2.3; 95% CI: 1.03–5.2) were independent predictors of disease recurrence in the multivariate analysis.

At least 52% of patients with discontinuous growth died of cervical cancer during the study period and 14% died of other causes compared with 38% of deaths from cervical cancer and 11% from other causes in the continuous group. In the multivariate analysis for OS, resection margins that were not tumour free (HR: 2.2; 95% CI: 1.03–4.7) and histological type adenocarcinoma (HR: 3.7; 95% CI: 1.7–10.8) were independent prognostic factors for death. Multivariate analysis of survival considering only death from cervical cancer as an event showed no independent prognostic factors.

Table 4 Follow-up data of patients with continuous and with discontinuous parametrial involvement

	Continuous (N=37)		Discontinuous (N=42)		
	Mean	**Min-max**	**Mean**	**Min-max**	**P-value**
Follow-up duration (mo)					
DFS	27	0-131	33	0-232	0.57
OS	48	0-256	46	0-241	0.839
	N	**%**	**N**	**%**	
< 2 years complete DFS data	10	27	7	17	
< 5 years complete OS data	13	35	7	17	
	N	**%**	**N**	**%**	
DFS					
Disease free	19	51	14	33	0.269
Disease recurrence	16	43	25	60	
Unknown	2	5	3	7	
OS					
Alive	18	49	13	31	
Died of cervical cancer	14	38	22	52	
Died of other disease	4	11	6	14	
Unknown	1	3	1	2	

DFS = disease-free survival; OS = overall survival

Characteristics and survival analyses of patients with different patterns of discontinuous growth

Patients with a tumour in the parametrial lymph nodes or lymph vessels had tumour-positive pelvic lymph nodes more often than patients with a parametrial tumour not located within the lymph system, but this difference was not statistically significant (82% vs. 67%, OR: 2.3; 95% CI: 0.4–11.6).

Multivariate survival analyses for DFS and OS in the discontinuous group (with the factors parametrial lymph system involvement, age, surgical procedure, resection margins, vaso-invasion of the primary tumour, tumour diameter of more than 4 cm or less than or equal to 4 cm, histological type, and pelvic lymph node status) showed tumour diameter of more than 4 cm (HR: 9.2; 95% CI: 2.3–36.0) and histology type adenocarcinoma (HR: 5.6; 95% CI: 1.1–29.3) as independent prognostic factors for disease recurrence. Both the factors were also the only independent predictors of death in the OS analysis (HR tumour diameter >4 cm 3.4; 95% CI: 1.04–11.5 and HR histological type adenocarcinoma 9.0; 95% CI: 2.0–41.1). Multivariate survival analysis, with only death from cervical cancer as an event, showed histological type adenocarcinoma as the only independent prognostic factor (HR: 8.0; 95% CI: 1.3–50.1).
As only 5 of the 42 patients (12%) had a primary tumour that did not show vaso-invasion, no statistically significant relationship between vaso-invasion and the different involvement patterns was found.

Four of the 6 patients (67%) with parametrial blood vessel involvement developed a distant metastasis during follow-up compared with 11 of the 36 patients (31%) without blood vessel involvement. In the multivariate regression analysis, with parametrial blood vessel instead of lymph system involvement and the earlier mentioned factors,

blood vessel involvement was the only independent prognostic factor for the development of distant metastasis (HR: 12.7; 95% CI: 1.6–103.3). Parametrial blood vessel involvement was not an independent predictor of overall disease recurrence (HR: 4.1; 95% CI: 0.99–16.6) or survival (HR: 5.1; 95% CI: 0.99–20.2).

Discussion

In our patient population, one-half of the parametrial tumour involvement found postoperatively was caused by continuous extension of the primary process into the parametria. In the other half, the tumour in the parametrium was separate from the primary process. In this discontinuous group, we showed the frequent presence of a tumour in lymph nodes and/or lymph vessels (79%) and even a rare appearance of a tumour in blood vessels as expected based on the known metastasis pattern of cervical cancer.

The frequencies in the blood or lymph vessel group might be even higher than we have shown because the small group of patients with tumours that seemed to be independent of other structures might consist of true tumour “foci,” but could also belong to the blood or lymph vessel group. As the foci were often relatively large, they might consist of tumour that has grown out of a vessel and completely destroyed the vascular endothelium; vessel walls were no longer recognizable on the slides.

The different involvement patterns in this study can be compared with the findings reported in the literature to a certain extent. Burghardt and Pickel (11) reported 24 tumour-positive parametria in 150 patients (16%; compared with 10% in our study), of whom 6 (25%) had continuous growth (compared with 47% in our study). Of the 18 patients (75%) with discontinuous growth, lymph nodes were involved in 6 patients (33%; compared with 50% in our study) and lymph or blood vessels in 12 patients (67%; compared with 67% in our study). In another study, Burghardt et al. (8) reported 86 of 359 patients with positive parametria (24%), of whom 24 patients (28%) had continuous growth. Candido et al. (12) found 12 of 30 patients (40%) with positive parametria, and the parametrial involvement was due to continuous growth in 9 of them (75%).

However, our findings are not fully comparable with all the figures reported in the literature for 2 main reasons. First, in the reported studies, blood vessels are not mentioned separately at all, or lymph and blood vessels are counted together. Immunohistochemical staining enabled us to differentiate between these 2 types of vessels. Second, we did not perform stepwise sectioning and staining of the entire parametrium because this material was not available from our patients. As one-third of our patients in the discontinuous group had more than 1 involvement pattern, we did not exclude the fact that other patterns or areas of microscopic continuous growth

might be found in the parametria of the discontinuous group after deeper sectioning. Furthermore, of the patients who had less radical surgery because of per operatively found lymph node involvement, only the proximal parametrium was investigated. We performed no additional staining of the parametria in which continuous growth was shown.

We compared clinical and pathologic characteristics of the patients with different involvement patterns and related them to the patient's postoperative survival. The difference in the FIGO stage between the continuous and the discontinuous group was reflected by the tendency toward a higher frequency of patients with a tumour diameter more than 4 cm in the continuous group. This difference was not statistically significant, but it seems valid that larger tumours more frequently extend into the vagina and the parametria. Although additional lymph system involvement might be present in the parametria of the continuous group, the significantly more frequent presence of positive pelvic lymph nodes in the discontinuous group seems to be understandable. Although recurrent disease and death (from cervical cancer) was more frequent in the discontinuous group, the Cox regression analysis showed that there was no independent relationship between the survival and continuous or discontinuous growth.

In accordance with the relationship between discontinuous involvement patterns and pelvic lymph nodes, tumour-positive pelvic lymph nodes tended to be more frequent in patients with parametrial lymph system involvement compared with patients without parametrial lymph system involvement in the discontinuous group. However, this difference was not statistically significant. The small number of patients with a primary process that did not show vaso-invasion hampers interpretation of the figures on that aspect in the discontinuous group.

Parametrial blood vessel involvement was an independent predictor for the development of distant recurrences, but with respect to general DFS and OS, the involvement patterns were not independent prognostic factors.

Tumour diameter more than 4 cm, resection margins containing tumour, and histological type adenocarcinoma (compared with squamous cell carcinoma) showed to be independent prognostic factors for DFS and/or OS in the whole group and/or in the discontinuous group. Unlike histological type, tumour diameter and resection margins that are not more than 5 mm free of tumour are known prognostic unfavourable factors that are considered in adjuvant treatment planning. Recently, a large study showed a worse DSF and OS after radical hysterectomy for histological type adenocarcinoma compared with squamous cell carcinoma.(14) Our results are in accordance with this finding. Our patient group was too small to analyse different involvement patterns and their relationship with survival in different groups of tumour diameter, resection margin status, and histological type.

In our study, we used the term “metastasis” for the tumour found in the parametrium. It might be argued that we could have used “spread” or “involvement” instead, because the significance of the tumour emboli is unknown (we found no survival differences). However, a tumour found outside a primary tumour, no matter how small, is usually referred to as a metastasis.

On the basis of our study, involvement patterns seem to fit into the known biological mechanisms of cervical cancer spread, and do not seem to be an independent predictor of postoperative survival. There are, however, 3 outcomes of this study that could be of clinical relevance:

The parametria are assessed preoperatively by physical examination and, if necessary, using imaging techniques. In some patients who are preoperatively staged as having no parametrial involvement, a tumour in the parametria during pathologic investigation can surprise the surgeon. Continuous growth was regularly identified in patients that were staged as stage 1b or 2a, which underlines the difficult task of staging. However, the finding that one-half of the parametrial involvement is caused by tumour deposits separate from the primary process, which might not be palpable explains a large part of the discrepancy between the physical pre- and pathologic postoperative examinations.

Furthermore, although a tumour diameter of more than 4 cm tended to be more frequent in the continuous group, the mean tumour diameter in patients with continuous and discontinuous involvement patterns was similar. The range in diameter was wide in both the groups. For example, 1 patient with a tumour only 10 mm in diameter had a tumour in the parametrial lymph system. We found no characteristics that might help to preoperatively identify patients with small tumours who are at risk for parametrial continuous or discontinuous parametrial tumour growth.

Finally, a tumour in parametrial blood vessels was related to the development of distant haematogenous tumour metastases. The survival analyses in the small number of cases with distant metastasis in our study group did not show an independent effect of parametrial blood vessel involvement on DFS and OS. In addition, considering the CIs, blood vessel involvement might, however, prove to be a predictor of DFS and OS in a larger group. Chemotherapy could be considered for this select group of patients.

Some of our findings suggest that the prognosis and best treatment options might be different for the different patterns of parametrial spread. Further investigation in prospective studies is necessary. We showed the very frequent presence of tumour in the lymph system, and the presence of tumour in parametrial blood vessels in parametria that were found to be involved during the pathologic investigation of the surgical specimen. As only a few sections of the parametria are examined during the

regular postoperative pathologic examination, the presence of tumour in parametrial nodes or vessels might be more frequent than currently expected, and a tumour might be present distant from the continuous tumour front. Future studies should include serial sectioning and immunohistochemical staining of the parametria of surgically treated cervical cancer patients to further investigate the frequency of the patterns of tumour spread and their relationship with survival.

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Chapter 7

Abdominal scar recurrences of cervical cancer: incidence and characteristics

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Abstract

Tumour recurrence in the surgical scar after radical hysterectomy for cervical cancer has been reported, but the incidence is unknown. Facts about patient and tumour characteristics and follow-up are lacking. The objective of this study was to analyse the incidence and characteristics of cervical cancer scar recurrences.

All patients who were surgically treated for cervical cancer in our centre between 1984 and 2007 were reviewed for scar recurrences. For each case, 5 random controls were selected. Clinical characteristics were compared between the cases and controls.

Eleven (1.3%) of 842 patients developed a scar recurrence. Mean time between surgery and scar recurrence was 16 months (range, 2-45 months). For 8 patients (73%), the scar recurrence was the first disease recurrence. Five patients (45%) died, and 2 (18%) were lost to follow-up. Mean time between scar recurrence and death was 9 months. Ninety-one percent of the cases had recurrent disease besides the scar recurrence during follow-up. The case group had a higher percentage of advanced FIGO stage and postoperatively found involvement of parametria or resection margins and tumour diameter greater than 4 cm, whereas lymph nodes were more often involved in the control group.

The incidence of scar recurrences after primary surgery for cervical cancer was 1.3%. Time to development was variable, and prognosis was poor. Besides higher FIGO stage and concurrent unfavourable pathological characteristics, we found no outstanding characteristics of patients with scar recurrence. Scar recurrences go hand in hand with recurrent disease at other locations and seem a manifestation of tumours with extensive metastatic potential.

Introduction

Cervical cancer mainly spreads via the lymphatic pathway. Haematogenous metastases are uncommon and occur mainly in the liver, lungs, and bones. Skin metastases are rare, with an incidence ranging from 0.1% to 1.3%, (1,2) and scar recurrences seem to be even more uncommon. Scar recurrences can be localized in a laparotomy scar (including caesarean section site) or at a laparoscopic port site, but also in an episiotomy scar. The mechanism by which scar recurrences develop is unknown.

The mechanism for distant metastases was described by the "seed and soil" theory.(3) Cells can spread through the body via blood or lymph vessels. Although most circulating cells do not survive, a small amount of cells could develop into a metastasis. Not all malignant cells possess the characteristics necessary for metastasizing. Changes necessary to feature this process are thought to develop over time. Certain tumour types have a tendency to metastasize to specific organs, indicating that sites of metastasis are determined by both the characteristics of the neoplastic cells and the microenvironment of the host tissue.(3)

Healing of a surgical wound involves an inflammatory reaction of the tissue. Tissue trauma and subsequent inflammation create a favourable environment for implantation and growth of malignant cells.(3-5) This process is called inflammatory oncotaxis.(6) During the healing process, an increased permeability of the vessel wall can enhance the movement of malignant cells into the wound site. In addition, the clumping of leukocytes, platelets, and fibrin might cause circulating neoplastic cells to be trapped in arterioles or capillaries. The clot provides a barrier and nutrition. Tumour cells might travel to the wound by lymphatic or haematogenous pathways, spill during the operation, or spread through the abdominal cavity in the peritoneal fluid.(3;7)

By combining the concepts of seed and soil and inflammatory oncotaxis, we can understand why a surgical wound is prone for nestling and growth of both iatrogenic implanted and circulating cervical tumour cells. Despite the plausibility of the theories, scar recurrences are rare. Apparently, only a very small part possesses both the capacities to escape the hosts' immune system and the ability to grow out into a metastasis.

Although numerous case reports on scar recurrences of cervical cancer exist, the incidence is unknown,(8-51) and facts about patient and tumour characteristics and course in large patient populations are lacking. The objective of this study was to evaluate the incidence of laparotomy scar recurrences after surgery for cervical cancer, to analyse patient characteristics, and to compare them with a random sample of all patients who were surgically treated for cervical cancer.

Materials and methods

Patients

Clinical data of all cervical cancer patients who have been treated in the Leiden University Medical Center (LUMC) between 1984 and 2008 were recorded in a database. The source population of our study consisted of all patients who underwent surgery, with the intention to perform on whom a radical hysterectomy or trachelectomy. This group includes inhabitants of Surinam who were surgically treated in our centre between 1989 and 2005. In that period, the LUMC cooperated with Surinam, and surgery for cervical cancer was done in the Netherlands. The data of all the patients were reviewed for tumour recurrence in the abdominal surgical scar. Selection criteria were recurrences in or near the scar, which were visible or palpable on the abdomen, and were cytologically or histologically confirmed as a metastasis of the primary cervical tumour. Metastases in the rectus abdominus muscle that did not grow into the subcutis were not included. Eleven women had a scar recurrence and were selected in our case group. For each case, 5 controls were randomly selected from the source population.

Original treatment

Surgical treatment consisted primarily of radical abdominal hysterectomy (or trachelectomy) and pelvic lymphadenectomy. All procedures were performed by vertical midline incision. The procedure was converted to a less radical procedure called te Linde operation (Rutledge type 1) in case of advanced tumour spread outside the cervix necessitating postoperative radiation. Patients received adjuvant radiotherapy in case of lymph node metastasis, parametrial involvement, or non-radical surgical resection margins (<5 mm free of tumour). Additional inclusion criteria for receiving adjuvant radiotherapy from 1997 onward were presence of at least 2 of 3 of the following unfavourable prognostic factors: vaso-invasion, tumour diameter greater than 4 cm, and invasion depth greater than 15 mm. In individual cases of severe lymph node involvement, platinum-based chemoradiation was offered.

Evaluation

Clinical characteristics and follow-up data of the cases and controls were distracted from the database and medical records. For the cases, the follow-up period lasted from the surgical procedure to the end of the study (2007). For the controls, follow-up was reviewed from the surgical procedure to the first event (recurrence, death, or lost to follow-up) or until the end of the study.

Changes in treatment modalities in the LUMC were evaluated by studying the surgical reports. Archived slides of cytological and histological specimens of the scar recurrences, stained with haematoxylin-eosin, were reviewed by a pathologist (G.J.F.).

Statistical analysis

Statistical analysis was performed with the SPSS 14.0 package (SPSS Inc, Chicago, Ill). Five controls per case were selected with the SPSS function for random selection of cases. For the comparison of the cases and controls, the [chi]2 test was used to test the association between discrete or categorical variables in the univariate analysis and the t test to compare means. We chose a significance level of 95%. Hazard ratios were calculated by logistic regression analysis.

Results

A total of 842 patients underwent surgery as the treatment for cervical cancer. Of those, 11 patients developed a scar recurrence. Accordingly, the incidence of scar recurrences after surgery for cervical cancer was 1.3%. The distribution of the 11 cases over the years is shown in Figure 1.

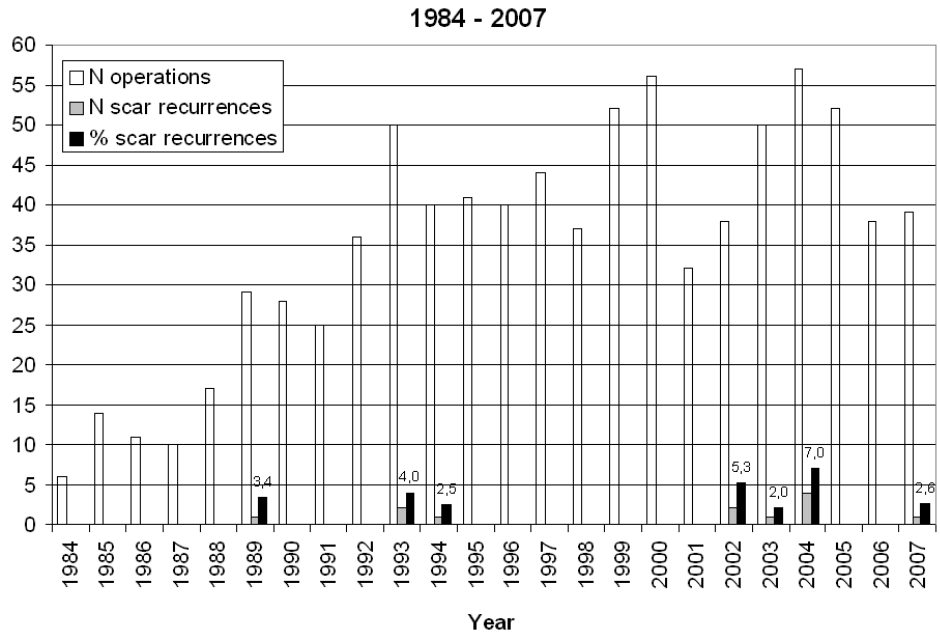

Figure 1 Representation of the distribution of the scar recurrences over the years, and of the numbers in proportion to the total numbers of radical hysterectomies performed in our hospital each year during the studied period (1984-2007)

Possible important changes in treatment modalities were the use of distilled water to track small bleeding vessels to control haemostasis since 1991, no more closing of the visceral or parietal peritoneum in 1999, and stopping the routine application of postoperative drainage, also in 1999. The indication for postoperative radiation was changed in 1997 as described in Methods. Treatment schedules of radiotherapy and chemotherapy changed or were adjusted according to international guidelines. All patients were operated on by the same team of gynaecologic oncologists.

Table 1 shows the general and treatment characteristics of the 11 cases. The age of the patients at primary treatment ranged from 26 to 65 years (mean, 42 years). Four patients (36%) were of Surinamese origin. Scar recurrences were diagnosed in 2.6% of all Surinamese patients and 1.0% of all Dutch patients who were surgically treated for cervical cancer. The FIGO (International Federation of Gynaecology and Obstetrics) stages in the 11 patients with a scar recurrence were 1b1-2b, with 64% at advanced stage (>=1b2).

Eight patients (73%) had a radical hysterectomy, of which one had a debulking operation the next year via the same abdominal incision site, 2 (18%) a te Linde operation, and 1 (9%) had a radical cervical stump resection after a supravaginal hysterectomy for a benign condition in the past. According to the treatment guidelines, the peritoneum was closed in all patients who were operated on before 1999 and not closed in those who had surgery after 1999. Distilled water was used in 6 (60%) of the 10 patients who were treated since its introduction. Operating time ranged from 150 to 220 minutes (mean, 189 minutes). After 5 (46%) of the 11 procedures, a drain was left behind. Seven patients (64%) received adjuvant therapy.

Table 2 lists the findings of the pathological examination of the operation specimens. Nine tumours (82%) were of the squamous cell type, 1 (9%) adenocarcinoma, and 1 (9%) mixed adenosquamous. With respect to unfavourable prognostic factors, 4 scar recurrence patients (36%) had infiltrated parametria, resection margins were less than 5 mm free of tumour in 4 cases (36%), and 2 patients (18%) had positive lymph nodes. Altogether, 5 patients (46%) had at least 1 of these unfavourable prognostic factors. Of the cases without these "hard" factors, 1 case (9%) had 2 or more of the characteristics: tumour diameter greater than 40 mm, vaso-invasion, or infiltration depth greater than 15 mm. Another 2 patients had 1 of these relative disadvantageous characteristics. Three patients (27%) had no known unfavourable prognostic factors.

Follow-up data are shown in Table 3. Mean time between surgery and development of the scar recurrence was 16 months (median, 11 months). For 8 patients (73%), the abdominal wall recurrence was the first recurrence of the disease. These recurrences were diagnosed within 2 years after the surgical procedure. Overall, 55% of the scar recurrences were diagnosed within the first year. Three patients (27%) had been

diagnosed with a recurrence earlier. The scar recurrences of these patients were diagnosed 27 to 45 months after the surgery. Other metastases diagnosed simultaneously with the abdominal wall recurrence were present in 4 patients (36%), of whom one had a non-radical operation. After the scar recurrence, 5 patients (45%) were diagnosed with another metastasis. Three patients (27%) developed no subsequent recurrences.

Table 1 (Pre-) operative characteristics of 11 patients with a scar recurrence

ID	Year	Age	Origin	FIGO stage	Type of surgery	Closure of peritoneum	Aquadest	Operating time (min)	Drain
1	1989	63	NL	2a	Te Linde	Yes	No	180	Yes
2	1993	29	S	2b	Te Linde	Yes	No	150	Yes
3	1993	34	S	2b	RH	Yes	No	210	Yes
4	1994	35	S	1b2	RH	Yes	Yes	210	Yes
5	2002	35	NL	1b1	RH	No	Yes	220	No
6	2002*	60	NL	1b1*	RH, debulking*	No*	No*	210*	No*
7	2003	65	NL	1b1	Cervical stump resection	No	Unknown	180	Yes
8	2004	41	NL	1b2	RH	No	Yes	180	No
9	2004	44	S	1b2	RH	No	Yes	180	No
10	2004	32	NL	1b2	RH	No	Yes	150	No
11	2007	26	NL	1b1	RH	No	Yes	210	No

ID = patients subsequently numbered; NL = the Netherlands; S = Surinam; Year = year of hysterectomy; Age = age at time of operation (yrs); RH = radical hysterectomy; Aquadest = use of intra abdominal distilled water lavage
* radical hysterectomy in 2002 and debulking in 2003: (same incision site) data of first procedure were used

Table 2 Pathological findings

ID	Histological type	Parametria	Resection margins	Lymph nodes	Tumour diameter	Vaso invasion	Infiltration depth
1	Squamous	Infiltrated	< 5 mm free	Neg	> 40 mm	Yes	11-15 mm
2	Squamous	Infiltrated	< 5 mm free	Pos	21-40 mm	Yes	11-15 mm
3	Squamous	Infiltrated	Tumour free	Neg	> 40 mm	No	> 15 mm
4	Squamous	Tumour free	Tumour free	Neg	> 40 mm	No	11-15 mm
5	Squamous	Tumour free	Tumour free	Neg	21-40 mm	No	11-15 mm
6	Adeno	Tumour free*	Tumour free*	Neg*	0.1-20 mm*	No*	6-10 mm*
7	Squamous	Infiltrated	< 5 mm free	Neg	21-40 mm	Yes	> 15 mm
8	Squamous	Tumour free	< 5 mm free	Pos	> 40 mm	Yes	> 15 mm
9	Squamous	Tumour free	Tumour free	Neg	21-40 mm	No	0.1-5 mm
10	Adeno squamous	Tumour free	Tumour free	Neg	> 40 mm	No	11-15 mm
11	Squamous	Tumour free	Tumour free	Neg	> 40 mm	No	> 15 mm

* radical hysterectomy in 2002 and debulking in 2003 (same incision site); data of the first procedure were used

Table 3 Follow-up data

ID	Adjuvant therapy	Previous* recurrence (time to)	Time to scar recurrence (mth)	Concurrent metastasis	Therapy	Subsequent metastases	Diseased	Time to death (mth)	Follow-up period (mth)
1	RT	Yes (3 mth)	27	No	Excision	No	Yes	19	46
2	RT	No	2	Yes	Excision	Unknown	Yes	2	4
3	RT	No	4	Yes	Excision	Unknown	Yes	7	11
4	RT	No	21	No	Excision	Yes	Unknown	Unknown	21
5	None	No	11	No	Excision + RT	Yes	No	n/a	73
6	None	Yes (15 mth)	45 (30)**	No	Excision	Yes***	No	n/a	69
7	RT	No****	10	Yes	Chemotherapy	No	Yes	4	14
8	Chemo + RT	No	14	Yes	Chemotherapy	Yes	Yes	14	29
9	None	No	6	No	Excision + RT	Unknown	Unknown	Unknown	13
10	None	Yes (5 mth)	33	No	Excision	No	No	n/a	53
11	RT	No	5	No	Excision + RT	Yes	No	n/a	11

RT = radiotherapy, n/a = not applicable
* before scar recurrence
** radical hysterectomy in ' 02, debulking in ' 03: time between hysterectomy and scar recurrence (debulking and scar recurrence)
*** = recurrence of scar recurrence

Of these 3 patients, it is not known whether they had another metastasis after the scar recurrence. The therapy for the scar recurrences (and coexisting metastases) was solely surgical excision in 6 cases (55%) and excision combined with radiotherapy in 3 cases (27%), and 2 patients (18%) received chemotherapy. Five of the patients with scar recurrence (45%) died due to cervical cancer, 4 (36%) survived up to now, and of 2 Surinamese patients, we had no further survival data since they were lost to follow-up shortly after the treatment for the scar recurrence. The mean period between the diagnosis of the abdominal wall recurrence and death was 9 months (median, 7 months). Of the 4 surviving patients, 2 were disease-free at the end of the study (53- and 73-month follow-up duration, respectively), and 2 had progressive disease (11- and 69-month follow-up duration).

Division of the 24-year study period in 3 equal periods of 8 years (1984-1992, 1992-2000, 2000-2008) shows 1 scar recurrence (0.7%) in 140 patients in 1984 to 1992, 3 (0.9%) of 340 patients in 1992 to 2000, and 7 (2.0%) of 362 in 2000 to 2008.

The scar recurrences occurred at different locations in the surgical scar. A preferential localization could not be distinguished. Pathological review of the paraffin-embedded biopsy or excision specimens of the scar recurrences did not show specific abnormalities. Although clearly of epithelial origin and resembling the tumour cells of the primary tumour, the scar recurrences were so much advanced at time of diagnosis that it was impossible to differentiate between, for example, lymphovascular or haematogenous origin or implantation during the operation with neoangiogenesis.

Table 4 shows the characteristics of the 11 cases and 55 random controls. Cases had a higher FIGO stage compared with controls (hazard ratio, 3.7; 95% confidence interval, 1.0-14.4). Other clear differences were a higher percentage of Surinamese patients, parametrial infiltration, resection margins less than 5 mm free, and tumour diameter greater than 4 cm in the cases. Wertheim procedure, adenocarcinoma, and positive lymph nodes were more frequent in the controls. In general, cases more frequently had 1 or more unfavourable prognostic characteristic. Data on vaso-invasion and infiltration depth were frequently not available in the control group. Recurrences at locations other than the scar occurred more frequently in the cases. Six (55%) of the cases developed a recurrence before or concurrent with the scar recurrence, and at least 10 (91%) had a recurrence other than the scar recurrence at some point during the follow-up period. Twenty-seven percent of the controls developed a recurrence.

Table 4 Data of the 11 cases and 55 controls taken randomly from the study population

	Cases	**Controls**
	Mean	**Mean**
Age (yrs)	42	44
	N (%)	**N (%)**
Origin		
The Netherlands	7 (64)	48 (87)
Surinam	4 (36)	7 (13)
FIGO stage		
1a	0	5 (9)
1b	0	2 (4)
1b1	4 (36)	31 (56)
1b2	4 (36)	9 (16)
2a	1 (9)	8 (15)
2b	2 (18)	0
Advanced stage (>/= 1b2)	7 (64)	17 (32)
Neo adjuvant therapy		
None	11 (100)	53 (96)
Chemotherapy	0	1 (2)
Radiotherapy	0	1 (2)
Type of surgery		
Te Linde	2 (18)	7 (13)
Wertheim	8 (73)	45 (82)
Trachelectomy	0	1 (2)
Cervical stump resection	1 (9)	1 (2)
Supravaginal hysterectomy	0	1 (2)
	Mean	**Mean**
Operating time (min)	189	203
	N (%)	**N (%)**
Histological type		
Squamous	9 (82)	39 (71)
Adeno	1 (9)	11 (20)
Other	1 (9)	5 (9)
Parametria		
Tumour free	7 (64)	42 (76)
Infiltrated	4 (36)	9 (16)
Unknown	0	4 (7)
Resection margins		
Tumour free	7 (64)	42 (76)
< 5 mm tumour free	4 (36)	12 (22)
Unknown	0	1 (2)
Tumour diameter		
< 4 cm	5 (46)	34 (62)
> 4 cm	6 (55)	18 (33)
Unknown	0	3 (6)
Vaso-invasion		
No	7 (64)	16 (29)
Yes	4 (36)	20 (36)
Unknown	0	19 (35)
Infiltration depth		
<15 mm	7 (64)	27 (49)
> 15 mm	4 (36)	13 (24)
Unknown	0	15 (27)
Lymph nodes		
Negative	9 (82)	37 (67)
Positive	2 (18)	17 (31)
Unknown	0	1 (2)
Adjuvant therapy		
None	4 (36)	25 (46)
Radiotherapy	6 (55)	24 (44)
Chemoradiation	1 (9)	6 (11)
Recurrence*		
During total follow-up period	8 (73)	15 (27)
Before or concurrent with the scar recurrence	6 (55)	

* other than the scar recurrence

Thirty-five (64%) of the controls had a follow-up at more than 5 years or died within 5 years after surgery. Five controls (9%) were lost to follow-up within 5 years, and 15 (27%) had their surgery less than 5 years ago. Forty-three controls (78%) had a follow-up at more than 2 years or died within 2 years. The availability of follow-up of the controls and cases was similar.

Discussion

Although 44 case reports describing 52 cases of scar recurrences of cervical cancer have been published (Table 5), the incidence of scar recurrences was hitherto unknown.(8-51) We report a large well-defined patient population showing an incidence of laparotomy scar recurrences after surgery for cervical cancer of 1.3%.

A few studies report the incidence of incision-site recurrences after laparotomy for other malignancies. For gynaecologic malignancies, figures are not available. The incidence of scar recurrences after surgical treatment for cervical cancer as reported in the current study is similar to the incidences of scar recurrences after open colectomy for large-bowel cancer and pancreatic cancer, which ranged between 0.6% and 4.6%, depending on the definition of scar recurrence, inclusion criteria, treatment and follow-up protocol, and detection method of metastases.(52-55)

The variety in time between the surgical procedure and the occurrence of the scar recurrence in our cases adds to the assumption that different mechanisms of origin, maintenance, and growth are involved in the development of scar recurrences. The case reports in literature also show this wide time range between surgery and diagnosis of scar recurrence (Table 5). We propose the existence of "dormant" tumour cells in the surgical scar as an explanation for late recurrences. Tumour dormancy is a state of temporary mitotic arrest in tumour cells, preventing further growth or metastasis.(6;56-61) The dormancy of tumour cells could be caused by an equilibrium state in the body, with tumour growth being controlled by the host immune system. In case of immunosuppression, the tumour cells can grow expansively or attain new capacities that allow them to escape the immune system and form a metastasis.(62)

Table 5 Scar recurrences of cervical cancer reported in literature

Reference type	Year	Site	FIGO stage	Hist.	Concurr. metast.	Recurr. time
Greenlee	1981	AS	1b	Sq	Yes	7
Stenson	1990	AS	2b	Sq	Yes	7
Dhall	1991	AS	1b	Ad	U	8
Gunasekera	1994	AS	1b	Ad	Yes	204
Selo-Ojeme	1998	AS	1a	Ad	No	54
Sharma (2x)	2000/i	AS/i	1b/i	Sq/i	No/i	8/22
Srivastava	2005	AS	2a	Sq	Yes	42
Sachdev	2007	AS	2a	Sq	No	24
Paraskevaidis	2000	AS	1b	Sq	No	3
	2000	LPS	1b	Sq	Yes	13
Patsner	1992	LPS	1b	Sq	Yes	3
Kadar (2x)	1997/i	LPS/i	1b/3b	U/i	U/i	U/i
Naumann	1997	LPS	3b	Sq	Yes	5
Wang	1997	LPS	1b	Sq	Yes	2
Lane	1999	LPS	1b	Adsq	No	10
Lavie	1999	LPS	1a	Ad	No	9
Kohlberger	2000	LPS	1b	Sq	No	19
Lecuru	2000	LPS	1b	Sq	Yes	48
Gregor	2001	LPS	2b	Sq	No	3
Tjalma	2001	LPS	3b	Sq	Yes	15
Agostini	2003	LPS	2b	Sq	Yes	7
Picone	2003	LPS	2b	Ad, Cc	Yes	5
Martinez-Palones	2004	LPS	3b	Ad	No	7
Park	2008	LPS	2b	Ad	Yes	1
Singh	1976	UCS	u	Sq	No	
Copas	1995	DS	2a	Sq	Yes	6
Behtash	2001	DS	2a	Sq	U	60
Iavazzo	2007	DS	2a	Sq	No	14
Barranger	2004	SIS	1b1	Ne	Yes	9
Bader	2006	SIS	1b1	Ad	Yes	12
Burgess	1987	ES	1b	Sq	U	18
Copeland (2x)	1987/i	ES/i	1b/i	Ad/i	No/i	3/6
Gordon	1989	ES	1b	Sq	No	1
Dam van (2x)	1992/i	ES/i	2a/1b	Sq/Ad	U/i	6/3
Khalil	1993	ES	3b	Sq	U	3
Cliby (4x)	1960-1993	ES	1b	Sq	Yes	2
	1960-1993/i/i	ES/i/i	1b/i/i	Sq/i/i	No/i/i	24/3/1
Broek van den	1995	ES	CIS	Ad	Yes	1.5
Sood	2000	ES	2a	U	U	5
Goldman	2003	ES	1b	Sq	No	66
Heron	2005	ES	1b1	Vg, Ad	No	42
Neumann	2007	ES	1b2	Adsq	Yes	0.5
Baloglu	2007	ES	3a	Sq	No	8
Hoopmann	2003	PT	u	Sq	No	4
Amanie	2004	DVT	u	Ad, En	No	17
Pradhan	2006	CSS	2b	Sq	Yes	n/a*

Recurr. time = time between surgery or delivery and diagnosis of scar recurrence (months); AS = Abdominal scar; LPS = Laparoscopic port site; UCS: Urinary conduit stoma site; DS = Drain site; SIS = Schuchardt incision scar; ES = Episiotomy site; PT = perineal tear; DVT = Delivery-induced vaginal tear; CSS = Caesarean section scar; u = unknown; Sq = squamous; Ad = adeno; Adsq = adenosquamous; Cc = clear cell; Ne = neuroendocrine; Vg = villoglandular; En = endometrioid; i = idem.
* scar recurrence occurred 20 mth after diagnosis of cervical cancer and treatment by radiotherapy, but in a 26 years old scar

More than half of our cases had an advanced disease (FIGO stage ≥1b2), which was emphasized by the fact that parametrial involvement, not radically free resection margins, and tumour diameter greater than 4 cm were relatively frequently found postoperatively. Based on our findings, scar recurrences do not seem to be just single and treatable events. All but one (who was lost to follow-up after 13 months) of the

cases sooner or later developed other metastases. We believe that scar recurrences are not likely to be the cause of other metastases. The scar recurrences of the 3 patients for whom the scar recurrence was not the first disease recurrence were all diagnosed at more than 2 years after the primary surgery. Furthermore, at least 45% of the patients with a scar recurrence in our study group died, and all within 19 months after the scar recurrence was diagnosed.

The wide variety in time to development of scar recurrences is similar to "normal" metastases, and the percentage of scar recurrences developing within 2 years after surgery corresponds with the development of local recurrences. Altogether, scar recurrences seem to be part of the presence of more extensive metastatic disease and seem to be similar to other metastases.

A limitation of the study was the small number of cases because of the rarity of the phenomenon scar recurrences. However, clear differences were observed between cases and controls. It was not possible to assess the effect of changes in treatment modalities (the use of distilled water, ceasing separate closure of the peritoneum, application of a postoperative drain, and addition of postoperative radiation criteria) because of their almost synchronic times of introduction and because all patients were treated similarly.

Monitoring the clinical outcome of our patients, we had the impression that the problem of scar recurrences was increasing over time. Although the number of events was small, our study results indicate an increase in incidence in the last period on the basis of the available data.

The higher percentage of scar recurrences among the Surinamese patients is likely to be a result of the higher tumour stage because of the absence of a screening program for cervical cancer in Surinam. Higher stage was the only specific feature in this group. Ethnicity is less probable to play a role because the Surinamese population is a melting pot of different ethnical origins.

The phenomenon port-site recurrence after laparoscopic procedures has been investigated and reported elaborately before.(63;64) Still, true incidences are unknown for most types of malignancies. Reported incidences are 0% to 4.6% for colon and rectal cancer surgery: 4.6% in an international survey (65) and 0% to 0.9% (colorectal, 0.9%; colon, 0.7%; rectum, 0%) in a systematic review (66); 0.8% for upper gastrointestinal cancer surgery (67); and 0.1% for urological oncologic laparoscopy (68); and up to 17% for gallbladder cancer.(65) For gynaecologic cancer, true incidences of port-site recurrences are unknown, although incidences of 1% to 9% for ovarian cancer have been reported.(69;70) Ovarian cancer is mentioned as high risk for port-site recurrences, but this seems to be largely based on the number of case reports and

might be a reflection of more advanced tumour stages at the time of surgery. There are no data on the true incidence of port-site recurrences after laparoscopy for cervical or endometrial cancer. Although it is interesting to compare the safety of laparoscopic and laparotomic procedures with respect to scar recurrences, our findings cannot be compared with the previously mentioned incidences. The data of all reported incidences have been gathered in different ways. Furthermore, different mechanisms of tumour spread might be involved in laparoscopic and laparotomic procedures because of procedural differences. However, also in port-site recurrences, the biology of the cancer cells is suggested to be more important than technical characteristics.(63;64;71;72) Randomized trials with multivariate analyses would be necessary to compare safety with respect to scar recurrences between laparoscopy and laparotomy.

It is difficult to give advice on the prevention of scar recurrences, based on our findings. Within the scar recurrence group, a strong heterogeneity was found on all investigated characteristics. The role of iatrogenic factors as compared with that of tumour, stage, host, or treatment characteristics remains unclear.

We would like to comment on one specific change in treatment modality. Since 1999, we have ceased to close the peritoneum separately. One could speculate about the facilitation of loose tumour cell migration toward the scar without the protection of the closed peritoneum. This argument has been postulated to explain the occurrence of port-site metastases after laparoscopy. An animal study and a clinical experiment found an increased risk for the development of scar recurrences if only the skin was closed after surgery in an area with tumour as compared with the separate closure of all layers. The used methodology, however, hampers concluding from the clinical study.(70;73)

We do not advocate changes in the standard treatment, but underscore the conventional carefulness to avoid any direct contact between the tumour and unaffected (traumatized) tissue, especially in patients with advanced stage of the disease. Yet, even when we succeed in this difficult task, tumour cells will still be present in the peritoneal cavity, lymph and blood vessels, and bone marrow (74-83) and could be attracted to the wound site by inflammatory oncotaxis.

Based on our findings, we strongly recommend extra carefulness when a scar recurrence is discovered. Thorough physical examination and the use of imaging techniques are extremely important to exclude metastases at other sides. Perhaps chemotherapy in addition to local excision should be considered in all patients with scar recurrence. However, chemotherapy could be unfavourable if, as given to consider by Melief,(84) immunosuppressive radiation or chemotherapy could possibly enhance the escape of dormant tumour cells from the equilibrium state.

Although we have been able to collect detailed information on scar recurrences, many questions remain unanswered. We believe that future research should focus on genetic characteristics of tumour cells and immune control. Different tumour clones in primary tumours, and their capabilities to spread and grow, can be studied by DNA analysis.

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Chapter 8

Barrel Index of bulky cervical tumours and intra uterine fluid determined by MRI as additional prognostic factors for survival

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Abstract

To investigate whether morphologic characteristics determined by MRI can discriminate between bulky cervical tumours with a favourable or worse prognosis, MRI examinations were performed in 24 patients with cervical cancer stage ≥1b2. The ratio between tumour width and length (Barrel Index: BI) and the presence of intra uterine fluid retention were related to survival in a multivariate regression analysis. BI and intra cavital fluid were predictors of survival, independent from tumour diameter and other known important factors for survival. A cut-off value of 1.40 for the BI proved to be the best prognostic factor with respect to recurrence and death: the hazard ratios of BI > 1.40 as compared to BI ≤ 1.40 were 18.9 (95% CI 2.8 to 125.6) for recurrent disease and 16.4 (95% CI 2.9 to 93.9) for death by cervical cancer. The hazard ratios of intra cavital fluid retention were 73.6 (95% CI 5.3 to 1016.4) and 48.1 (95% CI 4.7 to 491.6) for recurrence and death respectively. The morphologic characteristic Barrel Index and the presence or absence of intra cavital fluid as determined by MRI might have predictive value for survival in patients with bulky cervical tumours.

Introduction

The staging procedure of cervical cancer is based on the FIGO staging system, which does not routinely include MRI-, or CT-scan.(1) Accordingly, although used more and more frequently, not all clinics with available MRI or CT use these imaging modalities in the routine work-up of cervical cancer patients. Tumour extension (i.e. tumour diameter and infiltration of surrounding tissue like the parametria, vagina, bladder and rectum) is the most important factor in the FIGO staging system and is considered to be the deciding factor for the choice of the primary treatment.(2)

Due to poor prognostic properties bulky cervical tumours are assigned to a separate class in the FIGO system (1b2 or above). Morphology however, is not accounted for.(3; 4) A prospective study of 400 surgically treated bulky (diameter > 4 cm) cervical cancer patients showed that morphologic characteristics of the tumour are of prognostic significance. Patients with a barrel shaped tumour had a significantly worse disease free and overall survival after primary surgery compared to patients with the same FIGO stage but with an exophytic growing tumour. The authors of that study concluded that surgical treatment should be considered for patients with bulky exophytic cervical cancer(5). There is no general agreement about the optimal treatment of bulky cervical tumours. Morphology could play a role in the selection of a subgroup that is suitable for primary surgery.

The purpose of this study was to determine whether MRI can discriminate between patients with morphologically favourable bulky cervical tumours and patients with bulky cervical tumours with a worse prognosis. MRI allows accurate visualization and assessment of extension of the tumour in surrounding tissues, without the need for intravenous contrast.(6) We performed a retrospective observational study in patients with surgically treated bulky cervical cancer of whom MRI of the female pelvis was performed prior to surgery. The ratio between tumour width and length and the accumulation of fluid in the uterine cavity were related to disease free and overall survival of the patients.

Materials and Methods

Review board approval

All data were obtained in accordance with the medical ethical guidelines of our hospital.

Patients

One hundred and eight patients were surgically treated for cervical cancer stage 1b2 or above in our centre between 1984 and 2000. Twenty-four of these patients had a pre-operative MRI scan and were included in the study.

Clinical assessment

The included patients were evaluated pre operatively by the standard staging procedure of our gynaecologic oncology department, which included complete physical and gynaecological examination under anaesthesia, routine blood- and urine analysis, chest radiography, and ultrasound to exclude ureteral dilatation. Additionally, a MRI scan was performed. The indications for the MRI scan were high suspicion of tumour extension into the parametria, bladder, or rectum, and lymph node metastases, based on physical examination under anaesthesia. The MRI scans were performed on a 1.5 T scanner (Philips Intera 1.5T, Best, The Netherlands), using a phased array torso coil. All patients were scanned in supine position. The imaging protocol consisted of axial T1-weighted fast spin echo images (TR/TE = 575/8 msec, 24 slices, 4 mm thickness, field of view 200 mm, matrix 320 x 256), and axial and sagittal T2-weighted fast spin echo images (TR 4800 msec, TE 150 msec, 24 slices, 4 mm thickness, field of view 200 mm, 320x 256 matrix), through the pelvic region. The images were analysed on a dedicated workstation (Philips Viewforum, Best, The Netherlands).

Treatment

In our centre, the standard treatment for cervical cancer stage 1b and 2a is radical abdominal hysterectomy and pelvic lymphadenectomy(7). For one patient with FIGO stage 2b the treatment was individualized to chemo therapy followed by radical hysterectomy. Post-operative pathologic assessment included histological type, infiltration depth, involvement of the parametria and resection margins, tumour diameter, and lymph node metastases. Patients received adjuvant radiotherapy in case of ≥ 1 tumour positive lymph node, and if parametrial involvement or non-radical surgical resection margins (< 5 mm free of tumour) were found. An additional criterion for adjuvant radiotherapy from 1997 onwards was the presence of at least two of the following prognostic unfavourably factors: tumour diameter > 4 cm, invasion depth > 15 mm, and vaso-invasion. In individual cases of severe tumour extension platinum-based chemoradiation was offered.

Measurements

The maximal cranio-caudal length of the cervical tumour (increased signal on T2 weighted images compared to normal cervical tissue) parallel to the cervical axis and

the maximal width perpendicular to the cervical axis were measured (Figure 1). Subsequently the ratio between tumour width and length was calculated and expressed as the 'barrel index' ($BI = \frac{width}{length}$). A barrel shaped tumour was expected to cause fluid retention in the uterine cavity by obstruction of the endocervical canal. We therefore visually assessed the presence of fluid in the lumen of the uterus. Because differentiating between fluid and endometrial glands and stroma can be difficult, we defined two categories: 'no fluid or just a stripe of fluid', and 'more than a stripe of fluid'. Presence of intra cavital fluid was recognized by its' biconcave shape. The two groups are further referred to as 'without fluid retention' and 'with fluid retention'. Figure 2 shows an example of a uterine cavity with fluid retention (contrary to the example in Figure 1). All measurements were independently performed by three different radiologists (AŠ, MW, and IL) who were blinded for the follow-up status of the patient.

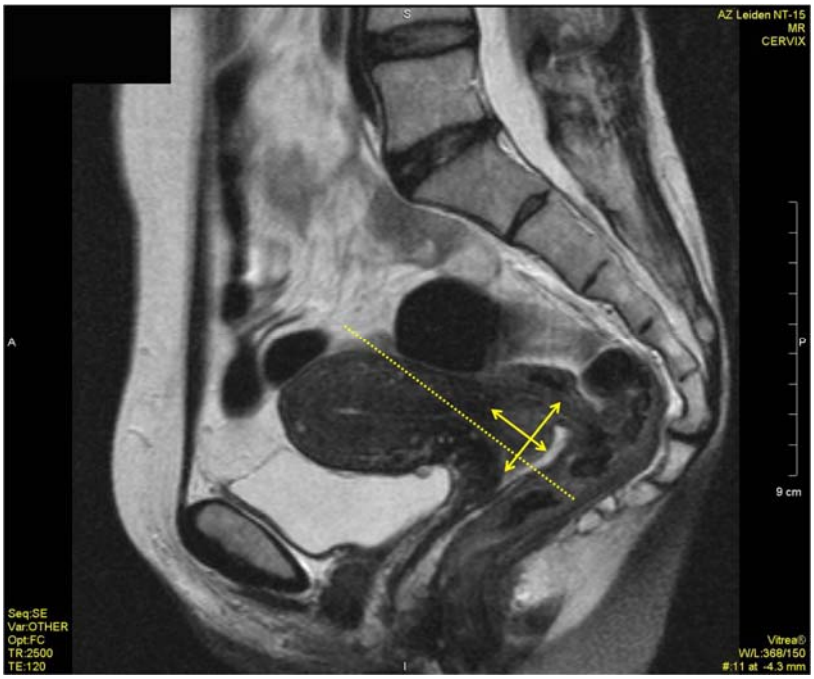

Figure 1 Sagittal T2-weighted MR image showing the cervical axis (dotted line) and the directions in which tumour length (arrow parallel to cervical axis) and width (arrow perpendicular to cervical axis) were measured for the determination of the barrel index. No intra uterine fluid retention

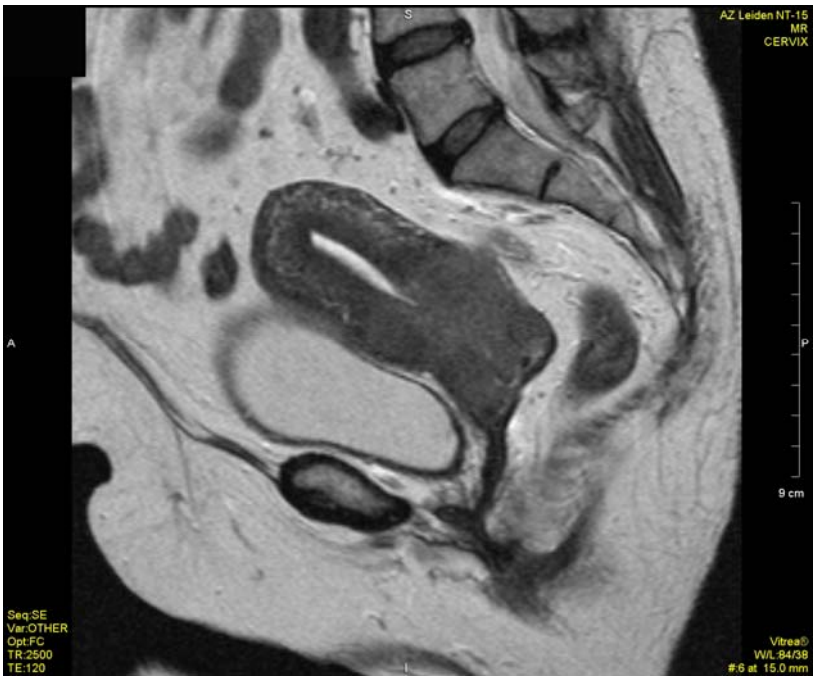

Figure 2 Sagittal T2-weighted MR image of cervical tumour patient with intra uterine fluid retention

Follow-up

Follow-up data and patient characteristics were extracted from the medical records. The follow-up period for this study extended from the individual date of surgery to November 2007.

Statistical analysis

Cox regression was used to analyse the effect of the barrel index and fluid retention on disease free survival (DFS) and overall survival (OS). First we performed univariate analysis for varying cut-off points of the barrel index to determine the optimal cut-off point for the barrel index in the subsequent analyses. The multivariate analysis included BI or fluid retention, and the known disadvantageous prognostic factors age, positive lymph nodes, invasion depth > 15 mm, tumour positive parametria, and resection margins < 5 mm free of tumour. Student's t-test was used to compare the mean tumour width between the patients with and without intra cavital fluid retention. For the statistical analysis with respect to prognosis, the measurements of one investigator (AŠ) were used. Cohen's kappa coefficient was calculated to evaluate the inter observer concordance of the MRI determinations (BI and presence of intra cavital fluid).

Results

General characteristics

General characteristics, pathology results and results with respect to survival after radical hysterectomy of the 24 patients are summarized in Table 1. The group consisted of 16 (67%) Dutch and 8 (33%) Surinamese patients who were referred to the Netherlands for cervical cancer treatment. Two patients received neo adjuvant chemotherapy, which was started after the MRI scan. The follow-up period ranged from 0.8 to 131.6 months. Mean DFS was 31.2 months, and mean OS 44.4 months. One patient died in the first month after treatment by another cause than cervical cancer and was excluded from the survival analyses. Three other patients were lost to follow-up after follow-up periods of 4.0, 4.6, and 21.3 months respectively. Fourteen patients (61%) had a recurrence. All patients with recurrent disease died of cervical cancer.

Table 1 Characteristics of 24 patients with bulky (>40 mm) cervical tumours

	Mean	**Min - Max**
Age (yrs)	50.8	31 - 75
	N	**%**
Place of residence		
Netherlands	16	67
Surinam	8	33
FIGO stage		
1b2	16	67
2a	7	29
2b	1	4
Neo adjuvant therapy		
Chemotherapy	2	8
Disadvantageous pathological parameters		
Positive lymph node	9	38
Resection margins 5 mm free of tumour	13	54
Parametria tumour positive	3	13
Invasion depth > 15 mm	19	79
	Mean	**Min - Max**
Tumour size (mm)	57.3	45 - 80
	N	**%**
Adjuvant therapy		
Radiotherapy	19	79
Chemo radiation	2	8
Recurrence		
Local	4	17
Regional	2	9
Distant	8	35
Deceased		
Yes, by cervical cancer	14	58
Yes, by other cause	1	4
	Mean	**Min - Max**
DFS (mth)	31.2	0.8 - 110.1
OS (mth)	44.4	0.8 - 131.6

N = number; DFS = disease free survival; OS = overall survival

Barrel index

Univariate Cox regression analysis with varying barrel indices ranging from 1.10 to 1.90 showed that a cut-off point of 1.40 for the BI was the best prognostic factor with respect to recurrence and death in our sample. Ten of 23 patients (44%) had a BI > 1.40, the rest of the barrel indices were 1.40 or smaller. The group of patients with a BI > 1.40 consisted of 7 patients (70%) with FIGO stage 1b2, 2 (20%) with 2a, and 1 (10%) with 2b. The group with BI ≤ 1.40 consisted of 9 patients (64%) with stage 1b2, and 5 (36%) with 2a. Eight patients with a BI > 1.40 (80%) and 6 (46%) with a BI ≤ 1.40 had a recurrence. Table 2 shows the results of the multivariate survival analysis based on this cut-off point. The hazard ratio (HR) of BI > 1.40 as compared to BI ≤ 1.40 was 18.9 (95% CI 2.8 to 125.6) for recurrent disease, and 16.4 (95% CI 2.9 to 93.9) for death by cervical cancer. Exclusion of the two patients that received adjuvant chemoradiation from the analysis increased the HR's of BI > 1.40 to 32.2 (95% CI 3.3 to 312.9) for DFS and to 20.8 (95% CI 2.9 to 151.7) for OS. The inter observer concordance of the MRI measurements of BI > / ≤ 1.40 by the three radiologists was moderate to substantial (kappa = 0.50 to 0.74) (Table 3).

Table 2 Cox regression analysis of the shape of the cervical tumour (BI)

DFS **Prognostic factors**		
	HR	**95% CI**
Age	0.9	0.9 to 1.0
Positive lymph nodes	0.1	0.01 to 0.5
Invasion depth > 15 mm	3.7	0.6 to 22.7
Parametria tumour positive	47	3.9 to 562.6
Resection margins < 5 mm	1.1	0.3 to 4.2
Barrel Index > 1.40	18.9	2.8 to 125.6
OS **Prognostic factors**		
	HR	**95% CI**
Age	0.9	0.9 to 1.0
Positive lymph nodes	0.1	0.01 to 0.5
Invasion depth > 15 mm	3.6	0.6 to 21.3
Parametria tumour positive	51.4	4.3 to 610.6
Resection margins < 5 mm	0.9	0.2 to 3.7
Barrel Index > 1.40	16.4	2.9 to 93.9

Table 3 Inter observer concordances

BI > / ≤ 1.40 **Kappa**			
	Observer 1	Observer 2	Observer 3
Observer 1	x	0.74	0.5
Observer 2	0.74	x	0.58
Observer 3	0.5	0.58	x
Intra uterine fluid retention **Kappa**			
	Observer 1	Observer 2	Observer 3
Observer 1	x	0.92	0.67
Observer 2	0.92	x	0.75
Observer 3	0.67	0.75	x

Intra cavital fluid retention

In 14 of the 24 patients (58%) high intensity fluid was present in the uterine cavity. There was a trend towards lower stages in the fluid retention group (11 patients (79%) with 1b2, and 3 (21%) with 2a) as compared to the group without fluid retention (5 patients (50%) with 1b2, 4 (40%) 2a, and 1 (10%) 2b). In the 23 patients included for the survival analyses, 11 of 14 patients (79%) with fluid retention, and 3 of 9 patients without fluid retention (33%) had a recurrence. The hazard ratio of fluid retention as compared to a stripe or no fluid was 73.6 for recurrent disease (95% CI 5.3 to 1016.4) and 48.1 for death by cervical cancer (95% CI 4.7 to 491.6) (Table 4). Exclusion of the two patients that received adjuvant chemoradiation from the analysis decreased the HR for DFS of fluid retention to 43.8 (95% CI 3.2 to 602.2) for DFS and increased the HR for OS of fluid retention to 54.8 (95% CI 4.4 to 674.2). The inter observer concordance of the estimation of high intensity retention material in the uterine cavity on MRI was substantial to almost perfect (kappa = 0.67 to 0.92) (Table 3).

Table 4 Cox regression analysis of intra cavital uterine fluid retention

DFS **Prognostic factors**		
	HR	**95% CI**
Age	0.9	0.9 to 1.0
Positive lymph nodes	1.6	0.3 to 8.4
Invasion depth > 15 mm	0.2	0.01 to 2.2
Parametria tumour positive	24.2	1.9 to 304.4
Resection margins < 5 mm	1	0.2 to 5.6
With intra cavital fluid retention	73.6	5.3 to 1016.4
OS **Prognostic factors**		
	HR	**95% CI**
Age	1	0.9 to 1.1
Positive lymph nodes	1.1	0.2 to 5.6
Invasion depth > 15 mm	0.3	0.03 to 3.2
Parametria tumour positive	15.9	1.3 to 192.8
Resection margins < 5 mm	0.6	0.1 to 2.9
With intra cavital fluid retention	48.1	4.7 to 491.6

BI and fluid retention with regard to tumour size

Six of the 10 patients (60%) without fluid retention had a BI ≤ 1.40 based on MRI measurements, and 6 of 14 patients (43%) with fluid retention had a BI > 1.40.

The largest tumour width measured on MRI in the group without fluid retention was 59 mm, whereas 57% of patients with fluid retention had a tumour width > 59 mm, with a maximum of 87 mm. The difference between the mean tumour width of the group with fluid retention (63 mm) and without fluid retention (50 mm), was significant (mean difference = 12.5 mm, 95% CI = 2.7 to 22.2).

Inclusion of the maximal tumour width in the multivariate survival analysis for BI > / ≤ 1.40 resulted in a decrease of the HR from 18.9 to 11.3 (95% CI = 1.4 to 92.6) for DFS and from 16.4 to 12.2 (95% CI = 1.8 to 85.2) for OS. In both survival analyses, however, the BI remained an independent prognostic factor. A similar decrease of the HR for fluid retention was observed when the maximal tumour width was included in that multivariate analysis: decrease of the HR from 73.6 to 26.4 (95% CI = 2.2. to 315.5) for DFS and from 48.1 to 24.8 (95% CI = 2.5 to 241.4) for OS. Fluid retention also remained an independent prognostic factor.

Discussion

The results of this observational study on growth pattern of cervical tumours in relation to recurrence and survival suggest that the morphologic characteristic BI and presence or absence of intra cavital fluid as determined by MRI may have prognostic value.

A division in groups based on the ratio between tumour length and width (BI) was an independent prognostic factor for recurrence and overall survival in our study. The relation to survival was independent from the known prognostic factor tumour diameter. In previous studies a barrel shaped growth pattern and tumour size were also found to be independent disadvantageous prognostic factors.(5;8) The results of these studies support our findings. To the best of our knowledge this is the first study in which the BI of cervical tumours determined by MRI was found to be a prognostic factor for survival.

The results of our study suggest that presence or absence of fluid in the uterine cavity is a strong prognostic factor for recurrence and overall survival. The hazard ratios for recurrence and overall survival were even higher than for BI. Intra uterine retention material could not conclusively be related to morphology based on MRI assessment, but it had a clear relation with tumour width as measured on MRI. Although intra uterine fluid accumulation is a quite common finding in transvaginal sonography among asymptomatic postmenopausal women, accumulation of fluid in the uterine cavity on MRI and transvaginal sonography imaging has been related to gynaecologic cancer.(9-12) As far as we know, this is the first study in which this finding has been related to growth pattern and to prognosis. Given the substantial to almost perfect inter observer concordance of this parameter on MRI and the strong relation we found in our study to prognosis, retention material in the uterine cavity is a promising prognostic parameter. More and larger studies are needed to confirm our findings.

In our study we found that tumour diameter is also a prognostic factor for recurrence and overall survival after surgical treatment of cervical cancer. Previous reports confirm this finding.(8) Multivariate survival analysis including tumour diameter, BI and fluid

retention, however, showed that only BI and fluid retention remained independent risk factors. These findings suggest that tumour diameter, BI and fluid retention are related but that BI and fluid retention are stronger independent prognostic factors.

Our study has several limitations. One limitation is that in daily practice in our centre MRI imaging is no routine part of the staging procedure for cervical cancer. In our study group a MRI scan was performed based on clinical grounds. Our studied population might therefore not be a representative sample of the average population of patients with bulky cervical cancer. This probably explains the very high hazard ratios we found for BI and fluid retention for recurrence and overall survival and the unusual finding that lymph nodes appear to be protective in some of the analyses. Furthermore follow-up was not fully documented in 3 of the 23 patients (13%) that were included in the survival analyses. When these individuals were removed completely from the analyses the results did not change substantially (data not shown). We therefore do not think that the results of our study are much influenced by the individuals that were lost to follow-up during the study. Another limitation is the small study size. Despite the small study size our results were highly significant, suggesting that the BI and presence or absence of intra cavital fluid are strong prognostic factors. Furthermore it has already shown that BI is a prognostic factor. The additional value of our study is that our results show that the prognostic factor BI can be determined with MRI prior to therapy. Larger and prospective studies are needed to confirm our results.

In conclusion, the results of our study support the reported finding that morphology of bulky cervical tumours is predictive for prognosis. The morphologic characteristic BI and intra cavital fluid as assessed by MRI might be helpful in identifying a subgroup of individuals with bulky cervical cancer with a better prognosis and who therefore are eligible for primary surgical therapy.

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Chapter 9

Loss of heterozygosity and copy number alterations in flow-sorted bulky cervical cancer

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Abstract

Treatment choices for cervical cancer are primarily based on clinical FIGO stage and the post-operative evaluation of prognostic parameters including tumour diameter, parametrial and lymph node involvement, vaso-invasion, infiltration depth, and histological type. The aim of this study was to evaluate genomic changes in bulky cervical tumours and their relation to clinical parameters, using single nucleotide polymorphism (SNP)-analysis.

Flow-sorted tumour cells and patient-matched normal cells were extracted from 81 bulky cervical tumours. DNA-index (DI) measurement and whole genome SNP-analysis were performed. Data were analysed to detect copy number alterations (CNA) and allelic balance state: balanced, imbalanced or pure LOH, and their relation to clinical parameters.

The DI varied from 0.92-2.56. Pure LOH was found in ≥40% of samples on chromosome-arms 3p, 4p, 6p, 6q, and 11q, CN gains in >20% on 1q, 3q, 5p, 8q, and 20q, and losses on 2q, 3p, 4p, 11q, and 13q. Over 40% showed gain on 3q. The only significant differences were found between histological types (squamous, adeno and adenosquamous) in the lesser allele intensity ratio (LAIR) ($p=0.035$) and in the CNA analysis ($p=0.011$). More losses were found on chromosome-arm 2q (FDR=0.004) in squamous tumours and more gains on 7p, 7q, and 9p in adenosquamous tumours (FDR=0.006, FDR=0.004, and FDR=0.029).

Whole genome analysis of bulky cervical cancer shows widespread changes in allelic balance and CN. The overall genetic changes and CNA on specific chromosome-arms differed between histological types. No relation was found with the clinical parameters that currently dictate treatment choice.

Introduction

Prognostic factors for cervical cancer

Cervical cancer is one of the most frequent gynaecological cancers worldwide. Following the surgical treatment of cervical tumours, prognostic factors for survival include the clinical parameters FIGO stage, tumour diameter, tumour in the parametria, tumour positive pelvic lymph nodes, vaso-invasion, and infiltration depth. Histological type is also related to prognosis, and is evaluated both pre- and postoperatively.(1-4) Although parameters can be partly determined pre-operatively by clinical examination, imaging, or the pathological evaluation of biopsy specimens, most parameters are only definitively established following the post-operative pathological examination of surgical specimens. Presence or absence of these factors is of prognostic relevance and is therefore used to select both the primary treatment, and to decide whether adjuvant chemotherapy and/or radiotherapy are necessary.

Surgical treatment is considered to be the optimal primary treatment for small diameter cervical tumours (<4 cm, FIGO stage <1b2). Locally extended tumours (FIGO 2b or higher) are primarily treated by chemo-radiation. There is, however, no worldwide agreement on the optimal primary treatment for bulky cervical cancer (diameter > 4 cm, FIGO ≥ 1b2-2b), although radiotherapy or surgery are options.(5-13) Recently, our group reported a possible additional prognostic factor for bulky cervical tumours. Patients with barrel-shaped (lateral extension ≥1.5 x cranio-caudal extension) bulky tumours showed a worse disease-free and overall survival after surgical treatment, when compared to exophytic (all other) tumours. Primary surgical treatment, rather than radiotherapy or chemo-radiation, has been proposed as the optimal treatment for patients with exophytic bulky tumours.(14)

The ability to select more homogenous subgroups of patients with cervical tumours may help in the selection of the most suitable treatment strategy for individual patients. Identification of patients with specific genetic patterns might be a way to achieve this goal. Genetic changes could be objectively assessed, pre-operatively, in tumour biopsies, potentially providing a more accurate prediction of stage and clinical behaviour than the physical examination of the patient. Furthermore, genetic profiling could provide information on the genes or pathways responsible for tumour growth and metastasis.

Genetic profiling

The progression of normal cells to cancer is accompanied by changes in DNA, and genetic profiles have been established for several types of cancer. These profiles have been largely determined using arrayCGH, and have therefore been limited to copy

number changes. In this study, we used single nucleotide polymorphism (SNP) arrays to determine the genetic profile of flow-sorted tumour populations. This approach has the advantage of also determining allele-specific changes, in addition to copy number alterations (CNA), in pure tumour cells. In order to include loss of heterozygosity (LOH) in the analysis, we developed the lesser allele intensity ratio (LAIR) approach, which allows the assessment of discrete allele specific copy numbers (CN) for all genomic locations.(16) This method allows the classification of the discrete total CN as both the sum of two alleles and as the balance state, which can then be divided into 3 classes: balanced, imbalance, and LOH.

The statistical analysis of differences in genetic profiles between groups of tumours has proven to be difficult. The nature of the genetic changes in tumours causes strong correlations between measurements from neighbouring probes, correlations that are not properly handled in commonly used statistical tests. In this study we introduce a statistical method based on the global test (17), which performs multiple testing correction correctly in the presence of strongly correlated values. Another advantage of the global test is that it can test the hypothesis that groups of samples are the same on a whole genome level, and can zoom in on chromosome arms when a difference between groups is found.

Aim of the study

The purpose of this study was to identify genetic changes associated with one or more prognostic factors in cervical cancer patients. Our approach was to use SNP array analysis on flow-sorted FFPE tumour tissue from bulky cervical cancers.

To our knowledge, this is the first large scale, whole genome SNP array study of this stage of cervical tumours, and no publication has yet described a genomic profile of cervical tumours based on the SNP array analysis of pure tumour tissue. Additionally, this is the first whole genome SNP array study of a large group of bulky cervical tumours in relation to genetic changes in balance state and CN, and their relationship to unfavourable prognostic factors.

Materials and methods

Samples

Tissue from 107 cervical carcinomas, as well as paired normal (non-affected) endometrial and/or lymph node tissue, was obtained from the FFPE tissue bank of the Department of Pathology, Leiden University Medical Center (LUMC). Samples were handled in accordance with the medical ethical guidelines described in the Code Proper

Secondary Use of Human Tissue established by the Dutch Federation of Medical Sciences (www.federa.org). Our study group consisted of patients living in the Netherlands and in Suriname. All patients presented with bulky cervical cancer, were classified as stage ≥1b2-2b according to FIGO, and received primary surgical treatment at the LUMC between January 1984 and November 2000. A large number of clinical parameters have been characterized in these patients, but for this study we choose to investigate seven clinical parameters known to be of prognostic value: tumour diameter, histological type, parametrial involvement, pelvic lymph node status, vaso-invasion, infiltration depth, and growth pattern. The growth pattern was defined as barrel-shaped if the lateral extension of the tumour was ≥1.5 x the cranio-caudal extension of the tumour; otherwise the tumour was classified as exophytic. Histological typing (squamous, adeno, adenosquamous, or mixed tumours) was based on histochemical staining with H&E, periodic acid-Schiff (PAS) reagent, and Alcian blue for mucin detection. FIGO stage was not included in the analyses since the various postoperative characteristics are more accurate than the pre-operative staging.

Tissue sample preparation

Paraffin sections taken from all samples were H&E stained and reviewed by a pathologist (GJF). The tumour nodule was marked on the H&E section, which was used as a guide to trim the normal tissue from paraffin block prior to flow cytometric workup. The tumour negative status of blocks containing normal tissue (either tumour negative lymph nodes or endometrial tissue) was reviewed by histology and confirmed. Cell suspensions were prepared for flow-cytometry, as described in detail elsewhere.(18;19) Briefly, six to ten 60 µm sections were taken from each paraffin block. Sections were dewaxed and further processed until a cell suspension was obtained. Cells were then harvested, washed, counted and stored on ice prior to further processing.

Immunocytochemistry of cell suspensions

Immunocytochemistry has been described in detail elsewhere.(18;19) Briefly, five million cells were incubated in a mixture of monoclonal antibodies directed against keratin or vimentin. The following MAbs were used: anti-keratin MNF116 (DAKO, Glostrup, Denmark), anti-keratin AE1/AE3 (Millipore-Chemicon, Billerica, MA), and anti-vimentin V9-2b (diluted 1:5) (Antibodies for Research Applications BV, Gouda, The Netherlands). Cells were incubated with premixed FITC- or RPE-labelled secondary reagents (Goat F(ab2)' anti-mouse IgG1-FITC and goat F(ab2)' anti-mouse IgG2b-RPE [Southern Biotechnology Associates, Birmingham, AL]), and DNA was labelled with DAPI (Sigma-Aldrich, Zwijndrecht, Netherlands).(20)

Flow-cytometry and sorting

Using an LSRII (BD Biosciences, Erembodegem, Belgium) flow cytometer, a gate was created to collect 20,000 keratin-positive single cell events during acquisition. Standard filter sets were used for the detection of FITC, R-PE and DAPI fluorescence. A data file contained all events. The WinList 6.0 and ModFit 3.2.1 software packages (Verity Software House, Inc., Topsham, ME) were used for data analysis and DNA index (DI) calculation (median of G0G1 population of tumour cell fraction / median of G0G1 population of stromal cell fraction). Keratin-positive and vimentin-positive normal cells were collected separately, using a FACSAria I flow-sorter at 40 psi (BD Biosciences, Erembodegem, Belgium). In cases where flow-cytometry detected more than one population of keratin-positive tumour cells, both populations were sorted independently. The most prevalent DNA population was selected to undergo SNP array analysis.

DNA isolation was performed as previously described.(21) In cases where endometrial or lymph node tissue was not available, DNA from sorted tumour stroma cell fractions was used as a reference.(21)

SNP array

The Golden gate Linkage panel V, consisting of 4 arrays with a total of 6000 SNPs (Illumina, San Diego, USA), was used to analyse tumour-derived DNA, together with DNA from matched normal/non-affected tissue. Assays were performed as previously described (22). Samples were processed in 6 batches of 48 samples, and samples of the same patient were always processed in the same batch. A 7th batch was used to repeat assays with low quality. The samples were genotyped in Illumina Beadstudio 2.3. The reference genotype clusters were derived from the normal samples in the dataset, and genotypes and allele intensities were extracted. The beadarraySNP package was used for further data processing. The analysis steps are depicted in Figure 1. SNPs that deviated significantly from Hardy-Weinberg equilibrium (at a significance level of 0.05, divided by the number of SNPs analysed = 0.00001), and SNPs with a call rate lower than 95% in controls, were removed to prevent the possibility of genotyping errors. All assays with a median intensity of below 2000 for one of the alleles were rejected and repeated in batch 7. Normalization was subdivided in 4 steps: I) normalize intensities for dye effect - quantile normalization was applied to make the distribution of the intensities for both dyes identical, II) per sample between assay quantile normalization to equalize intensity differences, III) within sample normalization to scale the median intensity of each allele to 1, IV) per SNP between sample normalization using the reference samples (which scales the total intensity of SNPs and corrects the allele specific bias by using a linear model between the B-allele ratio and total intensity). Matched normal samples were used to select the informative heterozygous SNPs. The LAIR was calculated for all informative SNPs in the tumour samples. The LAIR value is

basically the B-allele ratio, the ratio of the intensity of the B-allele and the total intensity, mirrored on its symmetry axis at 0.5, and scaled to a value between 0 and 1. This makes it easy to compute averages and enables segmentation. In order to identify genomic regions with identical CN and balance state, the data were segmented using circular binary segmentation at the default settings of the DNAcopy R package.(23) For each tumour, first the signal intensity of each chromosome was segmented, followed by a sub-segmentation of the previously obtained segments of the LAIR.(16) By combining the continuous CN (signal intensity) and the LAIR value of a segment with the sample DNA index measured by flow-cytometry, we developed a new calling method that assigns an allelic state to each segment.(16) The allelic state can be summarized in several ways. In this study the allelic states were distinguished along 2 dimensions. For each segment, discrete CN and a balance state was assigned. The discrete CN assigned to segments is done in such a way that the average discrete CN values across all segments should be close to the sample DNA index. The allelic states were divided in 3 possible outcomes: balanced segments - with the same CN for both alleles and a LAIR value near 1; LOH - segments with only one allele present and a LAIR value near 0; imbalanced segments - with different CN for the two alleles and a LAIR value between 0 and 1.

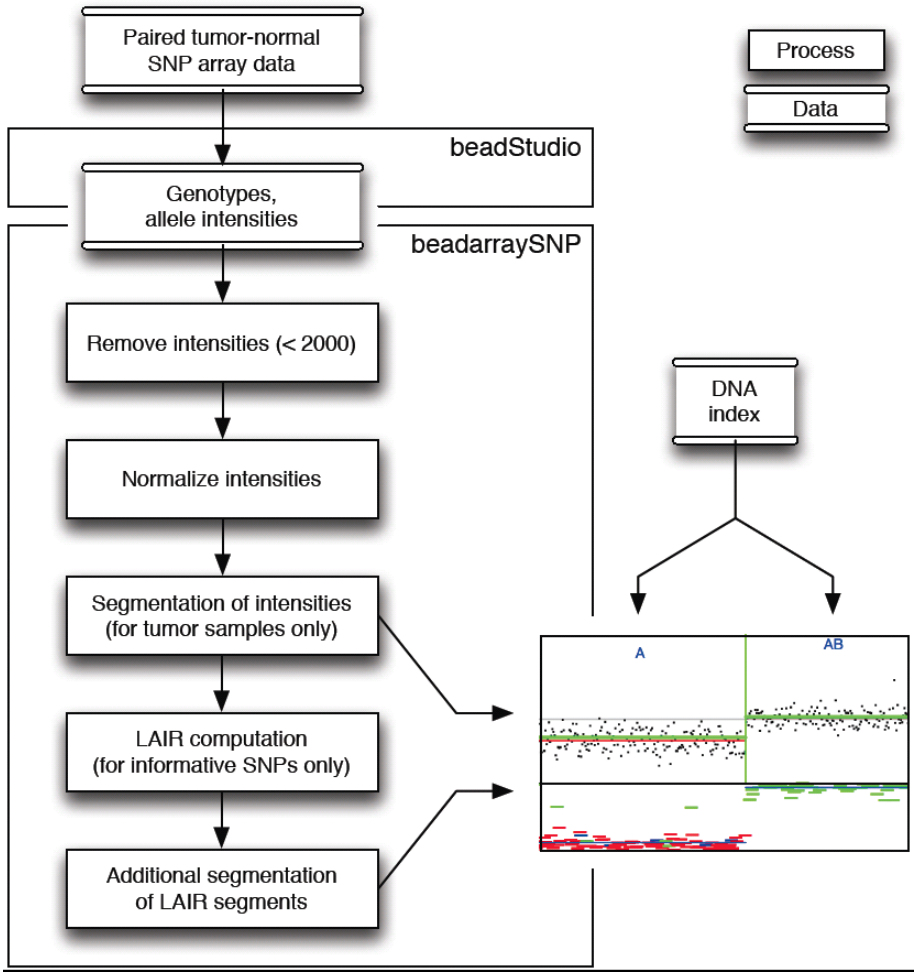

Figure 1 Analysis steps for SNP array data with the Illumina Beadstudio and the beadarraySNP package

Statistical analysis

Following the segmentation of all samples, the overlapping segments across all samples were reduced to unique segments. The global test was used to detect differences in genetic changes between groups of patients, both at the whole genome level and in chromosomal arms.(17) We tested differences in continuous and discrete CN, as well as LAIR and balance state. Continuous CN gains and losses were defined as deviating more than 15% from the sample average. The global test allows the use of confounders: DI was used as a confounder in the analysis of continuous CN, and ethnic group was used as a confounder for all tests. Ethnicity was defined by clustering the genotypes together

with HapMap samples of known ethnic origin. The tests were further localized by performing the global test on all chromosomal arms individually. Differences between groups were accepted as significant when the false discovery rate was lower than 0.05.(24)

All of our SNP-data can be found in the Gene Expression Omnibus: series GSE29143.

Results

Clinical data

From the 107 cervix carcinoma patients included in the study, sufficient DNA material was obtained for 82 matched tumour/normal pairs after flow sorting. One sample was removed after hybridization due to low data quality. Table 1 shows detailed information on the 81 patients analysed. As 96.3% of the tumours were found to have a tumour diameter larger than 40 mm, this parameter was not explored further.

Principal Components Analysis (PCA) was performed using the 4 original HapMap populations as reference panels, together with genotypes obtained from the patients' normal tissue. Three major genetic clusters could be distinguished in the four HapMap populations, with the Japanese and Chinese HapMap populations clustering together. Guided by this clustering, we classified the patients into 3 ethnic groups: European (EUR) for patients that cluster together with the CEU HapMap population, African (AFR) that cluster together with YRI, and Asian (ASI) that cluster together with the CHB and JPT populations. For more detailed information, see Supplemental material (Figure S1).

Table 1 Clinical data of 81 SNP analysed patients

	Mean	**Min - Max**
Age (yrs)	47	25 - 87
	N	**Percentage**
Place of residence		
Netherlands	47	58
Surinam	32	39.5
Other	2	2.5
Ethnic cluster		
European	45	55.6
African	25	30.9
Asian	11	13.6
FIGO stage		
1b2	59	72.8
2a	19	23.5
2b	3	3.7
Histological type		
Squamous carcinoma	52	64.2
Adenocarcinoma	3	3.7
Adenosquamous carcinoma	20	24.7
Other/mixed	6	7.4
Growth pattern		
Exophytic	40	49.4
Barrel shaped	41	50.6
Infiltration depth		
0 - 5 mm	4	4.9
6 - 10 mm	11	13.6
11 - 15 mm	16	19.8
> 15 mm	44	54.3
Tumour diameter		
21 - 40 mm	1	1.2
> 40 mm	78	96.3
Unknown	2	2.5
Tumour in parametria		
No	57	70.4
Yes	24	29.6
Tumour in lymph nodes		
No	47	58
Yes	34	42
Vaso-invasion		
No	36	44.4
Yes	39	48.1
Unknown	6	7.4

Samples

Most of the 81 matched tumour samples (89%) could be paired with normal/non-affected tissue. As normal tissue was not available in 9 cases, the tumour DNA was instead paired with the DNA from normal stroma cells obtained from the flow sorting procedure (21). Cell sorting detected the presence of more than one tumour cell population in 8 cases. For these cases, the most prevalent DNA population was selected to undergo SNP array analysis. The DNA-index (DI) of flow-sorted samples varied from 0.92 to 2.56. Figure 2 shows the DI density plot of the patient group.

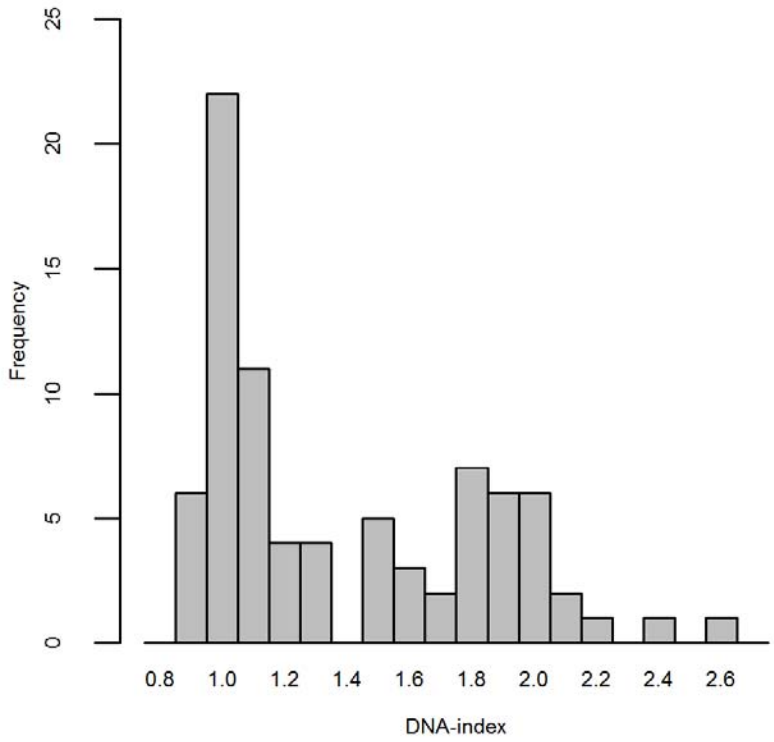

Figure 2 Distribution of the DNA index in 81 cervical tumour samples

Since we consider the DI to be an important factor in determining the CN profile of a tumour, DI was used as a confounder in the subsequent association analysis between continuous CN values and the 6 clinical parameters studied. DI was not used as a confounder for the analysis of discrete CN. As explained in the supplementary material, this factor is already taken into account in the translation of continuous to discrete CN. Ethnicity was used as a confounder in the analysis of the discrete and continuous CN, LAIR, and balance state, since genetic background is expected to influence the genetic profile of an individual.

Overall genetic pattern

Balance state

When looking at the balance state patterns generated by the analysis of the SNP array data, it can be observed that LOH is present in almost all chromosomal regions (Figure 3). In 10-20% of the patients, LOH was found on 28 chromosome arms. LOH was

particularly frequent on chromosome arms 3p, 4p, 6p, 6q, and 11q, where it was observed in more than 40% of all patients.

Copy number alterations

CNA using the continuous CN can be seen throughout the genome (Figure 4). More than 20% of the patients show gains on 1q, 3q, 5p, 8q, and 20q and losses on chromosomes 2q, 3p, 4p, 11q, and 13q. Gain on 3q was found in >40% of all samples.

Relation between clinical parameters and genetic changes

Balance state

Table 2A shows the results of the whole genome analysis of LAIR and the balance state. When the 22 autosomal plus the X chromosome were analysed together, only histological type showed statistically significant differences in LAIR value (p=0.035). No differences between the different clinical parameters were observed in balance state (p=0.050). Focusing on the chromosome arms individually showed that the difference between histological groups could not be attributed to a specific chromosome arm.

Copy number alterations

Table 2B shows the result of the whole genome analysis for changes in CN. Continuous CN values showed statistically significant differences only for histological types (p=0.011). The discrete CN profiles of patients with and without lymph node metastasis were significantly different (p=0.032). However, the DNA index (DI) is an important parameter in the translation of discrete CN from the continuous CN. When DI was included as a confounder in the analysis of discrete CN, a difference was found between histological types (p=0.019), while the difference between patients with and without lymph node involvement was no longer significant (p=0.637) - see also supplementary data. We then zoomed in on individual chromosome arms when analysing the clinical parameters that showed a difference. Squamous tumours showed greater losses on 2q (FDR=0.004), while adenosquamous tumours were found to have more gains on 7p, 7q, and 9p (FDR=0.006, FDR=0.004, and FDR=0.029 respectively). For discrete CN, the differences between groups with and without lymph node involvement could not be attributed to any of the chromosome arms in particular. Figure 5 shows the differences in continuous CN for histological type on the different chromosome arms.

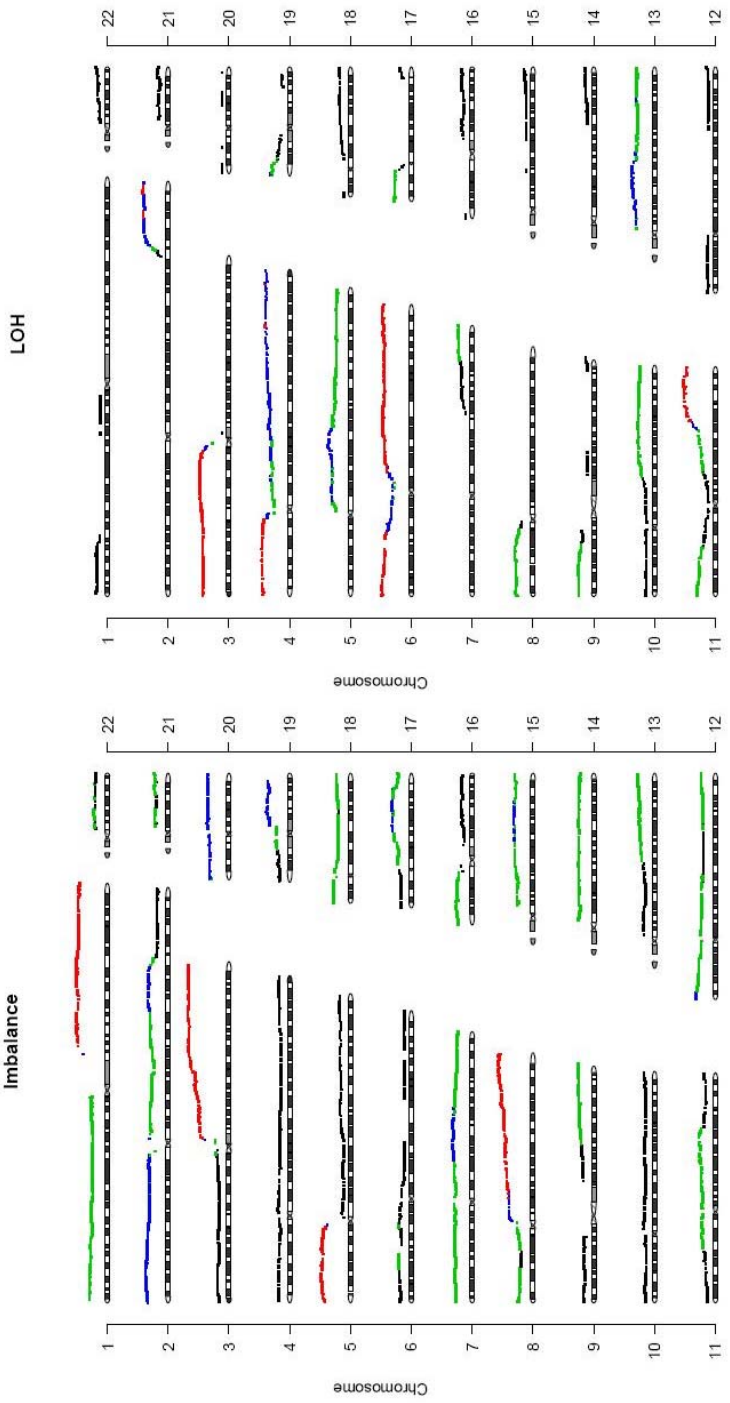

Figure 3 Frequency of imbalance and LOH per chromosome. The colours indicate the frequency of LOH; black: > 10 %, green: > 20%, blue: > 30%, red: > 40%

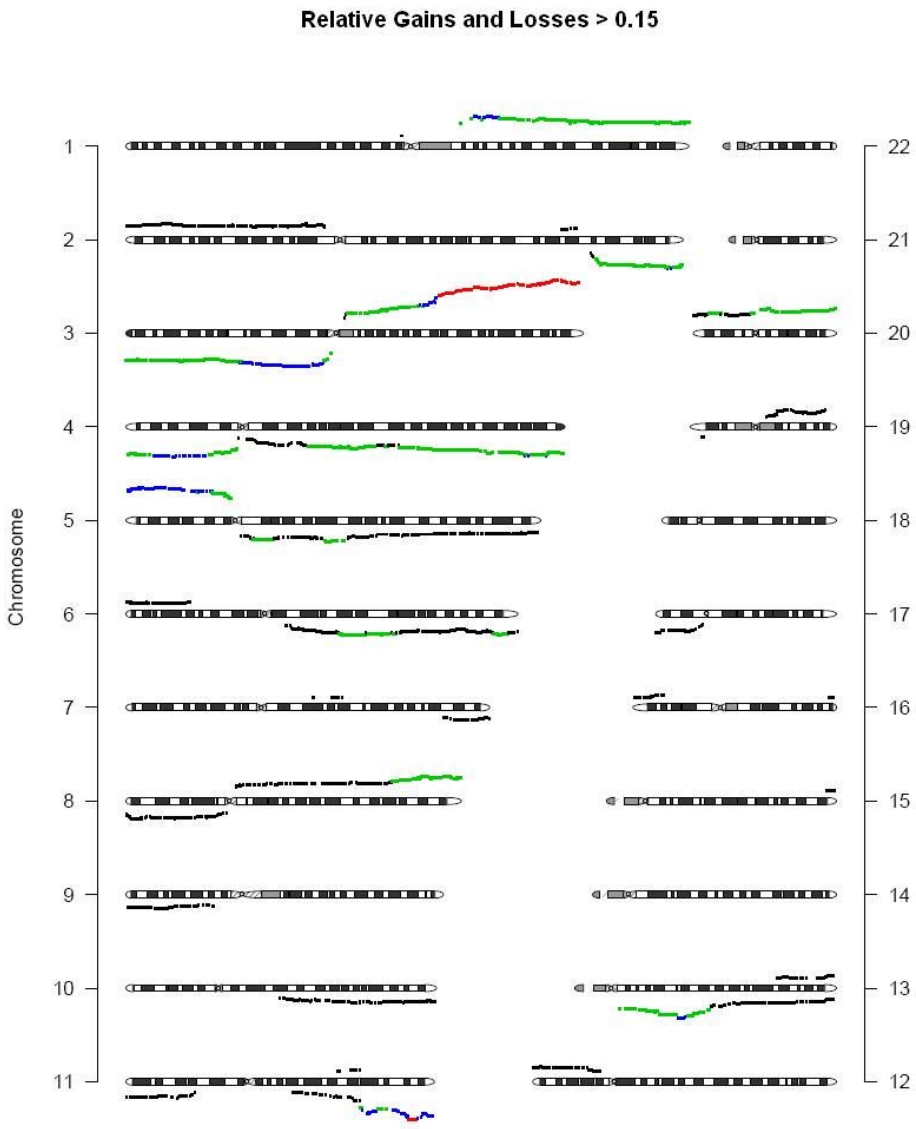

Figure 4 Frequency of gains and losses. Gains are depicted on top of the ideograms, while losses are depicted below. The colours indicate the frequency within the dataset; black: > 10 %, green: > 20%, blue: > 30%, red: > 40%. Gains and losses were identified when the continuous CN deviated more than 15% from the sample average.

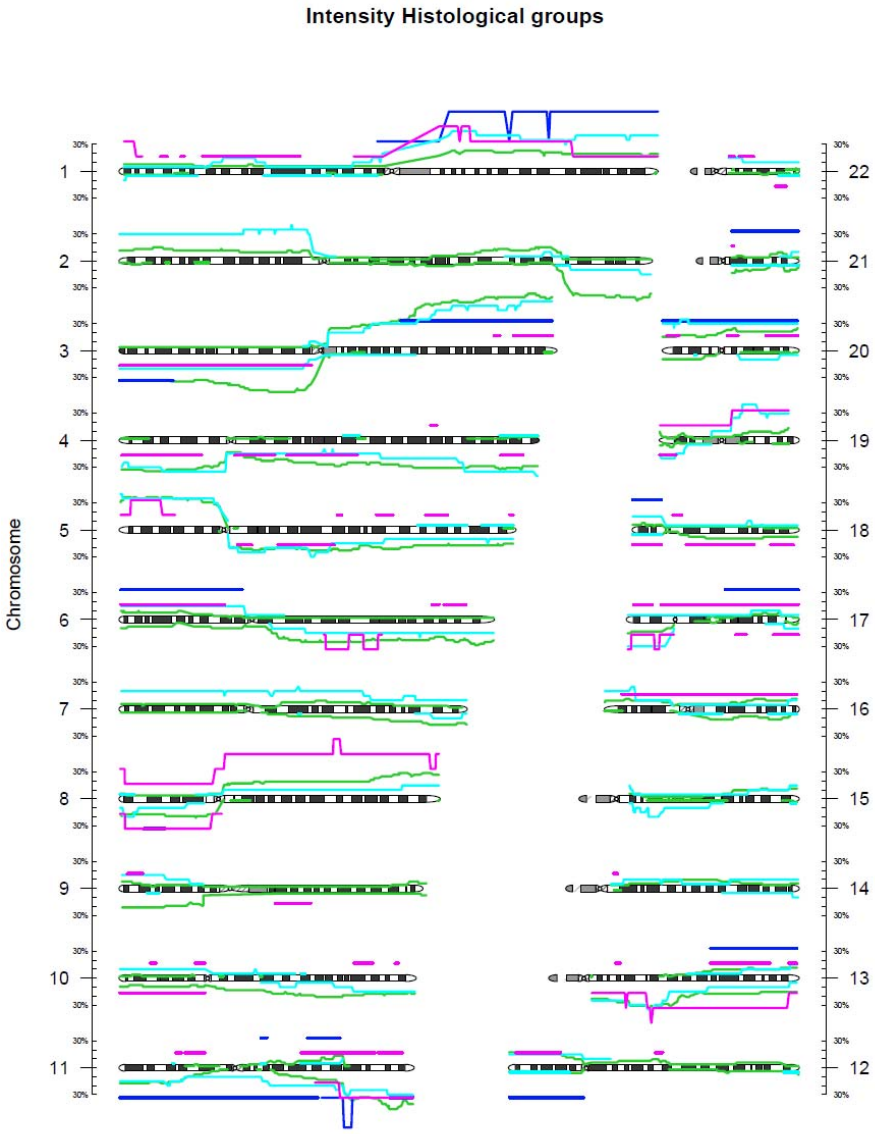

Figure 5 Frequency of gains and losses in 4 histological groups. Gains are depicted on top of the ideograms, while losses are depicted below. Green: squamous tumours, blue: adenocarcinoma, light blue: adenosquamous tumours, pink: mixed type.

Table 2 Whole genome analyses of genetic changes and clinical parameters

A. Whole genome analysis of balance *		
	LAIR	**Allelic Balance**
	P-value	**P-value**
Clinical parameter		
Growth pattern	0.244	0.184
Histological type	**0.035**	0.050
Infiltration depth	0.651	0.509
Lymph nodes	0.626	0.683
Parametria	0.928	0.769
Vaso-invasion	0.146	0.301

B. Whole genome analysis of Copy Number *			
	Continuous variables	**Discrete variables**	**Extra confounder DI**
	P-value	**P-value**	**P-value**
Clinical parameter			
Growth pattern	0.346	0.209	0.396
Histological type	**0.011**	0.363	**0.019**
Infiltration depth	0.397	0.967	0.338
Lymph nodes	0.614	**0.032**	0.637
Parametria	0.695	0.383	0.654
Vaso-invasion	0.213	0.744	0.083

* confounder: Ethnic cluster

Discussion

Using SNP array analysis, we have shown extensive genome-wide LOH and CN changes in bulky cervical cancer. To our knowledge, no previous studies have applied genome-wide genetic profiling to such a large number of bulky cervical cancers (FIGO stage 1b2-2b).

SNP arrays were used to assess genome-wide LOH and CNA, and known problems in SNP array analysis of tumour material were overcome by combining several techniques. Although high-density SNP array platforms require high quality DNA, prohibiting the use of archival formalin-fixed paraffin-embedded (FFPE) tissue, the 6000 SNP Illumina GoldenGate assay has been shown to be reliable for genotyping, LOH detection, and measurement of CN on the fragmented DNA obtained from archival FFPE tissue.(22;25) As many bulky cervical cancers were only available as FFPE material, the use of the GoldenGate SNP array allowed us to access a much higher number of samples.

The malignant cells of a tumour co-exist with normal cells, e.g. connective tissue, blood vessels, or infiltrating immune cells. Given this admixture of tumour and normal cells, the genetic analysis of DNA isolated from whole tissue sections is commonly affected by a dilution of the measured intensity signal.(26;27) As the use of pure tumour cell populations improves the detection of genomic changes, we first isolated pure cancer cells using FACS, with the additional advantage of allowing the measurement of the cell DNA content.

The calling method used (see methods) assigned a discrete CN and a balance state (balance, imbalance and LOH) to each segment of a sample. Using both a discrete CN and a balance state per segment enables a more intuitive interpretation of the genetic changes observed. The discrete CN values, as we defined them, also gave us an absolute DNA content per segment. An example would be a segment of CN 3 in a tetraploid genome (DI = 2) - this is a gain, and not the loss it would have been interpreted as if only the continuous (relative) CN value of that segment had been taken into account. Because we were interested in establishing the contribution of not only relative (current practice in the field) but also absolute CN changes in tumour genesis, we performed statistical tests on both the continuous and the discrete CN values.

Regarding allelic balance state, we found LOH most frequently (in >40% of all samples) on chromosome arms 3p, 4p, 6p, 6q, and 11q. CNA were even more frequent: gain was predominantly (>20% of all samples) found on 1q, 3q, 5p, 8q, and 20q and loss on 2q, 3p, 4p, 11q, and 13q. Gain on 3q was found in >40% of all samples.

We also analysed the relation between genetic changes and the prognostic factors histological type, infiltration depth, lymph node status, extension to the parametria, vaso-invasion, and growth pattern. A statistically significant relationship was found in the whole genome analysis between histological type and changes in LAIR and continuous CN. A significant difference was found in the analysis of the discrete CN between groups with and without lymph node involvement. Even though the discrete CN did not show differences between the different histological types, we observed that when the DNA index was used as a confounder in the analysis the significant difference between the histological groups was restored (p-value goes from 0.363 to 0.019). This signifies that the discrete CN values contain ample information to distinguish the groups, but that relative changes in DNA compared to the average DNA content of a cell are more important for the difference between histological groups than the absolute allele count.

In order to specify the genetic changes that contribute to the differences in clinical parameters, we analysed chromosome arms individually. In the analysis of continuous CN changes, the histological group squamous carcinomas showed a statistically significant increase in loss on 2q, while adenosquamous carcinomas showed more losses on 7p, 7q, and 9p. Despite the whole genome difference in discrete CN between patients with and without lymph node involvement, we could not link this difference to a specific chromosomal arm. Thus, this difference cannot be used to extract a clinical parameter.

To our knowledge, no other large whole genome SNP array study of bulky cervical cancer has yet been reported. Because we did not find a relation between LOH and a clinical parameter in the chromosome arm analysis, we have not compared our findings

with earlier studies. CNA were found in previous studies using classical array-CGH, which examines only continuous CN changes (see Supplementary Table) (28-37). Some of those studies also investigated the relation between clinical parameters and genetic changes in cervical cancer (see Supplementary Table).(28;29;32;33;35) Rao et al. found no differences between squamous and adeno tumours stage 1b – 4b. This may be due to the small number of adenocarcinomas included (5 adenocarcinomas and 72 squamous carcinomas).(33) There were no adenosquamous tumours in this group. In an analysis of stage 1b - 3b cervical tumours, Wilting et al. reported significantly more gains in 9 squamous tumours as compared to 7 adenocarcinomas. Higher gains were predominantly found on 3q (35), although the method used to define the histological type was not described. The (locations of) the differences in genetic alterations between squamous and adenosquamous carcinomas in our study did not coincide with the findings of Wilting et al. , but there were no adenosquamous tumours in this group. Differences in patient characteristics and methods cause difficulties when attempting to compare the results of previous studies with both our results and with each other. The gains and losses that we found have been reported before, but not in all studies. The difference in findings between our and previous studies may be explained by differences in FIGO stage, sample size, and staining techniques to discriminate histological type. Dilution caused by the use of tumour tissue instead of pure tumour cells may also explain differences in results.

The prognostic relevance of histological type is still an object of debate and is not currently used for primary or adjuvant treatment choice.(1-4;38-40) We used additional PAS and Alcian blue staining to determine the histological type of the tumours, which can be hard to identify without this staining. In fact, no statistical differences could be defined when using only standard H&E staining to distinguish histological types. Conflicting results of studies on the effect of histological type on tumour behaviour and patient survival may also be due to differences in the classification of tumours, where no specific staining was applied. Future studies on genomic alterations are advised to take the different genetic profiles of histological types into consideration.

The regions with the most prominent differences contain many genes. The aim of this study was to find additional pre-operative parameters that could be used for treatment choice, without knowing the causative gene. This topic deserves further investigation.

The SNP array used in this study consisted of 6000 SNP markers distributed evenly over the genome, and the array was optimized to have a higher minor-allele frequency in the Caucasian (European) population. As a consequence, the average number of informative probes was higher for the Caucasian patients (2139 informative SNPs) than for the African (1796) and Asian (1712) patients. This may result in an underestimation of the genetic changes that take place in cervical cancer in these populations. Although it would have been be interesting to compare genetic changes and the relationship to

clinical parameters by ethnic background, the subgroups are too small to allow this. The same applies to subgroup analyses based on other characteristics.

Our results revealed genetic changes related to histological type, a clinical parameter not currently used for treatment choice. Our analysis showed no relation of genetic changes to clinical parameters that could be used to predict unfavourable post-operative prognostic characteristics or select subgroups of patients. It seems that, at present, the best evaluation of prognostic factors still comes from pre-, intra-, and post-operative findings of the gynaecologic oncologist and pathologist. As differences in DNA alterations between tumours of different histological types may have an impact on tumour behaviour, treatment response and survival, future research should include larger patient groups, and use staining techniques that can reliably distinguish histological type.

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Supplemental material

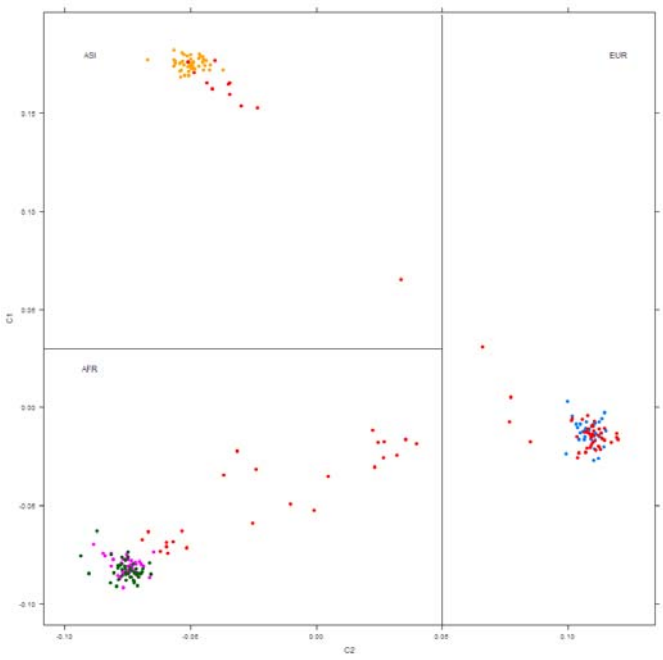

Figure S1 Classification of patient ethnicity in relation to HapMap populations. Red dots are patients, while blue, yellow, green, and pink dots correspond to European, African, Japanese and Chinese populations, respectively.

SNP arrays

Using SNP array data, we generated both continuous and discrete copy number (CN) values, as well as lesser allele intensity ratio (LAIR) values (ranging from 0 to 1) and balance states, for each of the 81 samples analysed. The continuous CN values and LAIR values are plotted against each other in figure S2, where colour is used to indicate balance state.

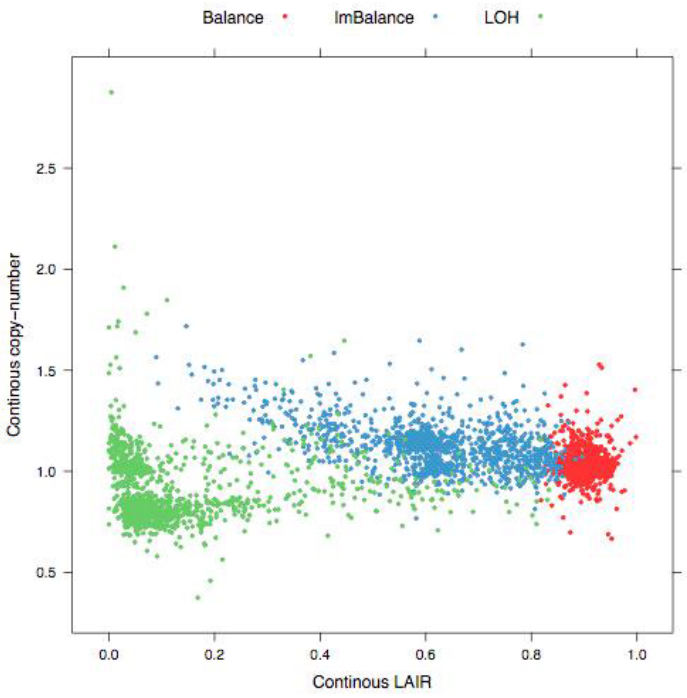

Figure S2 Balance state classification in relation to continuous CN and LAIR. Red dots indicate balanced state, blue dots imbalanced state and green dots indicate LOH.

In this plot, balanced segments (showing the same CN for both alleles) are expected to appear on the right side, with a LAIR value near 1. Segments with only one type of allele present (LOH) are expected to appear on the left and show a LAIR value near 0. Segments with different CN for the two alleles (imbalanced) are expected to appear in between these two extremes. Figure S1 shows many segments, classified with LOH, in the intermediate area rather than the left side. This is due to our calling procedure, which categorizes segments not only based on the LAIR values but also takes the discrete CN of the segment into consideration. Such shifts arise when an intermediate LAIR value for a segment with CN 1 is called as LOH.

We also plotted the continuous CN of each segment against its discrete CN (figure S3). The discrete CN assignment to segments is done in such a way that the average discrete CN values across all segments should be close to the sample DNA index (DI). As can be observed, the continuous and discrete CN show some correlation, but the ranges of continuous CN for the various discrete CN show significant overlap with each other, even after multiplication of the continuous CN by the sample DI. This can be explained

by a non-linear relationship between CN and the measured intensity on the array, complicated by the fact that, at least for some segments, there may be some heterogeneity within the sample, i.e. two or more cell populations with different CN. The average CN for such segments will not be an integer number.

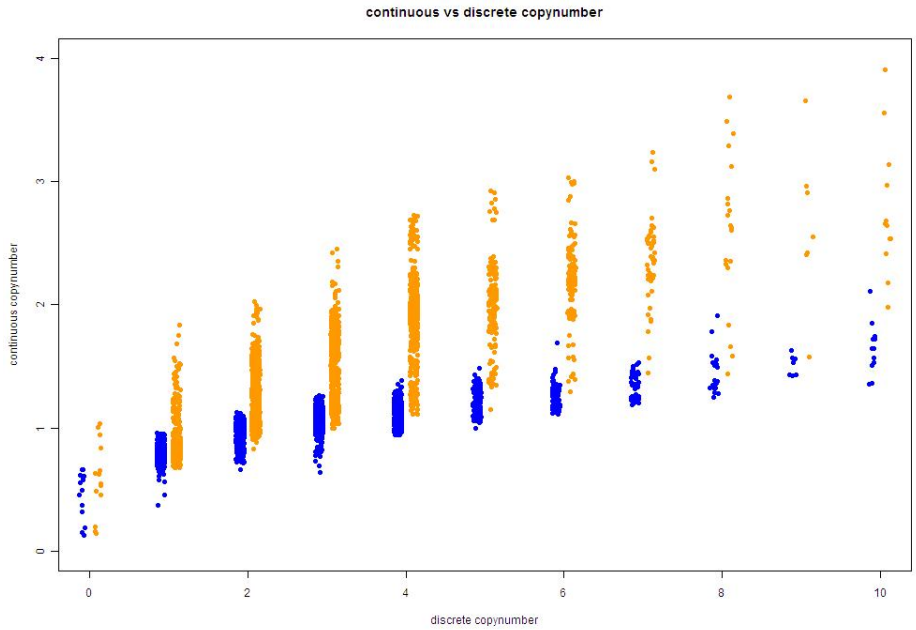

Figure S3 Relationship between discrete and continuous CN. The blue dots represent the continuous CN, while the orange dots represent continuous CN multiplied by sample DI.

Supplementary Table Main characteristics and results of arrayCGH studies on cervical tumours

Study	N	FIGO stage	Other notes	Tumour population	Main results
Current study	81	1b2 – 2b	Bulky tumours (> 4cm)	Pure tumour cells	Gain in >20% of all samples on 1q, 3q, 5p, 8q, and 20q. Gain on 3q in > 40%. Loss in >20% of all samples on 2q, 3p, 4p, 11q, and 13q. Squamous carcinomas showed significantly more losses on 2q. Adenosquamous carcinomas had more losses on 7p, 7q, and 9p.
Hesselmeyer et al.	30	2b - 4		FFPE tumour tissue	Gain on 1q (47%), 3q (77%), 5p (30%), 6p (27%), 20p and 20q (23%). Loss on 2q (33%), 3p (50%), 4p and q (33%), 8p (23%), and 13q (27%).
Dellas et al.	62	1b		FFPE tissue containing at least 75% tumour cells	Gain on 3q (15%), 17p (30%), 17q (27%), and 20q (16%). Loss on 3p (52%), 4p (44%), 4q (53%), 5q (40%), 6q (35%), 13q (45%), and 18q (37%). Deletions on 9p were more frequent in lymph node positive patients. Loss on 11p and 18q was related to a worse prognosis in lymph node positive patients. The clinical parameters invasion depth, vaso-invasion, and histological type were used as confounder but not analyzed separately.
Allen et al.	32	1b		Microdissection of FFPE tissue	Gain on 3q (44%). Loss on 3p (56%), 6q (22%), 10q (16%), and 11q (47%). Total number of gains and losses higher in lymph node positive patients. No significant differences between lymph node positive and negative patients in chromosome arm analysis.
Yang et al.	20	1b (N=16), 2a (3), 2b (1)	Adeno carcinomas, including intestinal and endometrioid type (N=5)	Frozen tumour tissue	Gain on 1q (25%), 1p (30%), 3q (70%), 11q (20%), and 17q (45%). Loss in 4 of 20 samples on 4q, 13q, or 18q.
Umayahara et al.	18	1a1-1b1	Squamous tumours	FFPE tissue containing tumour	Gain on 1p (33%), 1q (42%), and 3q (67%). Loss on 2q (33%), 3p (25%), 4p (25%), 4q (25%), 6p (25%),

					11p (25%), 11q (67%), and 17p (25%).
Rao et al.	77	1b – 4b	Adeno (N=5) and squamous (72)	Biopsies containing > 60% tumour cells	Gain on 1p (27%), 1q (20%), 3q (55%), 5p (30%), 8q (26%), 9q (25%), 13q (16%), 19p (17%), 20p (18%), and 20q (26%). Loss on 2q (57%), 3p (29%), 4p (30%), 4q (36%), 11q (36%), 13q (43%), and 17p (30%). No correlation with tumour diameter or histological type.
Hidalgo et al.	10	unknown		Biopsies containing > 70% tumour cells	Gain on 3q (59%), 5p (53%), 7p (53%), and 11p (47%). Loss in 47% of the tumours on 3p and in >29% on 1p, 4q, 8p, 9q, 13q, and 18q.
Huang et al.	24	1b – 2b			Gain on 1q (37%), 3q (47%), and 8q (20%). Loss on 2q (20%), 3p (33%), 6q (23%), and 11q (37%). Higher number of CNA in lymph node positive patients. Separate analysis of gains and losses showed no significant differences. More frequent gain on 3q and loss on 11q in lymph node positive patients.
Wilting et al.	16	1b – 3b and unknown (N=3)	Adeno (N=7) and squamous (9)	Frozen tissue containing > 70% tumour cells	CNA that were found in > 25% of all samples are reported: Gain on 1q (69%), 3q (69%), and 20q (63%). Loss on 8p (31%), 10q (31%), 11q (44%), and 13q (50% on one location and 38% on another location). Higher numbers of gains in squamous tumours, predominantly gain at 3q.
Lyng et al.	20	2b – 4b	Squamous (N=19) and adenosquamous (N=1) Study on intra-tumour heterogeneity	55 biopsies of 20 tumours	The most frequent gains were found on 1q, 3q, 5p, 8q, 19q, 20q and 22q (>50%) The most frequent loss on 4p (50%) and 13q (30%). The most frequent homogeneous aberrations: Gain on 3q (65%), 20q (65%) and 5p (50%).

Chapter 10

Summary and general discussion

Summary

This thesis describes studies on practical aspects of cervical cancer, concerning surgical considerations and aspects of tumour behaviour and tumour spread.

Chapter 1 gives an introduction on the thesis. The staging and treatment of low stage cervical cancer (FIGO 1a-2a) are described. Treatment choice for cervical cancer is primarily based on the clinical FIGO stage, and post-operative evaluation of prognostic parameters. Limitations of the staging system are addressed and possibilities for improvement are put forward. Recent advances in diagnosis and treatment are described, with an emphasis on modern techniques. The cohesion of the various studies that are described in this thesis is summarized. The thesis comprises studies on the surgical and biological aspects of cervical cancer. Emphasis is on distinctive tumour behaviour and relations with survival, aiming to contribute to treatment strategies tailored to the individual patient.

Surgical aspects

In chapter 2 a study on local recurrence rate, feasibility, and safety of non-nerve-sparing and nerve-sparing radical hysterectomy in cervical cancer patients stage 1a-2a is presented. The Leiden nerve-sparing technique has been routinely applied in the Leiden University Medical Center since 2001 because the damage that is caused to the pelvic autonomic nerves during traditional radical hysterectomy can lead to impaired bladder function, defecation problems, and sexual dysfunction. To clarify the debate about the possible threat of sparing the pelvic autonomic nerves to radicality, 246 patients with cervical cancer of stages 1a to 2a were analysed in a cohort study with 2 years of follow-up: 124 in the non-nerve-sparing group and 122 in the group where nerve-sparing was the intention-to-treat. Analysis of the patient data showed that the clinical characteristics of the treatment groups were comparable. Sparing the nerves unilaterally or bilaterally was possible in 80% of cases of the nerve-sparing group. Local recurrence rates in the non-nerve-sparing (4.9%) and nerve-sparing (8.3%) group were not significantly different. Mean local recurrence-free survival (LFS) within 2 years were 22.7 and 22.0 months, respectively. Univariate and multivariate regression analyses showed that nerve-sparing treatment was not an independent prognostic factor for local recurrence. With respect to per- and post-operative parameters, operating time and blood loss were less in the nerve-sparing group and mortality was equal (1 patient); the post-operative course of the nerve-sparing group was similar to the state-of-the-art of conventional radical hysterectomy. Based on the results of the study, the nerve-sparing technique for cervical cancer stages 1a to 2a is considered feasible and safe.

Chapter 3 describes the ‘Swift’ operation, a modification of the Leiden nerve-sparing radical hysterectomy that was developed in the LUMC, and routinely applied since 2007.

A report on the changes in the surgical approach and the results in the first 15 consecutive patients is presented. The report includes a stepwise explanation of the procedure. The Swift operation is more radical in the area of the uterosacral ligaments than the original nerve-sparing operation, and it dissects the hypogastric nerve free under direct vision. In the area of the parametria, it is more radical in the deep lateral part. The vascular parametrial tissue is dissected and separated ventrally from the ureters. From October 2006 to February 2007, 15 consecutive patients with cervical cancer stage 1a2 to 1b2 underwent the Swift operation. The extra operating time relative to conventional non-nerve-sparing radical hysterectomy amounted to 20 min, which was similar to the original nerve-sparing operation, and with no extra blood loss. The suprapubic catheter was removed after a median of five days. Up until the time that the report was written (February 2008), no recurrences were found in the patients. Based on the available results, the Swift procedure is easy to perform and offers advantages over the initial operation in terms of safety and radicality.

In chapter 4 the use of distilled water in the achievement of local haemostasis during oncologic surgery is described, and the possible impact on the peritoneum and on tumour cells is addressed. Distilled water is used to check on haemostasis at the end of pelvic oncologic operations. Nevertheless, reports about this procedure are lacking. This is regrettable because the method might be interesting for other surgeons. Also, discussion about possible favourable and unfavourable side effects is impossible without publications. After extensive pelvic surgery, e.g. oncologic operations or surgery for endometriosis, the surgeon can be confronted with a raw, oozing area in the pelvis. Stopping small venous bleeders to achieve adequate haemostasis is often a difficult task in these areas. In contrast to NaCl 0.9%, which gives a blurred view through an opaque fluid, distilled water causes rapid lysis of erythrocytes resulting in a transparent fluid in which a small source of bleeding is easily recognizable. A possible side effect of the lavage might be contribution to the formation of peritoneal adhesions by confusing the abdominal defence system. Systemic side effects are not to be expected. Although tumour cells might suffer from hypotonic distilled water lavage, the current use of distilled water at the end of surgery is probably too short and not effective to lyse tumour cells. The study explains the mechanism that causes the beneficial and possible negative effects of distilled water lavage to achieve haemostasis after extensive pelvic surgery. The study results support the on-going use of distilled water for this purpose.

Tumour behaviour and tumour spread

In 2005, an interesting theory concerning local tumour growth was postulated by Höckel et al. A topographically defined anatomical area, derived from common embryonic precursor tissue was described, and named the Müllerian ‘morphogenetic unit’ (MGU). The structures surrounding and surrounded by the MGU are derived from different embryological precursor tissue than the MGU itself. Höckel et al. postulated that, until

late in disease progression, local spread of carcinoma of the uterine cervix is restricted to the MGU. Hence it is very important to remove the complete MGU during surgery. Leaving (part of) the MGU in situ enhances the risk of recurrences according to this theory. Höckel et al. described the ‘total mesometrial resection’ (TMMR), developed on the basis of their findings and theory. Although the theoretical argumentation of the TMMR is extensive and the results of Höckel et al. with respect to survival are convincing, the translation of theory and embryological or cadaver-based findings into surgical practice can be difficult. In chapter 5 a closer look is taken at the MGU in vivo, to describe the possible difficulties in the translation of the theory of the MGU into clinical practice. MRI-scans were performed of healthy premenopausal women to visualise the MGU. A horseshoe-shape, like described by Höckel et al. as the shape of the MGU in the dorsal plane (ligamentous mesometrium, tails of the Swift), could be distinguished in most women. However, it is difficult to define exact borders of this area that might represent the MGU. The differences between the individuals are numerous, and body composition of the group of young women, with relatively low BMI's, might play a role in this. Furthermore, experiences with the excision of the MGU during the Swift operation in the LUMC are described.

A tumour in the parametria, either continuous with or separate from the primary malignancy, is an unfavourable prognostic factor in cervical cancer. The local spread of cervical cancer in the parametria has been described, but the mechanism of spread has not been determined in detail. In chapter 6 patterns of parametrial tumour involvement in radical hysterectomy specimens of cervical cancer patients are described, with emphasis on continuous and discontinuous growth and the effect on metastasising potential. The incidence of parametrial tumour deposits localised in blood or lymph vessels, or as isolated foci in the connective tissue, and the relationship of these involvement patterns with pathologic characteristics and prognosis, were investigated. In 79 of 763 surgically treated cervical cancer patients (10%), tumour was found in the parametria of the hysterectomy specimen. The available patient material was reviewed to discriminate between continuous and discontinuous parametrial tumour growth. The involvement pattern for discontinuous growth was specified on the basis of immunohistochemical staining with different specific markers. Fifty percent of the parametrial tumour involvement found postoperatively was caused by continuous extension of the primary process into the parametria. In the other 50%, the parametrial tumour was separate from the primary process. In this discontinuous group, a frequent presence of tumour in lymph nodes and/or lymph vessels (together 79%) was found, and even a rare appearance of tumour in blood vessels (14%). A tumour was further found in unspecified vessels in 2 patients (5%), and as isolated foci in 6 patients (14%). Fourteen patients (33%) had more than 1 involvement pattern. Positive pelvic lymph nodes were more frequent in the discontinuous group. The involvement pattern was no independent predictor of overall survival (OS). Parametrial blood vessel involvement was related to the development of distant metastases (HR: 12.7; 95% CI: 1.6–103.3).

The majority (79%) of parametrial involvement in the discontinuous group is caused by lymphatic metastases. Parametrial blood vessel involvement might be an independent predictor for the development of distant metastasis.

Chapter 7 describes the incidence of cervical cancer scar recurrences and the characteristics of the patients who developed this special manifestation of a metastasis, which is generally considered as a local recurrence. Tumour recurrence in the surgical scar after radical hysterectomy for cervical cancer has been reported, but the incidence is unknown and data about patient and tumour characteristics and follow-up are lacking. In the study that is described in chapter 9, the data of all patients who were surgically treated for cervical cancer in our centre between 1984 and 2007 was reviewed. All patients with a scar recurrence were selected and for each case, 5 random controls were selected. Clinical characteristics were compared between the cases and controls. Eleven (1.3%) of 842 patients developed a scar recurrence. The mean time between surgery and scar recurrence was 16 months (range, 2-45 months). For 8 patients (73%), the scar recurrence was the first disease recurrence. Five patients (45%) died, and 2 (18%) were lost to follow-up. The mean time between scar recurrence and death was 9 months. Ninety-one percent of the cases had recurrent disease besides the scar recurrence during follow-up. The case group had a higher percentage of advanced FIGO stage, postoperatively found involvement of parametria or resection margins, and tumour diameter greater than 4 cm, whereas lymph nodes were more often involved in the control group. Concluding, the incidence of scar recurrences after primary surgery for cervical cancer was 1.3%. The time to development was variable, and the prognosis was poor. Besides higher FIGO stage and concurrent unfavourable pathological characteristics, no particular characteristics of patients with a scar recurrence were found. Notable was the finding that scar recurrences go hand in hand with recurrent disease at other locations. Scar recurrences may be a manifestation of tumours with extensive metastatic potential rather than an effect of surgical handicraft at the time of the operation.

In chapter 8 a study on MRI-derived morphologic characteristics that might support the pre-operative clinical discrimination between bulky cervical tumours with a different response on primary surgical treatment is presented. Bulky cervical cancer (>4 cm; ≥stage 1b2) can be divided in barrel shaped or exophytic growing tumours. Although a survival difference after primary surgery between bulky cervical tumours with different growth patterns has been reported (our group), morphology is not accounted for in the FIGO staging system. In the study that is described in chapter 8, MR images of 24 patients with cervical cancer stage ≥1b2 were analysed to determine whether the shape of cervical tumours and the presence of fluid in the uterine cavity could discriminate between bulky tumours with a favourable or worse prognosis after surgery. The ratio between tumour width and length (Barrel Index: BI) and the presence of intra uterine fluid retention were related to survival in a multivariate regression analysis. A

relation with disease free and overall survival was demonstrated for both parameters. BI and intra cavital fluid were predictors of survival, independent from tumour diameter and other known important factors for survival. A cut-off value of 1.40 for the BI proved to be the best prognostic factor with respect to recurrence and death: the hazard ratios of BI >1.40 as compared to BI ≤1.40 were 18.9 (95% CI 2.8 to 125.6) for recurrent disease and 16.4 (95% CI 2.9 to 93.9) for death by cervical cancer. The hazard ratios of intra cavital fluid retention were 73.6 (95% CI 5.3 to 1016.4) and 48.1 (95% CI 4.7 to 491.6) for recurrence and death respectively. Thus, the morphologic characteristic Barrel Index and the presence or absence of intra cavital fluid as determined by MRI might have predictive value for survival in patients with bulky cervical tumours. The findings of the study suggest that growth pattern and intra cavital fluid estimation determined by MRI might be helpful in identifying a subgroup of individuals with bulky cervical cancer with a better prognosis, and therefore eligible for primary surgical therapy.

The study that is described in chapter 9 attempted to contribute to the assessment of parameters of tumour behaviour and tumour spread that are important for staging and treatment choice. Genomic changes in bulky cervical tumours and their relation to the clinical prognostic parameters barrel shaped or exophytic growth pattern, tumour diameter, parametrial and lymph node involvement, vaso-invasion, infiltration depth, and histological type, were evaluated using single nucleotide polymorphism (SNP) analysis. A whole genome SNP analysis was performed in patients with bulky cervical cancer tumours (stage 1b2-2b). Flow-sorted pure tumour cells and patient matched normal cells were extracted from 81 bulky cervical cancer patients. DNA-index (DI) measurement and whole genome SNP-analysis were performed. Data were analysed to detect copy number alterations (CNA) and allelic balance state: balanced, imbalanced or loss of heterozygosity (LOH), and their relation to the clinical parameters. The DI varied from 0.92 to 2.56. LOH was found in ≥40% of samples on chromosome arms 3p, 4p, 6p, 6q, and 11q, copy number (CN) gains in >20% on 1q, 3q, 5p, 8q, and 20q, and losses on 2q, 3p, 4p, 11q, and 13q. More than 40% showed gain on 3q. The only differences were found between histological types (squamous, adeno and adenosquamous) in the lesser allele intensity ratio (LAIR) ($p=0.035$) and in the CNA analysis ($p=0.011$). More losses were found on chromosome arm 2q (False Discovery Rate; FDR=0.004) in squamous tumours and more gains on 7p, 7q, and 9p in adenosquamous tumours (FDR=0.006, FDR=0.004, and FDR=0.029). Concluding, whole genome analysis of bulky cervical cancer shows wide spread changes in allelic balance and CN. The overall genetic changes and CNA on specific chromosome arms differed between histological types. No relation was found with clinical parameters that are currently used for treatment choice.

General discussion

In this thesis, studies on surgical and biological aspects of cervical cancer are presented. The aim of the studies was to strengthen the knowledge of these aspects of tumours of the uterine cervix, with emphasis on prognosis and survival. The findings might contribute to improving treatment strategies with an optimal balance between cancer cure and quality of life, tailored to the individual patient. In the general discussion, present implications of the results of the studies in this thesis, and possibilities for future advances are shortly addressed.

The study that is described in chapter 2 showed no statistically significant change in local recurrence rate and LFS after radical hysterectomy between the periods before and after introduction of nerve-sparing surgery for cervical cancer stages 1a to 2a. This finding of unaffected local and loco regional recurrence rate is emphasized by the comparison of the 2-year LFS and the 5-year OS (secondary end point of our analysis) of the 2 groups. The local recurrence rates and LFS within 2 years were consistent within the range of known data from the literature, for both treatment groups.(1-5) Comparing the per-operative and post-operative results and complications with the ‘state of the art’ showed that for mortality, secondary bleeding, wound, pelvic, and respiratory tract infection, and bowel obstruction in the nerve-sparing group of the study were similar to the state of the art. Results concerning postoperative urinary tract infections, thrombosis, operating time, and postoperative hospital stay were better in the study group.(6) With the methods used, a tendency to operate patients with more advanced tumours (more prognostically unfavourable characteristics) or a favourable effect of the on-going time on operation results could not be excluded. Although there was no reason to believe that these effects were major, comparing nerve-sparing and non-nerve-sparing surgery in a randomized controlled trial is suggested to give a definite answer to the question whether nerve-sparing is a threat to radicality. Furthermore, although the results of the study are promising, longer follow-up is recommended.

According to the theory of the MGU, the deep lateral parametrium as it is looked upon conventionally does not exist. As mentioned in chapter 3, after an extensive and deep lymphadenectomy in which the lymph-bearing tissue dorsally from the superior vesical arteries is also removed, hardly any tissue remains in the area that is called the deep lateral parametrium (apart from the autonomic nerves). The only tissue that can be found is directed in a more distal course towards the bladder and is to be regarded as bladder mesentery. Chapter 3 described the adjustment of the Leiden nerve-sparing radical hysterectomy based on the TMMR. In the Swift operation nerve-sparing is realized by meticulously separating the nerves from all adjacent tissue, resulting in the dissection of a complete tissue sheet that is consistent with the description of the MGU, and contains the uterosacral ligament, but is more extensive towards the rectum and the pelvic side wall than the original nerve-sparing operation. In the original operation,

nerve-sparing was acquired by separating the uterosacral ligament in a ligamentous and a nerve-containing part with dissection of only the ligamentous part of the uterosacral ligament, and resection in the lateral direction was less extensive. The pelvic autonomic nerves are not part of the MGU, but the tissue surrounding the nerves, extending towards half-way the circumference of the rectum and towards the pelvic side wall, is. By dissecting the pelvic autonomic nerves from the surrounding tissue and removing all parametrial and paracervical tissue as done in the Swift procedure, there is virtually no fear of compromised radicality anymore.

An attempt to visualise the MGU and its borders on MRI is described in chapter 5. The horseshoe-shape that was found resembled the description of the MGU in the dorsal plane. In most patients it was not possible to define the exact borders of this area on MRI. Concerning the complete removal of the MGU during surgery, surgeons' experiences with the Swift operation are also described in chapter 5. The borders of the MGU are in part recognizable as anatomical structures (proximal and intermediate MGU), partly by identifiable tissue borders (vascular mesometrium, part of the ligamentous mesometrium), or with the help of anatomical landmarks (dorsal part of ligamentous mesometrium; towards halfway the circumference of the rectum). The border between the distal part of the mesometrium and the bladder mesentery is regarded as the most difficult part because there are very few anatomical landmarks in this area where often, especially in obese patients, tissues seem to fuse, and bleeding hampers the recognition of clear borders. The MR images found provide no absolute evidence for the theory of the MGU, but are supportive for the hypothesis. This is consistent with other findings of studies concerning the anatomy of the MGU.(7) A randomised controlled trial (which takes the extent of lymph node dissection into account) is suggested as the best proof to support the theory of the MGU.

The randomized controlled trial that is suggested in chapter 2 and 5 could once and for all settle the debate about the threat of nerve-sparing to radicality. In view of ethical deliberations of those favouring and not favouring nerve sparing, this could be best accomplished in a multicentre setting. In the meantime, the difficulties in defining the borders of the area that might represent the MGU, do not exclude adequate surgery. On the basis of the theory of the MGU and the results with respect to recurrence and survival of both Höckel (TMMR) and the study that is described in chapter 2, nerve-sparing is safe from an oncologic point of view. Given the long-term morbidity after iatrogenic damage to the pelvic autonomic nerves, and the possibility to improve quality of life after the operation, we are convinced that a nerve-sparing approach should be an integral part of all radical hysterectomies in this group of patients. With respect to radicality, we strongly advise excision of the total MGU (as is done in the Swift operation and TMMR).

A less extensive adaptation, but a helpful method in oncologic surgery is the use of distilled water to achieve adequate haemostasis. The study that is described in chapter 4 was performed to deepen the knowledge about this empirical appliance of aquadest, to promote its use if found safe, and to explore possible positive side effects. Based on existing literature reports, it was concluded that the incubation time in the way that distilled water is used for haemostasis is too short and the numbers of lavages are too small to contribute effectively to tumour cell lysis in oncologic surgery. Nevertheless, free tumour cells in the pelvis might to some extent suffer from hypotonic distilled water lavage. Recently, a new study suggests that a single lavage of 15 minutes may reduce the sequelae of tumour spill during laparotomy.(8)

Besides using the best possible treatment techniques with the least sequelae for the group of cervical cancer patients as a whole, a more individually tailored treatment based on the unique combination of characteristics of the cervical tumour in a specific patient might further improve prognosis and survival. A better understanding of tumour behaviour and detailed findings in the pre- and post-operative course might help in defining smaller subgroups of patients who might benefit from a certain treatment strategy. The last four chapters of the thesis address those aspects.

The involvement patterns of tumour positive parametria as described in chapter 6 seem to fit into the known biological mechanisms of cervical cancer spread (mainly lymphatic). The different patterns were not found to represent an independent predictor of postoperative survival. The results of this study on continuous and discontinuous tumour growth underlined the difficult clinical staging of cervical cancer, and the difficulties for patient subgroup selection. Continuous growth was regularly identified in patients who were staged 1b or 2a. One-half of the parametrial involvement was caused by tumour deposits separate from the primary process. This kind of parametrial involvement is probably not palpable during the physical pre-operative examinations, and tumour biopsies cannot predict this. No characteristics that might help to pre-operatively identify patients with small tumours who are at risk for parametrial continuous or discontinuous tumour growth could be identified. With respect to possibilities for subgroup selection, the finding that tumour in parametrial blood vessels was related to the development of distant (haematogenous) tumour metastases deserves further investigation. The survival analyses in the small number of cases with distant metastasis in the study group did not show an independent effect of parametrial blood vessel involvement on disease free survival (DFS) and OS. Considering the CI's, blood vessel involvement may, however, prove to be a predictor of DFS and OS in a larger group. If this is the case, adjuvant chemotherapy may be considered for this select group of patients. The prognosis and best treatment options might be different for the different patterns of parametrial spread. Further investigation in large prospective studies is necessary, including serial sectioning and immunohistochemical staining of the parametria with markers for blood- and lymph vessels.

The study of scar recurrences in cervical cancer patients that is described in chapter 7, showed, besides the formerly unknown incidence, patient characteristics that may have an implication for treatment choice. The variety in time between the surgical procedure and the occurrence of the scar recurrence in the cases suggests that different mechanisms of origin, maintenance, and growth are involved in the development of scar recurrences. More than half of the cases studied had an advanced disease (FIGO stage ≥1b2), and parametrial involvement, not radically free resection margins, and tumour diameter >4 cm were relatively frequently found in the patients with a scar recurrence. Based on the results of the study, scar recurrences do not seem to be just single and treatable events. The wide variety in time to the development of scar recurrences is similar to 'normal' metastases, and largely corresponds with the development of local recurrences. Scar recurrences seem to be part of the presence of more extensive metastatic disease and seem to be similar to other metastases. In the scar recurrence group, a strong heterogeneity was found on all investigated characteristics. The contribution of iatrogenic factors as compared to those of tumour, stage, host, or treatment characteristics was unclear. Ceasing to separately close the peritoneum is a specific change in treatment modality that may deserve further attention. It can be postulated that migration of loose tumour cell towards the scar is facilitated without the protection of the closed peritoneum. However, the results and design of the present study did not provide enough support to advocate the separate closing of the peritoneum, but the combination of the finding of more scar recurrences and the recent findings concerning tumour lysis by distilled water lavage may be a reason to advise the use of distilled water lavage at the end of all radical hysterectomies.(8)
Based on the findings of the scar recurrences study, extra attentiveness is recommended when a scar recurrence is discovered. Thorough physical examination and the use of imaging techniques should be used to exclude metastases at other sides. Perhaps chemotherapy in addition to local excision should be considered in patients with scar recurrences. Future research on genetic characteristics of different tumour clones in primary tumours, and their capabilities to spread and grow may be able to answer some of the many unanswered questions about scar recurrences.

The evaluation of bulky cervical tumour morphology on MRI in the pre-operative period that is described in chapter 8 resulted in defining the Barrel Index, and the finding of intra-cavital fluid. These morphological characteristics that were related to exophytic or barrel shaped growth pattern and to survival might be a helpful tool for the pre-operative discrimination between barrel shaped or exophytic growth pattern. The findings have to be confirmed in a larger patient group of unselected patients. Particularly interesting, would be to investigate whether intra-cavital fluid and BI can be evaluated by ultrasound instead of MRI, especially in low resource settings where the vast majority of all cervical cancer is found.

Growth pattern of bulky cervical tumours was further investigated as one of the clinical parameters in the SNP analysis that is described in chapter 9. No genetic differences were found between barrel shaped and exophytic tumours. The SNP analysis was performed in an attempt to find genetic differences related to prognostic factors that could be helpful in the pre-operative selection of patient subgroups eligible for a specific treatment. Extensive genome-wide LOH and CN changes were found, and a clear difference between tumours of different histological types was observed. The prognostic relevance of histological type is still object of dispute and is currently not routinely used for primary or adjuvant treatment choice. Additional PAS and Alcian blue staining enhanced distinguishing different tumour types, and therefore distinguishing the genetic differences in histological subtypes. Lack of specific mucus staining techniques may explain the conflicting results of studies on the effect of histological type on tumour behaviour and patient survival. For the other evaluated parameters, no specific genetic differences were found. Ethnicity was used as a confounder in the analyses. It would be interesting to compare genetic changes and the relationship to clinical parameters between patients with a different ethnic background. The same applies to subgroup analyses based on other characteristics. These analyses require larger groups. Since the aim of the study was to find pre-operative parameters that could predict tumour characteristics, no further analyses to causative genes in the regions with the most prominent differences were performed. This topic deserves further investigation. Although a SNP profile that may help the pre-operative treatment choice was not found with the used methods, the existence of such a genetic profile for clinical parameters other than histological type is not ruled out. In view of the differences in appearance and survival, the different characteristics are not likely to be a coincidence. It is not clear in which stage of cancer progression the different characteristics (and genetic differences) appear.

The studies on the surgical and biological aspects of low stage cervical cancer in this thesis provide small steps forward to optimal treatment strategies tailored to the individual patient. Suggestions are given for improving treatment quality, with an optimal balance between cure and quality of life after treatment, based on characteristics of patient subgroups or individual patients. Deducing direct clinical use of the study results would be premature for most of the results, but advances in the understanding of biological mechanisms, specific tumour behaviour, and characteristics of subgroups and individuals were made, and possibilities for further research were identified. Further scientific research is essential in the constant search for better treatment options, tailored to the individual patient. In the meantime, patients have to be treated on the basis of the best available evidence. The combination of the findings of scientists, advances in novel techniques, and clinical expertise of experienced doctors has to warrant the best possible management of cervical cancer patients.

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Nederlandse samenvatting

Praktische aspecten van cervixcarcinoom

Samenvatting

Dit proefschrift beschrijft studies naar praktische aspecten van cervixcarcinoom en bevat chirurgische overwegingen en aspecten van tumorgedrag en tumorverspreiding.

Hoofdstuk 1 geeft een inleiding op het proefschrift. De stadiëring en behandeling van laagstadium cervixcarcinoom (FIGO 1a-2a) worden beschreven. De keuze van de behandeling van cervixcarcinoom wordt voornamelijk gebaseerd op het klinische FIGO-stadium en postoperatieve evaluatie van prognostische parameters. Beperkingen van het stadiëringssysteem worden behandeld en mogelijkheden voor verbetering worden geopperd. Recente ontwikkelingen op het gebied van diagnose en behandeling worden beschreven met de nadruk op moderne technieken. De onderlinge samenhang van de verschillende studies die in dit proefschrift worden beschreven wordt samengevat. Dit proefschrift bestaat uit studies naar de chirurgische en biologische aspecten van cervixcarcinoom. De nadruk ligt op onderscheidend tumorgedrag en de relatie met overleving, met als doel een bijdrage te leveren aan behandelingstrategieën op maat voor de individuele patiënt.

Chirurgische aspecten

In hoofdstuk 2 wordt een studie naar lokaal recidief percentage, uitvoerbaarheid en veiligheid van niet-zenuwsparende en zenuwsparende radicale hysterectomie bij cervixcarcinoompatiënten stadium 1a-2a gepresenteerd. De Leidse zenuwsparende techniek wordt routinematig toegepast in het Leids Universitair Medisch Centrum sinds 2001, omdat de schade die tijdens de traditionele radicale hysterectomie wordt toegebracht aan de autonome bekkenzenuwen kan leiden tot een verminderde blaasfunctie, defaecatieproblemen en seksuele disfunctie. Om het debat over de mogelijke bedreiging van het sparen van de autonome bekkenzenuwen voor de oncologische radicaliteit op te helderen, werden 246 patiënten met cervixcarcinoom stadium 1a-2a geanalyseerd in een cohortstudie met 2 jaar follow-up: 124 in de niet-zenuwsparende groep en 122 in de groep waar zenuwsparende de intention-to-treat was. Analyse van de patiëntgegevens liet zien dat de klinische karakteristieken van de behandelgroepen vergelijkbaar waren. De zenuwen uni- of bilateraal sparen was mogelijk in 80% van de gevallen in de zenuwsparende groep. Locaal recidief percentages in de niet-zenuwsparende (4,9%) en zenuwsparende (8,3%) groep waren niet statistisch significant verschillend. De gemiddelde lokaal recidief-vrije overleving binnen 2 jaar waren respectievelijk 22.7 en 22.0 maanden. Univariate en multivariate regressie analyses toonden aan dat zenuwsparende behandeling geen onafhankelijke prognostische factor was voor het optreden van een lokaal recidief. Met betrekking tot peri- en postoperatieve parameters, operatieduur en bloedverlies waren minder in de zenuwsparende groep en de mortaliteit was gelijk (1 patiënt); het postoperatieve verloop in de zenuwsparende groep was vergelijkbaar met de ’state-of-the-art’ van

conventionele radicale hysterectomie. Op basis van de resultaten van de studie wordt de zenuwsparende techniek voor cervixcarcinoom stadium 1a-2a uitvoerbaar en veilig geacht.

Hoofdstuk 3 beschrijft de 'Swift' operatie, een aanpassing van de Leidse zenuwsparende radicale hysterectomie, die werd ontwikkeld in het LUMC en routinematig wordt toegepast sinds 2007.
Een verslag van de veranderingen in de chirurgische aanpak en de resultaten van de eerste 15 opeenvolgende patiënten wordt gepresenteerd. Het verslag bevat een stapsgewijze uitleg van de procedure. De Swift operatie is radicaler in het gebied van het ligamentum sacro-uterinum dan de oorspronkelijke zenuwsparende operatie en prepareert de nervus hypogastricus vrij onder direct zicht. In het gebied van de parametria is de Swift radicaler in het diepe laterale deel. Het vasculaire parametriale weefsel wordt losgemaakt en ventraal gescheiden van de urineleiders. Van oktober 2006 tot februari 2007, ondergingen 15 opeenvolgende patiënten met cervixcarcinoom stadium 1a2-1b2 de Swift operatie. De extra operatietijd ten opzichte van conventionele niet-zenuwsparende radicale hysterectomie bedroeg 20 min, wat vergelijkbaar was met de originele zenuwsparende operatie en zonder extra bloedverlies. De suprapubische katheter werd verwijderd na een mediane duur van vijf dagen. Tot op het moment dat het rapport werd geschreven (februari 2008) waren geen recidieven gevonden bij de patiënten. Op basis van de beschikbare resultaten is de Swift procedure eenvoudig uitvoerbaar en biedt deze voordelen ten opzichte van de initiële operatie wat betreft veiligheid en radicaliteit.

In hoofdstuk 4 wordt het gebruik van gedestilleerd water voor het bereiken van lokale hemostase tijdens oncologische chirurgie beschreven en wordt de mogelijke impact op het buikvlies en op tumorcellen behandeld. Gedistilleerd water wordt gebruikt om de hemostase te controleren aan het einde van oncologische chirurgie in het kleine bekken. Toch ontbreken verslagen over deze procedure. Dat is jammer omdat de methode interessant kan zijn voor andere chirurgen. Ook discussie over mogelijke gunstige en ongunstige bijwerkingen is onmogelijk zonder publicaties. Na uitgebreide bekkenchirurgie, bijvoorbeeld oncologische operaties of chirurgie voor endometriose, kan de chirurg geconfronteerd worden met een rauw, sijpelend gebied in het bekken. Het stoppen van kleine veneuze bloedingen om adequate hemostase te bereiken is vaak een moeilijke opgave in deze gebieden. In tegenstelling tot NaCl 0,9%, dat een wazig beeld geeft in een ondoorzichtige vloeistof, veroorzaakt gedestilleerd water een snelle lysis van erytrocyten resulterend in een transparante vloeistof waarin een kleine bloedingsbron makkelijk herkenbaar is. Een mogelijke bijwerking van de lavage kan bijdrage aan de vorming van peritoneale adhesies zijn, door het verstoren van het abdominale afweersysteem. Systemische bijwerkingen zijn niet te verwachten. Hoewel tumorcellen te lijden zouden kunnen hebben van de lavage met het hypotone gedestilleerd water, is het huidige gebruik van gedestilleerd water aan het einde van de

operatie waarschijnlijk te kort en niet effectief om tumorcellen te lyseren. De studie legt het mechanisme uit dat de positieve en mogelijke negatieve effecten van gedestilleerd water lavage voor het bereiken van hemostase na uitgebreide bekkenoperatie veroorzaakt. De studieresultaten ondersteunen het blijvende gebruik van gedestilleerd water voor dit doel.

Tumorgedrag en tumorverspreiding

In 2005 is een interessante theorie betreffende lokale tumorgroei gepostuleerd door Höckel et al. Een topografisch gedefinieerd anatomisch gebied, afgeleid van gemeenschappelijk embryonaal voorloper weefsel, werd beschreven en de Müllerian 'morphogenetic unit' (MGU) genoemd. De structuren rondom en omgeven door de MGU zijn afgeleid van ander embryologisch voorloperweefsel dan de MGU zelf. Höckel et al. opperen dat de lokale verspreiding van cervixcarcinoom, tot ver in de ziekteprogressie, beperkt blijft tot de MGU. Daarom is het erg belangrijk om de volledige MGU verwijderen tijdens chirurgie. Het in situ laten van (een deel van) de MGU verhoogt het risico op recidieven volgens deze theorie. Höckel et al. beschreven de 'total mesometrial resection' (TMMR), ontwikkeld op basis van hun bevindingen en theorie. Hoewel de theoretische onderbouwing van de TMMR uitgebreid is en de resultaten van Höckel et al. met betrekking tot overleving overtuigend zijn, kan de vertaling van theorie en embryologische of kadaver-gebaseerde bevindingen naar de chirurgische praktijk moeilijk zijn. In hoofdstuk 5 wordt de MGU in vivo nader bekeken, om de mogelijke problemen bij de vertaling van de theorie van de MGU naar de klinische praktijk te beschrijven. MRI-scans werden gemaakt van gezonde premenopauzale vrouwen om de MGU te visualiseren. Een hoefijzer-vorm, zoals door Höckel et al. beschreven als de vorm van de MGU in het dorsale vlak (ligamentaire mesometrium, staarten van de Swift), kon worden onderscheiden in de meeste vrouwen. Het was echter moeilijk om exacte grenzen van dit gebied, dat de MGU zou kunnen representeren, te definiëren. De verschillen tussen de individuen waren talrijk en de lichaamsbouw van de groep jonge vrouwen, met relatief lage BMI's, zou hierbij een rol kunnen spelen. Verder worden de ervaringen met de excisie van de MGU tijdens de Swift operatie in het LUMC beschreven.

Een tumor in de parametria, continu met of afzonderlijk van de primaire maligniteit, is een ongunstige prognostische factor bij cervixcarcinoom. De lokale verspreiding van cervixcarcinoom in de parametria is beschreven, maar het verspreidinsmechanisme is niet in detail vastgelegd. In hoofdstuk 6 worden patronen van tumorbetrokkenheid van het parametrium in radicale hysterectomie preparaten van cervixcarcinoompatiënten beschreven, met de nadruk op continue en discontinue groei en het effect op metastasepotentieel. De incidentie van parametriale tumorafzettingen gelokaliseerd in bloed- of lymfevaten, of als geïsoleerde foci in het bindweefsel en de relatie van deze patronen van betrokkenheid met pathologische kenmerken en prognose werden

onderzocht. Negenenzeventig van 763 chirurgisch behandelde cervixcarcinoompatiënten (10%) had een tumor in de parametria van het hysterectomiepreparaat. Het beschikbare patiëntmateriaal werd herbeoordeeld om onderscheid te maken tussen continue en discontinue parametriale tumorgroei. Het patroon van betrokkenheid bij discontinue groei werd bepaald aan de hand van immunohistochemische kleuring met verschillende specifieke markers. Vijftig procent van de postoperatief gevonden parametriale tumorbetrokkenheid werd veroorzaakt door continue uitbreiding van het primaire proces in de parametria. In de overige 50% was de parametriale tumor los van het primaire proces. In deze discontinue groep werd een frequente aanwezigheid van tumor in lymfeklieren en/of lymfevaten (samen 79%) gevonden en zelfs een zeldzaam voorkomen van tumor in bloedvaten (14%). Een tumor werd verder gevonden in ongespecificeerde vaten in 2 patiënten (5%) en als geïsoleerde foci in 6 patiënten (14%). Veertien patiënten (33%) hadden meer dan 1 patroon van betrokkenheid. Positieve bekken lymfeklieren kwamen vaker voor in de discontinue groep. Het patroon van betrokkenheid was geen onafhankelijke voorspeller voor de algehele overleving. Parametriale bloedvat betrokkenheid was gerelateerd aan de ontwikkeling van metastasen op afstand (HR: 12.7, 95% CI: 1.6-103.3). De meerderheid (79%) van parametrium betrokkenheid in de discontinue groep werd veroorzaakt door lymfatische metastasen. Parametriale bloedvat betrokkenheid zou een onafhankelijke voorspeller kunnen zijn voor de ontwikkeling van metastasen op afstand.

Hoofdstuk 7 beschrijft de incidentie van cervixcarcinoom littekenmetastasen en de kenmerken van de patiënten die deze speciale manifestatie van een metastase ontwikkelen, die over het algemeen wordt beschouwd als een lokaal recidief. Recidieven van tumor in het operatielitteken na radicale hysterectomie voor zijn beschreven, maar de incidentie is onbekend en gegevens over patiënt- en tumorkenmerken en follow-up ontbreken. In het onderzoek dat wordt beschreven in hoofdstuk 9 zijn de gegevens van alle patiënten die chirurgisch werden behandeld voor cervixcarcinoom in ons centrum tussen 1984 en 2007 beoordeeld. Alle patiënten met een littekenmetastase werden geselecteerd en voor elke casus werden 5 willekeurige controles geselecteerd. Klinische kenmerken werden vergeleken tussen de patiënten en controles. Elf (1.3%) van de 842 patiënten ontwikkelden een littekenmetastase. De gemiddelde tijd tussen operatie en littekenmetastase was 16 maanden (bereik, 2 tot 45 maanden). Voor 8 patiënten (73%) was de littekenmetastase het eerste recidief van de ziekte. Vijf patiënten (45%) overleden en 2 (18%) raakten ‘lost to follow-up’. De gemiddelde tijd tussen littekenmetastase en overlijden was 9 maanden. Eenennegentig procent van de cases had tijdens de follow-up recidief van de ziekte buiten de littekenmetastase. De case groep had een hoger percentage gevorderd FIGO-stadium, postoperatief gevonden betrokkenheid van parametria of resectieranden en tumordiameter groter dan 4 cm, terwijl lymfeklieren vaker betrokken waren in de controlegroep. Concluderend was de incidentie van littekenmetastasen na primaire chirurgie voor cervixcarcinoom 1.3%. De tijd tot ontwikkeling was variabel en de prognose was slecht. Naast hoger FIGO-stadium

en bijgaande ongunstige pathologische kenmerken werden geen bijzondere kenmerken van patiënten met een littekenmetastasen gevonden. Opmerkelijk was de bevinding dat littekenmetastasen hand in hand gaan met recidieverende ziekte op andere plaatsen. Littekenmetastasen zouden een manifestatie van tumoren met een uitgebreid metastatisch potentieel kunnen zijn, in plaats van een effect van chirurgisch handwerk ten tijde van de operatie.

In hoofdstuk 8 wordt een studie over van MRI afgeleide morfologische kenmerken die de pre-operatieve klinische discriminatie tussen patiënten met een bulky cervixcarcinoom met een verschillende reactie op de primaire chirurgische behandeling zouden kunnen ondersteunen gepresenteerd. Bulky cervixcarcinoom (>4 cm; ≥stadium 1b2) kan worden onderverdeeld in tonvormige (barrel shaped) of exophytisch groeiende tumoren. Hoewel een verschil in overleving na primaire chirurgie is gerapporteerd (onze groep) tussen bulky cervixtumoren met verschillende groeipatronen, wordt de morfologie niet meegenomen in het FIGO stadiëringssysteem. In het onderzoek dat wordt beschreven in hoofdstuk 8, werden MRI-beelden van 24 patiënten met cervixcarcinoom stadium ≥1b2 geanalyseerd om te bepalen of de vorm van cervicale tumoren en de aanwezigheid van vocht in het cavum uteri kunnen discrimineren tussen bulky tumoren met een gunstige of minder gunstige prognose na chirurgie. De verhouding tussen tumorbreedte en -lengte (Barrel Index: BI) en de aanwezigheid van intra-uteriene vochtretentie waren gerelateerd aan overleving in een multivariate regressieanalyse. Een relatie met de ziektevrije en algehele overleving werd aangetoond voor beide parameters. BI en intracavitair vocht waren voorspellers van overleving, onafhankelijk van tumordiameter en andere bekende voor de overleving belangrijke factoren. Een cut-off waarde van 1.40 voor de BI bleek de beste prognostische factor met betrekking tot recidief en overlijden: de hazard ratio's van BI >1.40 ten opzichte van BI ≤1.40 waren 18.9 (95% CI 2.8-125.6) voor recidief en 16.4 (95% CI 2.9-93.9) voor sterfte door cervixcarcinoom. De hazard ratio's van intracavitaire vochtretentie waren 73.6 (95% CI 5.3-1016.4) en 48.1 (95% CI 4.7-491.6) voor resepctievelijk recidief en overlijden. Aldus zouden het morfologische kenmerk Barrel Index en de aan- of afwezigheid van intracavitair vocht zoals bepaald met MRI een voorspellende waarde kunnen hebben voor overleving van patiënten met bulky cervixtumoren. De bevindingen van de studie suggereren dat groeipatroon en intracavitair vocht bepaald met MRI behulpzaam kunnen zijn bij het identificeren van een subgroep van individuen met bulky cervixcarcinoom met een betere prognose en daarmee geschikt voor primaire chirurgische therapie.

De studie die wordt beschreven in hoofdstuk 9 trachtte bij te dragen aan de beoordeling van parameters van tumorgedrag en tumorverspreiding die belangrijk zijn voor stadiëring en behandelkeuze. Veranderingen in het genoom van bulky cervicale tumoren en hun relatie tot de klinische prognostische parameters barrel shaped of exophytisch groeipatroon, tumordiameter, parametrium en lymfklier betrokkenheid,

vaso-invasie, infiltratiediepte, en histologische type, werden geëvalueerd met behulp van single nucleotide polymorphism (SNP) analyse. Een whole genome SNP analyse werd uitgevoerd in patiënten met bulky cervixcarcinoom (stadium 1b2-2b). Flow-gesorteerde pure tumorcellen en normale cellen werden geëxtraheerd van 81 bulky cervixcarcinoompatiënten. DNA-index (DI) metingen en whole genome SNP-analyse werden uitgevoerd. De gegevens werden geanalyseerd om veranderingen in het aantal kopieën (copy number alterations: CNA) en de allelische balans-status te detecteren: gebalanceerd, ongebalanceerd of verlies van heterozygositeit (loss of heterozygosity: LOH) en hun relatie tot de klinische parameters. De DI varieerde van 0.92 tot 2.56. LOH werd gevonden in ≥40% van de samples op chromosoomarmen 3p, 4p, 6p, 6q, en 11q, copy number (CN) winst in >20% op 1q, 3q, 5p, 8q, en 20q, en verliezen op 2q, 3p, 4p, 11q, 13q en. Meer dan 40% toonde winst op 3q. De enige verschillen werden gevonden tussen histologische types (squamous, adeno en adenosquamous) in de lesser allele intensity ratio (LAIR) ($p=0.035$) en in de CNA-analyse ($p=0.011$). Meer verliezen werden gevonden op chromosoomarm 2q (FDR=0.004) in squameuze tumoren en meer winst op 7p, 7q en 9p in adenosquameuze tumoren (FDR=0.006, FDR=0.004, en FDR=0.029). Concluderend laat whole genome analyse van bulky cervixcarcinoom breed verspreide veranderingen in allelische balans en CN zien. De algehele genetische veranderingen en CNA op specifieke chromosoomarmen verschilden tussen de histologische types. Een relatie met klinische parameters die momenteel worden gebruikt voor de behandelkeuze werd niet gevonden.

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- John Gay

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Alcest, wilt gij den Zandberg op?
Zoo rijdt een eigen paard; geen huurknol haalt den top
- A.C.W. Staring