Tumour stroma in cervical cancer, novel prognostic parameters

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*Ter nagedachtenis aan mijn vader
Aan mijn moeder
Voor Marijne, Anne en Benjamin*

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Chapter 1

Introduction

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1.1 EPIDEMIOLOGY AND ETIOLOGY

1.1.1 Incidence

Cervical cancer has become the second most common cancer in women world-wide preceeding cancer of the breast and is the commonest cancer in the developing countries [Parkin et al., 1988]. Each year, approximately 465,000 new cases of invasive cervical cancer are diagnosed and 200,000 patients die from this disease. The incidence varies in different parts of the world; the highest incidence rates are observed in the developing countries, particularly in parts of Asia, South America and Africa. In Western Europe, the incidence rates are intermediate and the lowest rates are seen in Australia, North America and the Middle East.

In the developed countries, the incidence of cervical cancer is slowly decreasing due to better screening programs and a safer sexual behavior [Swanson, 1982; Devesa, 1986; Ayiomamatis, 1988]. In the Netherlands, next to cancer of the breast, cervical cancer is the second most frequent cancer in women between 30-44 years of age and the fourth most frequent cancer in the age group 15-29 years. The overall incidence is approximately 9.5 per 100,000 women and the mortality rate 3.3 per 100,000 women. In 1990, 757 new cases were diagnosed in the Netherlands, and 288 women died due to this disease [Cancer registry, 1990].

1.1.2 Etiology and Risk factors

Most cervical cancers develop from an area of cervical intraepithelial neoplasia (CIN) in the surface epithelium or underlying endocervical crypts, usually adjacent to the squamocolumnar junction. At least 30 percent of the patients with untreated carcinoma in situ develop invasive lesions within ten years [Petersen, 1956; Koss et al., 1963].

Causative factors in the development of cervical cancer have been the subject of study for several decades. In the absence of a plausible hypothesis on the natural history, a variety of possible risk factors have been mentioned in the literature. Several indicators of sexual behavior are known to be associated with cervical cancer, but the number of sexual partners and the age the first time they have intercourse have most consistently been related to this disease. For both invasive cervical cancer and cervical intraepithelial neoplasia, risk increases with the number of sexual partners and with increasingly younger age the first time of intercourse [La Vecchia et al., 1986a].

Many venerally transmitted agents have been implicated in the etiology of cervical cancer. Human papilloma viruses (HPV) are site-specific DNA viruses commonly found in the genital tracts of sexually active males and females [Gissman, 1984].

The so-called oncogenic HPV types 16 and 18 are frequently observed whereas the types 30 to 35 are occasionally observed in cervical intraepithelial neoplasia and invasive carcinoma [Lorincz et al., 1987; Fuchs et al., 1988].

Transfection with oncogenic HPV-DNA can immortalize human keratinocytes and further growth of these cells in nude mice can produce histologic patterns identical to severe dysplasia in the cervical epithelium (CIN) [Woodworth et al., 1990]. Invasive cancer cells show increased levels of E6 and E7 proteins encoded by oncogenic HPV types. These proteins antagonize the tumour suppressor proteins, p53 and pRB, thereby disregulating cell growth [Werness et al., 1990]. These in vitro studies suggest a role of HPV DNA in cervical cancer tumourigenesis, however, the exact contribution of HPV towards progression of CIN to invasive cancer has not been established.

HPV will be discussed more extensively in chapter 3.4.

Other infectious agents such as Herpes Simplex Virus, Chlamydia Trachomatis, Trichomonas and Epstein-Barr virus did not show consistent patterns of association with cervical cancer, although, some etiological role of these micro organisms is still suggested [Pasca et al., 1975; Schachter et al., 1982; Bistoletti and Lidbrink, 1988; Thompson et al., 1994].

Several reports describe a significant increase in risk of cervical cancer associated with cigarette smoking [Hellberg et al., 1986; la Vecchia et al., 1986b]. This association is plausible because tobacco constituents and a high level of mutagenicity have been found in cervical mucus of smokers [Sasson et al., 1985; Holly et al., 1986].

The causal relationship of contraceptives and diet is controversial, although several reports do suggest an association with cervical cancer [Thomas et al., 1985; Beral et al., 1988; la Vecchia et al., 1988]. Especially the relation between vitamin A and epithelial tumours has been investigated. Women with CIN or cervical cancer showed to have a lower dietary intake of vitamin A than controls [la Vecchia et al., 1988], but a causative role of vitamin A could not be established.

1.2 DIAGNOSIS AND STAGING

The diagnosis of cervical cancer must be confirmed by a biopsy of the cervical area.

Staging can be defined as the clinical estimation of the extent of disease. The current staging system of the International Federation of Gynecology and Obstetrics is presented in Table 1.1 [FIGO, 1986]. This staging system is based on pretherapeutic clinical findings and only the subclassifications of stage I (IA1, IA2) require histopathologic assessment. Clinical staging, in accordance to FIGO guidelines, involves examination under general anaesthesia, cystoscopy, proctoscopy, an intravenous pyelogram, chest X-ray, and skeletal X-rays if indicated.

Table 1.1: Definitions of clinical staging for cervical cancer according to the International Federation of Gynaecology and Obstetrics. (1986)

Stage I.	The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded).
Stage IA.	Preclinical carcinomas of the cervix, that is, those diagnosed only by microscopy.
Stage IA1.	Minimal microscopically evident stromal invasion.
Stage IA2	Lesions detected microscopically that can be measured. The upper limit of the measurement should not show a depth of invasion of more than 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates, and a second dimension, the horizontal spread, must not exceed 7mm. Larger lesions should be staged as IB.
Stage IB.	Lesions of greater dimensions than stage IA2 whether seen clinically or not. Performed lymph vascular space involvement should not alter the staging but should be specifically recorded so as to determine whether it should affect treatment decisions in the future.
Stage II.	The carcinoma extends beyond the cervix but has not extended on to the pelvic wall. The carcinoma involves the vagina, but not the lower third.
Stage IIA.	No obvious parametrial involvement.
Stage IIB.	Obvious parametrial involvement.
Stage III.	The carcinoma has extended on to the pelvic wall. On rectal examination, there is no cancer-free space between the tumour and the pelvic wall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or nonfunctioning kidney.
Stage IIIA.	No extension to the pelvic wall.
Stage IIIB.	Extension to the pelvic wall and/or hydronephrosis or non-functioning kidney.
Stage IV.	The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum. A bullous edema as such does not permit a case to be allotted to stage IV.
Stage IVA.	Spread of the growth to adjacent organs.
Stage IVB.	Spread to distant organs.

Initial staging forms the basis of the FIGO staging, and should not be changed at a later date. When it is doubtful as to which stage a particular patient should be allotted, the earlier stage is mandatory.

1.3 ANATOMIC PATHWAYS OF SPREAD

Cervical cancer, like other cancers, has the ability to grow in an uncontrolled fashion and is able to metastasize. Three anatomic pathways of spread are well recognized:

1. Direct invasion.

The major pathway of direct invasion is laterally into the parametria and finally to the structures of the lateral pelvic wall. This type of spread often involves the distal ureter. Caudally and cranially, cervical cancer spreads to the vaginal stroma of the upper vagina and to the lower uterine segment respectively.

2. Lymphatic involvement.

The primary regional nodal lymph groups to which cervical cancer metastasizes are the obturator, external iliac, internal iliac, common iliac and the sacral lymph nodes. After involvement of the regional lymph nodes, cervical cancer spreads out of the pelvis to involve the para-aortic and eventually the supraclavicular nodes. The frequency of pelvic and para-aortic lymph node involvement varies according to stage of disease which is summarized in table 1.2.

3. Hematogenous metastasis.

Spread via the hematogenous route usually occurs late when the disease has already extended beyond the true pelvis. The commonest sites of hematogenous metastasis are lungs (25%), liver (19%) and bone (16%) [Plentl and Friedman, 1971].

Table 1.2: Incidence of pelvic and para-aortal node metastasis by stage [Chung et al., 1980; Lagasse et al., 1980; Welander et al., 1981; Fuller et al., 1982; Burghardt et al., 1987; Larson et al., 1987].

Stage	% positive pelvic nodes	% positive para-aortic nodes
IB	10-15%	2- 5%
IIA	20-25%	10-16%
IIB	25-30%	15-20%
III	35-45%	25-33%
IVA	50-55%	40-60%

1.4 PATHOLOGY

Most cervical cancers develop from an area of intraepithelial neoplasia. The site of origin is no guide to its nature, for both squamous cell carcinomas and adenocarcinomas may occur either on the ectocervix and/or within the endocervix [Teshima et al., 1985].

Carcinomas developing on the ectocervix are commonly exophytic and tend to grow in either a polypoidal or papillary fashion. These tumours may infiltrate the cervical stroma only to a very minor degree, extending mainly into the vagina.

Carcinomas developing within the endocervical canal tend to expand the cervix to create a barrel-shaped deformity and spread through the cervical wall.

The various malignant neoplasms of the uterine cervix are listed in table 1.3. The first two categories, squamous cell carcinoma and adenocarcinoma, constitute the vast majority of cervical cancers. The conventional view of primary cervical cancers is that 90-95 percent are squamous cell carcinomas and that adenocarcinomas make up the bulk of the residu. This view is known to be incorrect; only 70 percent of the cervical cancers are purely squamous while approximately 15 percent are adenocarcinoma and 8-10 percent are adeno-squamous. This change has resulted from the inclusion of a mucin stain, preferably PAS/Alcian Blue, as part of the routine histopathological examination. The use of this staining has shown that categorization of cervical cancers using hematoxylin and eosin stained sections yields incomplete diagnosis and that many mucine-secreting neoplasms escape detection [Yajima et al., 1984; Benda et al., 1985].

Squamous cell carcinomas are traditionally divided into large cell keratinizing, large cell non keratinizing and small cell non keratinizing types [Reagan et al., 1957]. It is, however, more useful to classify squamous cell carcinomas as well differentiated (large cell tumours showing well marked keratinization), moderately differentiated (large cell tumours showing focal keratinization) and poorly differentiated (large or small cell tumours showing minimal evidence of keratinization) [Chung et al., 1981].

The majority of the squamous carcinomas are classified as moderately differentiated (60%), 15 percent of the tumours are well differentiated and 25 percent poorly differentiated.

Verrucous carcinoma is a rare variant of well-differentiated squamous cell carcinoma. This lesion is a slow growing but a potentially lethal neoplasm.

Most cervical adenocarcinomas arise from the glandular surface mucosa or the mucus secreting endocervical glands. The degree of differentiation is, similar to squamous cell carcinoma, classified in well, moderately and poorly differentiated tumours. The most differentiated form of cervical adenocarcinoma was designated as adenoma malignum in the past and is now known as minimal deviation adenocarcinoma.

Table 1.3: Malignant tumours of the uterine cervix [Fu and Woodruff, 1994].

Squamous cell carcinoma

well differentiated, keratinizing
moderately differentiated, focally keratinizing
poorly differentiated, large cell, non-keratinizing small cell, non-keratinizing
verrucous carcinoma
papillary squamous cell carcinoma (transitional cell)

Adenocarcinoma

endocervical adenocarcinoma (minimal deviation adenocarcinoma)
papillary serous adenocarcinoma
endometrioid adenocarcinoma
clear cell adenocarcinoma
mesonephric adenocarcinoma
enteric adenocarcinoma

Mixed cell carcinoma

adenosquamous carcinoma (glassy cell carcinoma)
adenoid cystic carcinoma

Small cell carcinoma

neuroendocrine tumours
adenoid basal carcinoma
subcolumnar reserve cell carcinoma

Undifferentiated carcinoma**Metastatic carcinoma****Malignant melanoma****Malignant non epithelial tumour**

sarcoma
lymphoma

Less frequent histologic varieties of adenocarcinoma include papillary serous adenocarcinoma, which arises from the surface epithelium of the endocervix; enteric adenocarcinoma with conspicuous mucus production, identical with adenocarcinoma of the large bowel; endometrioid carcinoma, in all respects similar to endometrial adenocarcinoma. Clear cell adenocarcinoma has been

reported in young women with a history of prenatal exposure to diethylstilbestrol (DES). This lesion arises from tubo-endometrial type gland cells within the endocervical mucosa.

Mixed cell tumours are thought to represent biphasic neoplastic differentiation in the pluripotential stemcells. The tumour is composed of a mixture of squamous epithelial cell cords and glandular structures. Very poorly differentiated adenosquamous carcinoma are often classified as glassy cell neoplasms [Arends et al., 1984]. This type of lesion has an extremely aggressive clinical course with poor response to therapy.

Small cell carcinoma are lesions with mixed patterns of differentiation or no differentiation and are believed to arise from subcolumnar reserve cells of the endocervix.

Malignant melanoma, sarcoma and lymphoma are very rare primary cervical tumours.

1.5 TREATMENT

The regular modalities for primary treatment of cervical cancer are surgery, radiotherapy, or a combination of both. The principle considerations for selecting treatment include the extent and size of the cancer and the general health of the patient.

Radiotherapy can be used in all stages of disease and surgery is limited to patients with stage I and IIA disease. Patients with poor general health at high risk for general anesthesia are preferably treated by radiotherapy. Old age and obesity are not deciding factors. With the present anaesthesiological techniques, elderly patients tolerate radical surgery almost as well as their younger counterparts [Lawton and Hacker, 1990].

The cure rate and both the frequency and severity of complications are the main considerations for settlement on choice of treatment.

1.5.1 Surgery

Surgical therapy for cervical cancer stage IB and IIA consists of radical hysterectomy, bilateral pelvic lymphadenectomy and para-aortic lymph node palpation. The goal is both to resect the primary tumour and to eliminate the paths by which it spreads in the pelvis and beyond. This can be done only by systematically removing all accessible lymph nodes and channels draining the cervix.

In most medical centers, radical surgery is the treatment of choice in stage IB and IIA. The advantages of radical surgery over radiotherapy are the opportunity to explore the entire abdomen and pelvis for evidence of disease, preservation of the ovaries, emotional satisfaction that the tumour has been removed and a better vaginal function [Adelusi, 1980; Schover et al., 1989].

Several surgical procedures have been used during the past century. The first radical abdominal hysterectomy was performed by Wertheim in 1889 [Wertheim, 1911]. Schauta improved the radical vaginal hysterectomy introduced by Schuchard in 1883 [Schauta, 1908]. In 1957, Sindram combined both the Wertheim and the Schauta procedure in one operation called Abdominal Vaginal Radical Uterus Extirpation and Lymphadenectomy (AVRUEL) [Sindram, 1959]. Both Meigs and Okabayashi modified the Wertheim procedure in different ways [Meigs, 1945; Okabayashi, 1921].

Nowadays, the modifications of Meigs and Okabayashi are the basis for the surgical procedures used in cervical cancer stage IB and IIA.

Many variations exist in the extent of the parametrial and the vaginal resection and in mobilisation of the ureter. Piver classified the different procedures to improve the comparison of the treatment results. Five types in increasing radicality were described [Piver et al., 1974].

At the University Hospital of Maastricht, the Wertheim-Meigs procedure initially starts with a pelvic lymph node dissection. The pelvic lymph nodes are intra-operatively evaluated using frozen sections. When the pelvic lymph nodes are involved by cancer, the surgical procedure is abandoned and patients are treated with full pelvic and intracavitary radiation therapy, postoperatively. When the pelvic lymph nodes are negative, the Wertheim-Meigs procedure is completed. This mode of treatment is also described by Potter and coworkers [Potter et al., 1990]. The treatment results of this procedure will be described in detail in chapter 5.

It has been shown by exploration of the entire abdomen (staging laparotomy) in patients with stage IB and IIA, that approximately 5-15 percent of the para-aortal lymph nodes are involved by tumour [Averette et al., 1972; Berman et al., 1984]. In experienced hands, severe operative morbidity of radical surgery is low. Major complications such as ureterovaginal fistulas, ureteral stricture and thrombo-embolism are less than 8 percent in most series [Webb and Symmonds, 1979; Riss et al., 1988; Kenter et al., 1989]. Approximately 30 percent of the patients have minor complications (voiding problems, constipation or lymph edema) after radical surgery.

Operative mortality has declined in the last decennia to approach zero due to safer surgical and anesthesiologic techniques. Survival following radical surgery is summarized in tabel 1.4. Recurrent disease is reported in 11-20% of the patients [Krebs et al., 1982; Larson et al., 1988].

1.5.2 Radiotherapy

Pelvic irradiation is a generally accepted treatment modality for cervical cancer either given alone or in combination with surgery. The two main modalities of radiotherapy are external photon beam irradiation and brachytherapy. External irradiation is used to treat the whole pelvis and the parametria including the

Table 1.4: Five year survival rates for cervical cancer stages IB and IIA treated by radical surgery

Reference	Stage	No. of Patients	Survival (%)
Delgado, 1978	IB	645	80
Van Nagell et al., 1977	IB	190	86
	IIA	43	79
Burghardt et al., 1987	IB	—	82
	IIA	—	77
Fuller et al., 1989	IB	295	86
	IIA	136	72
Lee et al., 1989	IB	237	86
	IIA	106	72
Tinga et al., 1991	IB	196	90
	IIA	77	80
This study	IB	83	83
	IIA	31	72

common iliac and para-aortic lymph nodes. Central disease is primarily irradiated with intracavitary sources. High energy photon beams are especially suited because of the thickness of the pelvis. They deliver a relatively low dose of irradiation to the bladder and the rectum and provide a more homogenous dose distribution in the central pelvis. Brachytherapy can be delivered with intracavitary techniques using a variety of intrauterine and vaginal applicators. Several isotopes ($^{137}\text{Cesium}$, $^{226}\text{Radon}$, $^{60}\text{Cobalt}$) are available for this method of treatment although $^{137}\text{Cesium}$ is mostly used because of its lower energy.

The optimal dose for cervical cancer stages IB and IIA with combined external and brachytherapy is approximately 45-55 Gy. Significantly more recurrences are reported if the central dose fell below that recommended [Hanks et al., 1983]. Different combinations of the irradiation modalities can be used but this may have no significant impact on outcome as long as the doses and volumes treated are similar.

Radiotherapy of the para-aortic fields is related to severe side effects in the upper abdomen. The complications, even deaths, after application of tumouricidal doses of radiation to the para-aortic field make this procedure questionable [Piver, 1987].

Major complications following radiotherapy are seen in 8-14% [Perez et al., 1983; Hanks et al., 1983; Montana et al., 1987]. Differentiation can be made between acute and late complications. Acute complications occur during or

Table 1.5: Percentages of radiotherapy complications for cervical cancer stage IB and IIA [Perez et al., 1983; Hanks et al., 1983; Montana et al., 1987].

Complication	%
castration	80-100
pelvic fibrosis	0.5
bladder reaction grade II-III	0.6-5.6
bowel reaction grade II-III	1.0-6.8

Table 1.6: Five year survival rates for cervical cancer stage IB and IIA treated by primary radiotherapy.

Reference	Stage	No. of Patients	Survival (%)
Perez et al., 1986	IB	312	85
	IIA	98	70
Horiot et al., 1988	IB	299	85
	IIA	315	76
Kim et al., 1989	IB	169	82
	IIA	83	76
Lanciano et al., 1990	IB	618	79

immediately after the completion of a course of treatment and are normally self-limiting. Late complications may be recognized three months but also years after therapy and are considered to be greatly dependent on the extent of vascular damage. Late complications after pelvic irradiation are castration, fibrosis of the pelvis and radiation reaction of the bladder and the bowel [Einhorn, 1975; Strockbine et al., 1979].

The frequency of the late complications are shown in table 1.5. Survival rates for patients with cervical cancer treated with radiotherapy are presented in table 1.6.

1.5.3 Combined Surgery and Radiotherapy

Combined surgery and radiotherapy in cervical cancer stage IB and IIA can be used as follows:

1. preoperative brachytherapy followed by radical hysterectomy to reduce central recurrences.

2. radical surgery followed by irradiation in patients at risk for recurrent disease.

Which patients should receive additional external radiotherapy is controversial. To clarify this problem, randomized prospective studies would be necessary, unfortunately, these studies can hardly be realized due to the large number of patients required. In order to solve this problem numerous retrospective studies investigating prognostic parameters have been published. Although most authors agree that postoperative radiotherapy is necessary for positive surgical margins, the use of radiation in patients with other high-risk factors is controversial.

In 1980, Morrow published a panel report of the Society of Gynecologic Oncologists discussing the benefit of postoperative pelvic radiation in patients with pelvic lymph node metastasis [Morrow, 1980]. According to the known data, no conclusions could be drawn regarding the efficacy of adjuvant pelvic irradiation. Although conflicting results have been presented, several papers support the contention that adjuvant pelvic irradiation for patients with pelvic lymph node metastasis adds no benefit to survival but only decreases the incidence of pelvic recurrences [Fuller et al., 1982; Larson et al., 1987; Alvarez et al., 1989; Kinney et al., 1989; Soison et al., 1990]. Most of these authors conclude that additional treatment is needed to improve survival.

Combined surgery and radiotherapy imply a greater risk for complications than with either treatment modality alone, particularly because of injury to the ureter or bladder.

The incidence of severe complications has been reported to be between 5% and 20%, depending on the dose and technique of irradiation, the type of surgical procedure (simple hysterectomy, transperitoneal or retroperitoneal approach) and on the extent of para-aortic lymph node dissection [Lepanto et al., 1975; Nelson et al., 1975; O'Quinn et al., 1980].

1.5.4 Chemotherapy

The role of chemotherapy in the treatment of cervical cancer has been limited for several reasons. Surgery and radiotherapy show good treatment results in patients with early stage disease and the majority of patients are diagnosed in these stages. Cervical cancer is relatively insensitive to cytotoxic agents and the response to chemotherapy could be adversely influenced by prior treatment. For example, both surgery and radiotherapy diminish blood flow and tissue permeability and result in lower drug concentrations in the recurrent tumour.

In addition, patients with recurrent disease have often reduced renal function due to ureteral obstruction and a reduced bone marrow tolerance due to prior irradiation. For all these reasons, only a few patients with cervical cancer are candidates for systemic chemotherapy.

Chemotherapy can be used in cervical cancer prior to local treatment (primary or neoadjuvant chemotherapy), postoperatively in patients at risk for recurrence (adjuvant chemotherapy) and in combination with radiotherapy to enhance the efficacy of the radiation (radiosensitizing chemotherapy).

Neoadjuvant chemotherapy has been reported in a large number of studies almost all using cisplatin in combination with other drugs. Response rates as high as 50-80% have been reported, however, none of the studies were prospective randomized trials [Giannone et al., 1987; Sardi et al., 1988; Elliot, 1989; Symmonds et al., 1989].

Two randomized trials using cisplatin combined with other drugs in patients with advanced disease showed no advantage in survival for the group treated with chemotherapy [Chauvergne et al., 1988; Souhami et al., 1988].

Adjuvant chemotherapy following radical surgery in patients with one or more high risk factors has shown some advantage in terms of better prognosis. Wertheim and coworkers treated 32 high risk patients stage IB and IIA with postoperative chemotherapy followed by pelvic radiation. The 28 months survival rate of this high risk group was favourably compared to the low risk patients [Wertheim et al., 1985]. Further randomized studies are necessary to evaluate the value of adjuvant chemotherapy.

1.5.5 Immunotherapy

Immunotherapy of human cancers can be applied by two basic approaches:

Passive immunotherapy circumvents deficits in immunocompetence and this therapy has used either the transfer of immune effector cells or the transfer of antibodies to patients with cancer. The growth of lymphocytes can be stimulated by recombinant IL-2 *in vitro*. If appropriate tumour associated antigen targets can be identified, the stimulation of specific autologous lymphocytes (tumour infiltrating lymphocytes, TIL) that are capable of lysing cervical tumour cells may be possible [Hilders et al., 1994]. Monoclonal antibodies conjugated with drugs or toxins and reactive with tumour associated antigens may provide new agents for serotherapy. Active immunotherapy makes an attempt to stimulate patients own immune response to destroy tumour cells. Systemic administration of bacterial immunostimulants to release cytokines by infiltrating leucocytes increases leucocytes numbers and natural killer activity with the regression of tumour nodules in ovarian cancer patients [Berek et al., 1985]. Cytokines play a role in tumour cell destruction and highly purified recombinant cytokines are now available to reduce toxicity.

Immunotherapy in cervical cancer is mostly used in an experimental setting and increased knowledge of tumour biology and the availability of pure antibodies and cytokines can facilitate the implementation of new therapeutic approaches.

1.6 CONCLUSIONS

Cervical cancer is an important and relatively frequently occurring disease with high incidence rates, especially in developing countries. Many risk factors have been investigated. The most likely being HPV infection. However, the exact cause of this disease remains unresolved. In stages IB and IIA, several treatment modalities are used, but neither radiation, surgery, nor a combination of both modalities offer an overwhelming advantage in survival.

Although comparison is difficult due to the different modes of therapy, 5 year survival has not changed during the last twenty years.

Complication rate, preservation of the ovaries and a better sexual function are the reasons for radical surgery as the first option in the early stages.

New therapeutic strategies including chemotherapy and immunotherapy may influence survival in the future but if we want to improve survival rates in patients with stage IB and IIA disease, patients at risk for recurrence have to be selected. Only a small group (20%) of these patients will develop recurrent disease after primary therapy and the goal must be to identify these patients shortly after diagnosis. Maximalisation of therapy in these patients probably improves survival and treatment can be minimized in the patients not at risk for recurrence. Several clinical and tumour characteristics helpful in selecting these patients have been described, and will, besides their usefulness, be discussed in the next chapter.

Chapter 2

Clinical and histopathological prognostic factors in cervical cancer: Review of the literature

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- 2.2 Clinical prognostic factors
 - 2.2.1 Stage
 - 2.2.2 Lymph node involvement
 - 2.2.3 Single versus multiple pelvic lymph node involvement
 - 2.2.4 Age
 - 2.2.5 Pregnancy
 - 2.2.6 Thrombocytosis
 - 2.2.7 Squamous cell carcinoma antigen
- 2.3 Histopathological prognostic factors
 - 2.3.1 Tumour size, tumour volume
 - 2.3.2 Histological grade and cell type
 - 2.3.3 Lymph vascular space involvement
 - 2.3.4 Depth of invasion into the cervix
 - 2.3.5 Parametrial invasion
- 2.4 Conclusions

2.1 INTRODUCTION

Prognosis, the prediction of the future of an individual patient with respect to duration, course and outcome of a disease, is important in medical practice.

The prognosis of patients with cervical cancer is influenced by a variety of factors. As in other malignancies, the major prognostic factors are the growth characteristics and the degree of spread of the tumour. These factors are closely related and can be assessed partly macroscopically and clinically, and partly microscopically.

Lymph node metastasis has been recognized for a long time to be the strongest predictor of recurrent disease and death in cervical cancer. In addition to pelvic lymph node involvement, a number of other tumour-associated characteristics have been reported to be predictive of outcome. Tumour size, lymph vascular space involvement, histological cell type and grade as well as depth of stromal invasion are, though not independently, all associated with prognosis, since they are also predictive of pelvic lymph node metastasis.

Recently, increased interest has been directed toward determining whether these tumour-associated factors may add independent information on the degree of risk over that of nodal involvement alone.

As expressed by Thomas and Dembo in 1991, only a number of the patients with recurrent disease has pelvic lymph node involvement. For example, of every one hundred patients with cervical cancer stage IB and IIA, 15 are likely to be node positive and 45% of these node positive patients will be cured. Eighty-five patients are node negative and of these 90% will be cured. In absolute terms, however, an equal number of eight recurrences occur in both the node negative group and the node positive group (Figure 2.1).

Of the node negative patients with recurrent disease, many patients have only pelvic recurrence without distant metastasis in comparison to the node positive patients with recurrent disease. Nearly all these patients have distant metastasis when the tumour recurs and, until now, cure has not been possible [Fuller et al., 1989].

In order to improve survival rates in both patients with and without pelvic lymph node metastasis, it is necessary to identify other parameters that select the patients at risk for recurrence. The aim would be to define a small subset of patients that accounts for the majority of relapses in the group of patients with negative pelvic lymph nodes and to identify the patients that are also at risk for recurrence in the group with positive pelvic lymph nodes.

This chapter discusses many characteristics of cervical tumours that are well established and proven to predict clinical course. Others that await further study, will be discussed as well.

In this context, it is important to realize that only one prospective study has been reported and only a few authors have used multivariate analysis to investigate the independency of the prognostic parameters.

Figure 2.1: Typical frequencies of pelvic lymph node metastasis, cure and absolute numbers of recurrences in patients with cervical cancer stages IB and IIA [Thomas and Dembo, 1991].

2.2 CLINICAL PROGNOSTIC FACTORS

2.2.1 Stage

As mentioned, the clinical extent of disease is expressed in groups designated stages I-IV. It is clear that a stepwise fall in survival from stage I to stage IV is observed, however, survival rates vary considerably from center to center (Table 2.2).

Clinical staging based on findings at palpation is relatively inaccurate and often does not correspond to the actual spread of disease as determined by surgical and pathologic examination. This applies especially to spread into the paracervical tissue (the distinction between IB and IIB). Where it is easy to establish the extension of cervical cancer into the vagina, parametrial invasion can only be detected clinically by a well-experienced gynecologist.

Van Nagell and coworkers were in 1971 among the first investigators to recognize and quantitate the discrepancies between clinical and surgical/pathological findings. Reported staging errors vary with stage and the spread of the cancer was accurately diagnosed in 67.9%-88.2% of the patients in stage IB, 47.3%-87.9% of those in stage IIA, and 24.4%-71.4% of those in stage IIB compared to postsurgical staging [Averette et al., 1972; Christensen et al., 1976; Zander et al., 1981; Matsuyama et al., 1984]. These results stress the difficulty of diagnosing parametrial invasion by physical examination. The discrepancies between clinical and surgical staging even achieve greater significance on survival when the impact of para-aortic node metastasis is examined.

Because of the inaccuracy of clinical staging and the importance of staging in the choice of treatment, it is almost impossible to compare the results of different series or the results of different treatment modalities objectively.

Table 2.2: Five year survival rates according to clinical FIGO stage.

Reference	N	Clinical stage				
		IB (%)	IIA (%)	IIB (%)	III (%)	IV (%)
Matsuyama et al., 1984	255	88	85	71	—	—
Einhorn et al., 1985	267	90	70	—	—	—
Ylinen et al., 1985	709	76	55	47	37	8
Burghardt et al., 1987	320	82	77	50	—	—
vd Graaf et al., 1988	294	77	83	55	36	11
Fuller et al., 1989	431	86	72	—	—	—
Lee et al., 1989	438	86	72	60	—	—
Tinga et al., 1991	384	90	80	49	33	10
This study	156	83	72	38	22	0

2.2.2 Lymph node involvement

The incidence of pelvic lymph node metastases in early cervical cancer is presented in table 2.3. The reported incidence varies from 8% to 31% in stage IB and 22% to 39% in stage IIA. The presence of lymph node metastasis is considered to be the most important prognostic factor in cervical cancer stages IB and IIA. In most reports, nodal involvement is a powerful predictor of outcome on single factor analysis and, therefore, it has become almost synonymous with high risk [Van Bommel et al., 1987]. Kamura and coworkers showed in a large retrospective study of 345 stage IB-II patients using multivariate analysis pelvic lymph node metastasis to be the most important prognostic factor [Kamura et al., 1992].

Surprisingly, Delgado observed in a well-controlled prospective study of the Gynecologic Oncology Group, a difference in disease free interval but no significant difference in survival between patients with and without pelvic lymph node involvement [Delgado et al., 1990]. This finding which differs from several other studies can partly be explained by the exclusion of patients with para-aortic lymph node metastasis, patients with tumour outside the uterus and patients with gross metastatic pelvic lymph nodes.

Table 2.4 compares survival of stages IB and IIA patients with and without pelvic lymph node metastasis.

Survival rates decrease from 82-94% for patients with negative pelvic lymph nodes to 44-72% when pelvic lymph nodes are involved.

Table 2.3: Incidence of pelvic lymph node metastasis in cervical cancer stages IB and IIA.

Reference	stage IB			stage IIA		
	total	pos	%	total	pos	%
Piver and Chung, 1975	145	39	27	95	32	34
Einhorn et al., 1985	100	8	8	50	15	30
Ylinen et al., 1985	242	31	13	54	21	39
Himmelman et al., 1985	265	29	11	79	26	33
Burghardt et al., 1987	122	38	31	8	2	25
Noguchi et al., 1987	218	36	17	101	32	32
Terada et al., 1987	362	47	13	—	—	—
Delgado et al., 1989	645	100	16	—	—	—
Fuller et al., 1989	285	42	15	133	29	
Lee et al., 1989	596	77	13	250	67	27
Inoue and Morita, 1990	484	105	22	96	22	23

Table 2.4: Survival related to pelvic lymph node status in patients with cervical cancer stages IB and IIA.

Reference	negative nodes		positive nodes	
	total No	survival (%)	total No	survival (%)
Piver and Chung, 1975	156	82	69	44
Einhorn et al., 1985	127	93	23	48
Himmelman et al., 1985	289	93	55	52
Burghardt et al., 1987	198	88	122	61
Noguchi et al., 1987	224	88	61	59
Fuller et al., 1989	347	85	71	50
Lee et al., 1989	295	85	48	58
Inoue and Morita, 1990	453	91	127	72
Kenter et al., 1991	148	94	65	65
This study	75	88	27	61

The incidence of involvement of the common iliac or para-aortic lymph nodes is low in cervical cancer stages IB and IIA and ranges from 1% to 10.6%. Survival rates vary from 17% to 50% when para-aortic lymph nodes are involved

[Martimbeau et al., 1982; Alvarez et al., 1989; Delgado et al., 1989; Lovecchio et al., 1990].

2.2.3 Single versus multiple pelvic lymph node involvement.

The number of positive lymph nodes is of prognostic value in cervical cancer. There is no discussion about the better prognosis for patients with only one positive lymph node compared to patients with 3 or more nodes involved. Probably, the number of positive nodes is related to prognosis more than the presence of nodal metastasis per se.

Table 2.5 shows the survival rates for patients with no, a single and multiple lymph node metastasis. In these studies, patients with two or more positive pelvic lymph nodes show a significant fall of 21-55 percent in survival rate in comparison with one positive pelvic lymph node [Matsuyama et al., 1984; Burghardt et al., 1987; Inoue and Morita, 1990; Tinga et al., 1990; Kamura et al., 1992]. Only Alvarez found a significant higher recurrence rate when at least three lymph nodes were involved [Alvarez et al., 1989]. He suggested that patients with small tumours and with one or two involved nodes have an excellent prognosis and are adequately treated with radical hysterectomy and pelvic lymphadenectomy.

From most studies it is apparent that the survival rate is worse in those patients with involvement of more than one lymph node, despite the effect of inconsistent treatment.

The recurrence rate increases significantly when two or more positive pelvic lymph nodes are found and in these patients adjuvant therapy is recommended. The highest level of the involved nodes is also of prognostic significance. Reported survival rates vary between 58-71% if only the primary lymph nodes

Table 2.5: The influence of number of pelvic lymph nodes with metastasis on the survival rate in cervical cancer stages IB, IIA and IIB.

reference	(stage)	N	Number of positive pelvic lymph nodes		
			0	1	2 or more
Tanaka et al., 1984	(IA-IV)	205	92%	62%	21%
Matsuyama et al., 1984	(IB-IIB)	54	—	62%	36%
Burghardt et al., 1987	(IB-IIB)	320	89%	64%	47%
Noguchi et al., 1987	(IB-III)	589	83%	64%	36%
Inoue and Morita, 1990	(IB)	94	89%	81%	55%
Tinga et al., 1990	(IB)	51	—	85%	24%
Kamura et al., 1992	(IB-IIB)	345	89%	61%	36%

(obturator, external iliac) are involved and between 0-23% if common iliac or para-aortic node metastasis is present [Martimbeau et al., 1982; Tanaka et al., 1984].

2.2.4 Age

Of all patients with cervical cancer, 18-25% of the patients is under 35 years of age [Smales et al., 1987; Elliot et al., 1989]. Several epidemiological reports show an increase in the incidence of invasive cervical carcinoma in young women. It has been suggested that young women with cancer of the cervix have a worse prognosis and therefore should be treated differently [Stanhope et al., 1980; Stehman et al., 1991]. Most other studies, however, find no difference in survival or even a better prognosis in young patients [Kjorstad, 1977; Gynning et al., 1983; Smales et al., 1987; Meanwell et al., 1988; Van der Graaf et al., 1988; Kodama et al., 1991].

In most reports, young patients have a more favorable stage distribution than older patients. Approximately 61-72% of the young patients were diagnosed as stage IB compared to 29-41% stage IB patients in the older group. The distribution on histologic cell type and grade are similar in younger and older groups of patients (Kjorstad, 1977; Carmichael et al., 1986; Smales et al., 1987; Chapman et al., 1988).

It can be concluded that, although there is a strong increase in the incidence of invasive cervical cancer in younger women, the natural history and the response to treatment are similar in all age groups.

2.2.5 Pregnancy

The incidence of cervical cancer associated with pregnancy ranges from one in 1000-5000 deliveries [Hacker et al., 1982; Orr and Shingleton, 1983]. The diagnosis is often delayed during pregnancy because bleeding is attributed to pregnancy related complications. Patients with invasion depth over 5 mm should be treated as having frankly invasive cervical carcinoma. The recommended treatment is classic cesarean section to avoid manipulation of the carcinoma followed by radical hysterectomy and pelvic lymph node dissection. The time of the surgical therapy should be discussed with the patients but it is probably insensible to delay therapy for longer than 4 weeks after diagnosis [Lee et al., 1981]. Dilatation of the cervix and vaginal delivery must be avoided.

The most important prognostic factor is stage and, when stratified for stage and tumour volume, survival of pregnant patients with cervical cancer is similar to that of nonpregnant patients [Creasman et al., 1970; Lee et al., 1981]. Probably, the natural history of cervical cancer is unaffected by the hormonal and vascular changes in pregnancy.

2.2.6 Thrombocytosis

Thrombocytosis may be found in association with a number of diseases and, over a century ago, Riess first observed the association with malignancy [Riess, 1872]. The specific mechanisms by which thrombocytosis is produced in cancer patients are unknown. One of the most plausible hypotheses is an increased production of thrombocytes by the megakaryocytes [Tranum and Haut, 1974], initiated through bone-marrow stimulating cytokines secreted by tumour cells [Constantini et al., 1990].

Thrombocytosis may adversely affect survival due to interactions between platelets and the cancer cells. Cancer cells have shown to cause activation of platelets *in vitro* and this activation may protect cancer cells against the immune system, as well as facilitate cancer cell adhesion to the vascular endothelium, an important step in the process of metastasis [Karpatin et al., 1981; Gasic et al., 1984].

Recently, Hernandez and coworkers reported thrombocytosis, defined as a platelet count greater over $400.10^9/L$, to be an independent poor prognostic factor in patients with cervical cancer treated with radiotherapy [Hernandez et al., 1992]. These findings were stressed by Rodriguez and coworkers in a study of 219 patients with cervical cancer stage IB [Rodriguez et al., 1994]. Lopez and coworkers, however, found a relation between advanced stage cervical cancer and thrombocytosis only with no significant difference in survival [Lopez et al., 1994].

2.2.7 Squamous cell carcinoma antigen (SCC)

Kato and coworkers reported in 1977 the purification of an antigen (TA-4) from tissue extracts of cervical squamous cell cancers [Kato et al., 1977]. Squamous cell carcinoma antigen is one of the 14 subfractions that can be separated in serum by isoelectric focussing. The SCC antigen can be detected in normal squamous epithelium, but only 1-4% of the healthy adults have a serum level above 2.5 ng/ml.

In patients with squamous cell carcinomas of the cervix approximately 60% have elevated SCC levels [Kato et al., 1982; Maiman et al., 1989; Duk et al., 1990]. The incidence of SCC elevation ranges from 30% in patients with stage I disease to 80-90% in patients with stage IV. Elevation of the SCC level in patients with stages IB and IIA disease at the time of diagnosis is a poor prognostic sign [Kato et al., 1983; Maiman et al., 1989]. This is probably caused by a larger tumour volume in patients with elevated SCC levels compared to patients with normal levels.

In addition, elevation of serial SCC levels after primary treatment, predicts the development of recurrent disease in 50% of the patients [Kato et al., 1984; Ngan et al., 1990].

Adenocarcinomas of the cervix may also be associated with elevation of SCC levels. Duk and coworkers reported SCC levels in 57% of the patients with adenosquamous carcinoma and in 29% of the patients with adenocarcinoma [Duk et al., 1989]. It can be concluded that serum SCC antigen correlates with stage and recurrent disease in patients with cervical cancer containing squamous cell components. In stages IB and IIA disease, serum SCC helps to identify patients at risk for recurrent disease.

2.3 HISTOPATHOLOGICAL PROGNOSTIC FACTORS

2.3.1 Tumour size, Tumour volume

Increasing tumour size is related to an increasing probability of positive pelvic lymph nodes as well as a higher risk for recurrent disease and death. However, even very small tumours (<1.0 cm) have an appreciable incidence (4%-21%) of metastases to pelvic lymph nodes, indicating that tumour size and metastatic potential are not perfectly correlated. The incidence of pelvic lymph node metastasis was found to increase to 30 percent in patients with tumours of at least 4 cm in diameter (Table 2.6).

Although tumour size itself is difficult to measure in cervical cancer, it is one of the corner stones of the FIGO classification since increasing tumour size points to a higher clinical stage [Burghardt et al., 1978]. Many investigators have tried to develop a method to measure the volume of the tumour exactly. However, tumour size is generally measured by clinical examination at the time of pre-therapeutic staging and exact measurement is only possible after surgical resection of the tumour.

Therefore, unfortunately, a great difference in measurement methods has been presented by several authors.

Fuller and coworkers measured the largest tumour diameter in patients with stages IB and IIA cervical cancer. They reported survival rates of 92%, 85%, 76%, and 62% for tumours less than 1 cm, 1-2 cm, 2-4 cm and more than 4 cm in diameter, respectively. The authors used regression analysis to correct for pelvic lymph node metastasis and found that independent of pelvic lymph node metastasis the increasing risk of recurrence persisted with increasing size of the cervical tumour [Fuller et al., 1989].

In a large study combining the results of three institutions, the volume of the tumours was measured in 1028 patients stages IB, IIA and IIB. Volumetry on hysterectomy specimens was based on axial diameters of the tumours or on computerized measurement of tumour areas combined with tumour diameters.

The 5-year survival rate of patients with small tumours (tumour volume less than 2.5 cm³) was 90.7% and fell to 79.2% for patients with a tumour volume of

Table 2.6: The influence of tumour size on the incidence of pelvic lymph node metastases and survival in cervical cancer stages IB and IIA.

Reference	tumour size (cm)	No. of patients	positive PLN (%)	survival (%)
Piver and Chung, 1975	<1	33	2	—
	<1-4	99	21	—
	>4	108	39	—
Fuller et al., 1989	<1	91	4	92
	1-4	243	20	83
	>4	92	31	62
Delgado et al., 1989 + 1990	<1	63	13	95
	1-3	262	16	86
	>3	152	23	68
Alvarez et al., 1991	<1	29	—	100
	1-4	169	—	68
	>4	32	—	43
Kamura et al., 1992	<2	108	—	98
	2-4	171	—	86
	>4	62	—	80

PLN: pelvic lymph nodes

2.5-10.0 cm³ and to 70.4% for patients with a volume of 10.0-50.0 cm³ [Burghardt et al., 1992].

In two studies using multivariate analysis, tumour size proved to be a statistically significant, independent parameter in predicting survival [Delgado et al., 1990; Kamura et al., 1992].

It can be concluded that patients with tumours less than 1 cm in maximal diameter have an excellent prognosis, with a 92-100% survival rate. For patients with large tumours (>4 cm) the reported metastasis rate increases to 23-39.8% and survival drops to 43-80% [Piver and Chung, 1975; Fuller et al., 1989; Delgado et al., 1989; Delgado et al., 1990; Alvarez et al., 1991; Kamura et al., 1992].

Overall, tumour size can be used as a crude predictor of metastatic potential, although the relationship is imperfect. Large tumours are more likely to have metastasis than smaller ones. However, very small tumours have pelvic lymph node metastasis at a clinically significant frequency. Nevertheless, women with small tumours have a very good longterm prognosis and the majority of studies show a strong association between tumour size and outcome.

2.3.2 Histological grade and cell type

Histological classification of malignant tumours is widely employed because it may be relevant for prognosis, it may define a different biological behavior and it may lead to an understanding of the histogenesis of a tumour. For cervical cancer, Reagan and Wentz based a classification system on cell type with potential prognostic relevance [Reagan et al., 1957; Wentz and Reagan, 1959]. Stendahl introduced another system considering not only the histological features of the tumour itself, but the nature of the interaction between the tumour and normal tissues [Stendahl et al., 1980]. A number of studies have been performed that have shown no significant correlation between survival and the degree of differentiation of squamous cell carcinoma or adenocarcinoma of the cervix [Gyndereson et al., 1974; Goellner, 1976; Crissman et al., 1987; Zaino et al., 1992; Stock et al., 1994]. Some authors reported a better survival rate in well differentiated lesions but this correlation was dependent on stage and pelvic lymph node metastasis [Baltzer et al., 1982; Fuller et al., 1988].

Recently, in a study of 445 patients with advanced cervical cancer treated with radiation therapy, a wide inter-observer variation between different pathologists with regard to the assessment of grade and cell type existed. No correlation between histological grade and survival was found [Stock et al., 1994]. Based on the reviewed literature, it is possibly true that histological grading has no prognostic value in cervical cancer. Table 2.7 illustrates the relationship between histological cell type and the incidence of nodal metastasis, recurrence rate and survival as reported in several studies. Most authors report a higher recurrence rate or a decreased survival in patients with an adeno(squamous) carcinoma compared to squamous carcinoma. The incidence of nodal metastasis does not differ significantly between the different cell types. Some studies suggest that adenocarcinoma may have a different pattern of metastasis and is prone to late recurrences and distant metastasis despite negative pelvic lymph nodes [Fuller et al., 1988]. Kamura showed in a multivariate analysis that adenocarcinoma has a worse outcome compared to squamous cell carcinoma, irrespective of other prognostic parameters [Kamura et al., 1992]. It can be concluded that literature data do not suggest that cell type and histological grade are of important prognostic significance.

2.3.3 Lymph vascular space involvement

Lymph vascular space involvement (LVS) is the localization of tumour cells or clusters of tumour cells into lymph and blood vessels. In routine analysis of cervical cancer specimens, the variability with which this parameter is looked for and reported varies between different pathologists.

Lymph vascular space involvement readily found in multiple fields on random view probably has other prognostic significance than one which is found only

Table 2.7: Relationship between histological cell type and the incidence of nodal metastasis, rate of recurrence and survival.

Reference	cell type	N	positive PLN(%)	Recur (%)	Surv (%)
van Nageil et al., 1977	S	75	20	24	77
	AS	7	0	14	86
Chung et al., 1980	S	88	26	17	85
	AS	10	20	20	80
Figge and Tamini, 1981	S	156	17	8	—
	AS	30	30	33	—
Burke et al., 1987	S	206	—	9	—
	AS	69	—	17	—
Fuller et al., 1988	S	357	18	—	83
	AS	48	10	—	71
Kamura et al., 1992	S	289	—	—	91
	AS	52	—	—	70

PLN: Pelvic Lymph Nodes; Recur: Recurrence rate; Surv: Survival rate; S: Squamous cell carcinoma; AS: Adenocarcinoma or Adenosquamous cell carcinoma.

after careful searching of many sections. Therefore, comparison of the different reports on this subject is difficult.

Several reports describe an increase in pelvic lymph node metastasis, a higher recurrence rate and a decreased survival if lymph vascular space involvement is present [Chung et al., 1980; Boyce et al., 1981; Burghardt et al., 1987; Delgado et al., 1989; Kenter et al., 1991]. In a prospective study of the Gynecologic Oncology Group, lymph vascular space involvement showed to be an independent prognostic parameter for 3-year disease free interval [Delgado et al., 1990].

In two studies, however, the significance of LVS on recurrence and survival was eliminated after stratification for pelvic lymph node involvement [Fuller et al., 1988; Kamura et al., 1992].

It can be concluded that the risk for pelvic lymph node metastasis is higher in patients with tumours showing lymph vascular space involvement but the independent prognostic value in both patients with or without pelvic lymph node involvement is controversial, probably due to difficulties in assessment of this parameter.

2.3.4 Depth of invasion into the cervix

Occasionally it may be difficult to examine the exact depth of invasion of a tumour and many different selection criteria are used for the determination of this parameter. Deep invasion is considered to be invasion greater than 10 mm or invasion greater than two-third of the thickness of the cervix. In most studies, a correlation between depth of invasion, pelvic lymph node involvement and survival is observed. The incidence of pelvic lymph node metastasis increased from 9-14% in patients with superficial invasion to 24-28% in patients with deep invasion [Delgado et al., 1989; Fuller et al., 1989; Zaine et al., 1992]. Fuller and coworkers observed a close relationship between tumour size and depth of invasion. After stratifying for tumour size and nodal status the correlation between depth of invasion and survival persisted [Fuller et al., 1989]. In a prospective study of 732 stage I patients, depth of invasion showed to be an independent prognostic factor [Delgado et al., 1990]. However, in a retrospective study, Kamura and coworkers did not find a correlation between depth of invasion and survival after multivariate analysis [Kamura et al., 1992].

Deep tumour invasion into the cervix may occur more frequently in large cervical cancers and the role of depth of invasion as an independent prognostic factor remains controversial.

2.3.5 Parametrial extension

The parametrium is the primary route for direct spread of cervical cancer, both by direct growth and via lymphatic channels. All lymphatic channels from the cervix pass through the parametrium in the direction of the pelvic wall. As mentioned above, clinical staging is based on the local spread of cervical cancer. In stages I and IIA, the parametria are free of tumour by manual palpation while stage IIB is characterized by parametrial infiltration or shortening.

Cancer cells can infiltrate into the parametrium via continuous or discontinuous spread or via lymphatic channel or lymph node involvement. The incidence of parametrial involvement is related to clinical stage and tumour size. In small tumours invading less than 20% of the cervix, 3.4% of the patients has parametrial involvement compared to 35% for tumours invading more than 80% of the cervix [Girardi et al., 1989]. When cancer cells infiltrate the parametria, the frequency of pelvic lymph node metastasis is high and ranges between 60-80 percent [Burghardt et al., 1987].

This is the major explanation for the poor prognosis of patients with parametrial involvement because the clinical impact of parametrial involvement alone is limited. Survival is almost similar for patients with and without parametrial involvement when pelvic lymph nodes are free of tumour [Burghardt et al., 1987].

2.4 CONCLUSIONS

In cervical cancer stages IB and IIA there is a need for prognostic variables to select in both the node negative and the node positive group the patients at risk for recurrence. Reviewing medical literature, a great number of reports concerning this subject have been published, however, nearly all studies are retrospective and conclusions are mainly based on single factor analysis. Most of the prognostic variables are probably interrelated and the relative prognostic importance is difficult to determine without multivariate analysis.

Recently, a few studies comparing several variables with multivariate analysis were described but the results are controversial. Pelvic lymph node and tumour size are the most constant independent parameters in predicting outcome in the different studies published. However, in the studies of the Gynecology Oncology Group, pelvic lymph node metastasis was not an independent variable predicting survival [Delgado et al., 1990]. Probably, cell type and histologic grade is only of prognostic significance in patients with negative pelvic lymph nodes. The prognostic value of lymph vascular space involvement and depth of invasion remains unclear. Both parameters are probably strongly interrelated with lymph node involvement. Age, pregnancy, histological grade and parametrial extension are not of independent prognostic value in cervical cancer stages IB and IIA. The role of trombocytosis and squamous cell carcinoma antigen in predicting outcome should be established in future studies.

The results of this review indicate that still little is known concerning the exact mechanisms leading to metastasis and recurrent disease in cervical cancer. Recently, it became clear that cell genetics, tumour viruses and tumour cell-tumour stroma interactions are important in the initiation of cancer and its subsequent behavior. Many of these factors will be discussed in chapter 3.

Chapter 3

Cancer cells and environmental influences

- 3.1 Introduction
- 3.2 The cell cycle
 - 3.2.1 Cell division
 - 3.2.2 DNA ploidy
- 3.3 Cell genetics
 - 3.3.1 Oncogenes
 - 3.3.2 Tumour suppressor genes
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- 3.5 Growth factors
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 - 3.6.1 Tumour stroma
 - 3.6.2 Basement membrane
 - 3.6.3 Angiogenesis

3.1 INTRODUCTION

Cancer cells proliferating in the absence of normal control mechanisms, are able to invade and colonize surrounding tissues and form secondary tumours or metastases. Even when a tumour has metastasized, all cancer cells are presumed to be derived from a single cell by cell division [Fearon et al., 1987]. This cell has undergone some heritable changes resulting in an alteration in the DNA sequence (genetic change) or a change in the pattern of gene expression (epigenetic change). In general, cancer is not caused by a single event, but is thought to result from the chance occurrence of several independent accidents in one cell with cumulative effects. The development of cancer involves many steps, each governed by multiple factors, some dependent on the genetic constitution of the individual, others dependent on the environment and way of life.

In this chapter, several factors that initiate and promote the development of normal cells to cancer cells and the progression of a tumour will be discussed very briefly. If possible, special reference to the development of cervical cancer will be made.

3.2 THE CELL CYCLE

3.2.1 Cell division

Cells reproduce by duplicating their contents and then dividing into two. The cell cycle is the fundamental means of this process and is traditionally divided into several distinct phases. The process of cell division consists of nuclear division (mitosis) followed by cytoplasmic division (cytokinesis). Mitosis starts with the condensation of the chromosomes and the formation of the mitotic spindle (prophase). The condensed chromosomes contain two chromatids held together at the centromere and are surrounded by the nuclear envelope. The prometaphase starts with the disruption of the nuclear envelope and microtubules of the mitotic spindle attach to protein complexes at each side of the centromere. The microtubules align the chromosomes in a plane between the spindle poles (metaphase) and anaphase begins as the separated chromosomes are pulled to the spindle pole. In the telophase the chromosomes arrive at the poles and a new nuclear envelope reforms around a group of chromosomes. The condensed chromatin expands and mitosis is at an end [McIntosh and Koonce, 1989]. During cytokinesis the cytoplasm divides by the action of a contractile ring of actin and myosin filaments forming midway between the spindle poles, a process called cleavage [Satterwhite and Pollard, 1992].

Replication of the nuclear DNA occurs during a specific part of the interphase, the S (synthesis) phase. The interval between the completion of mitosis and the beginning of DNA synthesis is called the G₁ (G=gap) phase and the interval

between the end of DNA synthesis and the beginning of mitosis is called the G2 phase. G1 and G2 provide additional time for growth to double cell mass before division [Murray and Hunt, 1993].

Control of the cell cycle is based on two families of proteins; the cyclin dependent protein kinases (Cdk) and activating proteins called cyclins that bind to Cdk molecules. These protein complexes triggers the essential processes of cell reproduction by special checkpoints. At least two protein complexes regulate the cell cycle; one at a late G1 checkpoint and the other late in the G2, just before mitosis and at these points the cell cycle can be halted [Murray and Kirschner, 1991].

3.2.2 DNA ploidy

For many years, it has been known that malignant tumours frequently show abnormalities in the number of chromosomes. DNA flow cytometry is used to measure the DNA content of cell nuclei in an attempt to study tumour biology [Schutte et al., 1985]. Although several studies concerning this subject in cervical cancer have been reported, the role of DNA ploidy as a prognostic factor remains controversial.

A relation between the frequency of pelvic lymph node metastasis and DNA ploidy was reported [Dyson et al., 1984]. Jacobsen and coworkers described the flow cytometric analysis of 171 cases with invasive cervical cancer treated with radiotherapy. They found a strong correlation between aneuploidy and recurrence rate [Jacobsen et al., 1985].

Unlike what was to be expected, most studies showed no difference in survival between patients with diploid and aneuploid cervical cancers [Rutgers and Peperzeel, 1987; Davis et al., 1989; Kenter, 1991; Connor et al., 1993].

3.3 CELL GENETICS

Cell proliferation can be regulated directly or indirectly; directly when a cell passes the checkpoints in the cell division cycle and indirectly by regulation of terminal cell differentiation or programmed cell death (apoptosis). In both pathways, regulatory genes can be classified into genes that stimulate an increase in cell number and genes that inhibit cell proliferation. Accordingly, two mutational routes toward uncontrolled cell proliferation and invasiveness are characteristic of cancer. First, a stimulatory gene can become hyperactive; the dominant mutated gene is called an oncogene and secondly an inhibitory gene can be made inactive; the lost recessive gene is called a tumour suppressor gene.

3.3.1 Oncogenes

The eukaryotic genome harbors a large and diverse set of genes, whose mutation, deletion or aberrant expression can lead to malignancy.

Modern techniques of molecular biology have allowed exploration of the cell at the molecular level using Northern, Southern, and Western-blot hybridizations which analyze RNA, DNA, and protein structures, respectively. When the gene of interest is present in small numbers, polymerase chain reaction can be used to expand the number of copies of the gene, yielding sufficient material for DNA sequencing.

Proto-oncogenes are cellular genes capable of transforming into oncogenes by various cellular mechanisms, such as transduction, translocation, point mutation, and gene amplification [Cooper, 1990]. In normal cells, most proto-oncogenes code for components that regulate the social behavior of cells. In particular, they regulate cell to cell signaling by secreted proteins, transmembrane receptors, protein kinases and gene regulatory proteins [Cantley et al., 1991].

The transformed oncogene results in aberrant production of the gene products that can be divided into several classes depending on their intracellular location and cellular function. These include peptide growth factors and receptors, protein kinases, guanosine triphosphate-binding proteins, and nuclear and cytoplasmic regulatory proteins.

A number of oncogenes (i.e. *ras*, *mos*, *abl*, *erbB* and *fes*) share a tyrosine kinase function. These oncogenes can be divided into transmembrane kinases that act as cell membrane receptors and membrane associated kinases that phosphorylate a wide variety of intracellular proteins.

The proto-oncogen *c-erbB-2*, also called *neu*, *NGL*, or *HER-2*, encodes for the receptor of epidermal growth factor (EGF) [Shechter et al., 1985]. When EGF binds to the extra-cellular domain of the receptor, the intracellular domain generates a stimulatory signal inside the cell. Mutation of *c-erbB-2* can result in a deletion of the extracellular EGF binding domain in such a way that the intracellular stimulatory signal is produced constantly, even if no EGF is present. Mutation of the *c-erbB-2* gene or over-expression of the *c-erbB-2* protein is detected in human breast cancer and a correlation with poor prognosis has been reported [Slamon et al., 1987].

In cervical cancer, Kihana and coworkers reported a correlation between over-expression of *c-erbB-2* protein and poor prognosis of patients with cervical adenocarcinomas [Kihana et al., 1994]. Similar results are reported for stage III patients treated with radiation therapy [Oka et al., 1994].

3.3.2 Tumour suppressor genes

Tumour suppressor genes are normal recessive genes with the ability to suppress the growth and metastasis of cells driven to uncontrolled proliferation by oncogenes.

Only a few tumour suppressor genes have been characterized of which the retinoblastoma (Rb) and p53 gene are the most frequently investigated. p53 tumour suppressor gene is located on the short arm of chromosome 17 and codes for a 375-amino acid nuclear phosphoprotein with a short half life time of several minutes [Finlay et al., 1989]. This normal or wild type p53 protein shows growth suppression activities, particularly leading to a reversible arrest in either the G1 phase or in the S phase of the cell division cycle [Casey et al., 1991]. This cell-cycle arrest may result in apoptosis, an irreversible process culminating in individualized cell death [Ulrich et al., 1992; Yin et al., 1992], or allow for repair of genomic aberrations [Bartek et al., 1990; Iggo et al., 1990]. All mechanisms of normal p53 function protect the cell against uncontrolled growth. People who inherit only one functional copy of the p53 gene from their parents are pre-disposed to cancer. Cells lacking both p53 genes carry on dividing, plunging into DNA replication without pausing to repair DNA lesions [Perry and Levine, 1993]. Mutation of the p53 gene has been found in several human tumours [Cattoretti et al., 1988; Van den Berg et al., 1989; Hollstein et al., 1991; Holm et al., 1993] and can be shown by immunohistochemical detection of the mutated gene product, which demonstrates an increased stability and a longer half life than the wild-type protein.

In a study of 192 stage III cervical cancers treated by radiation therapy, p53 immunoreactivity was observed in 26% of the tumours. No correlation was observed between patient survival and p53 protein expression [Oka et al., 1993]. Similar results were also found in a group stage III cervical cancers [Gitsch et al., 1992].

3.4 TUMOUR VIRUSES

Viruses are infectious particles that consist of a DNA or RNA molecule packaged in a protein capsid. Viruses can multiply only inside a host cell. A common outcome of a viral infection is the lysis of the infected cell and the release of infectious viral particules. In a small percentage of infections with DNA viruses, cells allow the virus to enter but not to replicate lytically. The viral chromosome can become integrated into the host cell genome and this genetic change may transform the cell in a cancerous equivalent. This proces is called virus mediated neoplastic transformation by DNA tumour viruses [Zur Hausen, 1991].

For one group of RNA viruses, the so-called RNA tumour viruses, the infection of cells leads simultaneously to a release of progeny virus from the cell surface

and a genetic change in the infected cell that make it cancerous [Varmus, 1988]. Both RNA and DNA tumour viruses transform cells and the presence of viral DNA in the cell causes the synthesis of new proteins that alter the control of cell proliferation. The genes that code for these proteins are also called oncogenes.

3.4.1 Human Papilloma Virus

The human papilloma virus is a member of the papovavirus family of double stranded circular DNA viruses, the genome of which is about 7900 base pairs. The virus characteristically infects the cells of the epidermis and replicates in the layer of maturing epithelium. Infected cells acquire a perinuclear zone or clear zone, referred to as koilocytosis.

More than 50 HPVs have been characterized. However, only 12 HPV types have been associated with malignancy.

In the cervix, infection with human papillomavirus (HPV) is associated with tumour development. HPV-DNA has been detected in 50-100 percent of the cervical cancers and the types 16, 18, 31 and 33 are the types most frequently found [Barnes et al., 1988; Fuchs et al., 1988; Wright and Richart, 1990; van Bommel et al., 1990].

HPV 18 has shown to be a most virulent HPV type, with the greatest transforming ability in vitro [Barness et al., 1990]. This type has been associated with a more rapid onset and progression of cervical cancer [Kurman et al., 1988; Walker et al., 1989].

The oncoprotein E6 encoded by HPV type 16 and 18 forms a complex with and promotes the degradation of the p53 protein [Werness et al., 1990]. In those cancers that lack expression of HPV proteins, mutant p53 has been detected. Consequently, interruption of growth regulation by p53 may occur in nearly all cervical cancers, either through infection with an oncogenic Human Papilloma Virus or through mutation of the host's endogenous p53 gene [Paquette et al., 1993].

Increasing evidence suggests a prognostic role for HPV DNA presence in cervical intra-epithelial neoplasia [Campion et al., 1986; Van Den Brule et al., 1991]. However, the prognostic significance in cervical cancer is controversial. Riou and coworkers observed an increased frequency of positive lymph nodes and a significant lower 2-year survival in patients with absence of HPV DNA compared to HPV positive patients [Riou et al., 1990]. Similar results were found in another study [Higgins et al., 1991]. Unlike this, in another study patients with HPV 16 positive tumours had significantly higher rates of parametrial and pelvic lymph node involvement compared to patients with HPV negative tumours [Girardi et al., 1992].

A relation between recurrence, survival and HPV status is observed in a minority of studies, particularly for HPV type 18 [Burnett et al., 1992]. However, most authors found no difference in survival between HPV positive and negative

cervical cancers [Meanwell et al., 1987; King et al., 1989; Kenter et al., 1991; Jarrel et al., 1992; Van Bommel et al., 1993].

3.5 GROWTH FACTORS

In order to grow and divide, cells must receive positive signals from other cells. Many of these signals are protein growth factors; polypeptides that interact with specific cellular membrane receptors resulting in the stimulation of target cell locomotion, proliferation and differentiation.

They act on the micro-environmental level through paracrine and autocrine mechanisms.

In cancer, the production of autostimulatory growth factors subvert normal growth-regulatory mechanisms and facilitate partial or complete growth autonomy. In addition, growth factors modulate the function of normal stromal cells generating a micro-environment favorable for tumour growth and invasion. Basic Fibroblast Growth Factor (bFGF) stimulates the proliferation of fibroblasts and binds to heparan sulfate proteoglycan in the extra cellular matrix, the latter serving as a reservoir for bFGF [Gospodarowicz et al., 1978; Vlodavsky et al., 1990]. bFGF has potent angiogenetic properties and is mitogenic for a variety of cells including fibroblasts and endothelial cells [Goldfarb, 1990]. In addition, bFGF activates plasminogen activator and the synthesis of collagenase type IV [Buckley Sturrock et al., 1989; Presta et al., 1989]. Together, all activities of bFGF attribute to tumour progression and metastasis.

Transforming Growth Factor- β (TGF β) synthesis and expression can be identified in a variety of normal and transformed cells. It exhibits a multitude of functions in cell growth regulation, tissue development, cell differentiation and adhesion [Rizzino, 1988].

With respect to cell proliferation, TGF β inhibits cell growth in the majority of normal and malignant cells of epithelial, endothelial, and lymphoid origin, whereas it stimulates growth of mesenchymal cells. In addition, TGF β has pleiotropic effects on tumour stromal cells, where it promotes extra cellular matrix formation [Rizzino, 1988] and upregulates the expression of adhesion molecules [Ignatz et al., 1989].

Epidermal Growth Factor (EGF) and Transforming Growth Factor α (TGF α) are structurally related polypeptides that compete for binding to the EGF receptor. TGF α is associated with neoplastic transformation and is predominantly expressed in solid tumour cells, especially of epithelial origin. Autocrine self-stimulation of TGF α has been demonstrated in ovarian carcinoma cells [Kurachi et al., 1991].

Platelet Derived Growth Factor (PDGF) is present in the α granules of platelets where it is released during clotting. PDGF is secreted by various transformed

cells and stimulates proliferation of fibroblasts and smooth muscle cells. Together with EGF, PDGF induces a mitogenic response in endothelial cells and is apparently important in angiogenesis [Hermansson et al., 1988].

In cervical cancer, squamous cell carcinomas frequently express epidermal growth factor receptor [Gullick et al., 1986; Berchuk et al., 1990]. Hayashi and coworkers could not identify a correlation between EGF receptor and nodal status in cervical squamous cell carcinomas [Hayashi et al., 1991]. In addition, overexpression of EGF receptor correlates with a poor prognosis [Pfeiffer et al., 1989] and this was particularly prominent in patients with adenosquamous carcinomas without pelvic lymph node metastasis [Hale et al., 1993].

3.6 THE EXTRACELLULAR MATRIX

The extracellular matrix (ECM) between cells is composed of an intricate network of macromolecules. These macromolecules can be subdivided into polysaccharid chains (glycosaminoglycans) which are usually linked to protein in the form of proteoglycans and in fibrous proteins of structural (collagen, elastin) and adhesive type (fibronectin and laminin).

The macromolecules are mainly produced by cells (fibroblasts) preserve in the ECM [Birk et al., 1991].

Proteoglycans have a diverse structure and are thought to play a major role in signalling between cells. They bind various protein growth factors thereby enhancing or inhibiting the activity of these factors. Moreover, plasma membrane proteoglycans such as betaglycan bind transforming growth factor β (TGF β) and present it to the TGF β receptor; It acts as a co-receptor collaborating with other cell surface receptor proteins in initiating the response of cells to some growth factors [Ruoslahti and Yamaguchi, 1991].

Collagens are fibrous proteins with a triple-stranded helical structure of three polypeptide chains called α chains. About 25 distinct collagen α chains have been identified, each encoded by a separate gene. Types of collagen can be classified into fibrillar (types I, II, III, V, XI) and network forming (types IV and VII) collagens. The major part of the latter ones are found in the basement membrane [Van der Rest and Garrone, 1991]. After being secreted, fibrillar collagens order with the mechanical help of fibroblasts into polymers and bind to the cell surface for support.

Elastin, another fibrous protein, forms a network of elastic fibers that gives the tissues resilience. It is a major component in skin, lungs and bloodvessels.

Fibronectin and laminin are examples of large, multidomain, adhesive glycoproteins found in the extracellular matrix as well as the basement membrane. Both proteins help organize the extracellular matrix and help cells adhere it [Hynes, 1986]. Fibroblasts are least specialized cells that play a central part in the support and repair of almost every tissue. They secrete a nonrigid extracel-

lular matrix rich of type I and type III collagen. The fibroblast can differentiate into several other mesenchymal cells regulated by the extracellular matrix, by hormones and growth factors.

3.6.1 Tumour stroma

The majority of epithelial cancers are composed of malignant epithelial cells and nourishing and supporting tissues, comprising the stroma. All variations, ranging from none to hardly any stroma in anaplastic tumours to abundant stroma in cirrhouc carcinomas, a process known as tumour desmoplasia, can be distinguished. The tumour stroma can be divided into two major categories based on their composition and location: basement membranes and interstitial connective tissue. Basement membranes separate organ cells, epithelia, or endothelia from the interstitial connective tissue and form the first barrier for invading tumour cells.

The interstitial connective tissue contains cells, such as fibroblasts, myofibroblasts, osteoblasts, chondroblasts or macrophages, depending from tissue type, located in a matrix composed of collagen fibers, glycoproteins and proteoglycans. Also, blood vessels and inflammatory cells are usually present in the tumour stroma.

In most types of carcinomas, the cancer cells are surrounded by a specific stroma that differs from normal connective tissue both by quantitative and qualitative changes [Van den Hooff, 1988]. It has been proposed that tumour desmoplasia represents a host response to prevent further cancer cell spreading and that inhibition of this response caused increased invasion and metastasis [Barsky and Gopalakrishna, 1987; Ahmed, 1990; Nakanishi et al., 1994]. On the other hand, the tumour stroma contributes to the ability of cancer cells to multiply outside their own compartment [Fidler, 1990; Clark, 1991] and is necessary for tumour growth beyond a certain size [Folkman, 1992].

In the last decade, it has become clear that tumour cell-stromal interactions play a pivotal role in tumour progression and metastasis. Disturbances in these interactions are manifested in many ways: gaps in the basement membrane [Liotta et al., 1980], uncontrolled growth of tumour stromal fibroblasts in vitro [Delinassios et al., 1983], the expression of stromal growth factor [Yee et al., 1989], transforming growth factor β controls both tumour cell proliferation and the synthesis of many stromal components [Moses et al., 1990; Sporn and Roberts, 1990], and tumour cells both stimulate and inhibit neovascularisation [Rastinejad et al., 1989; Dvorak et al., 1991].

Although several of these tumour cell-stromal alterations supply prognostic information, little is known concerning this subject in cervical cancer.

3.6.2 Basement membrane

Basement membranes (BM) are complex extracellular matrix structures, separating epithelium from the underlying stroma.

They provide physical support for the epithelial cells and consist of a network of type IV collagen fibrils to which the other constituents, including laminin, entactin and heparan sulphate proteoglycan, are attached [Martinez-Hernandez and Amenta, 1983; Leblond and Inque, 1989].

In normal tissues, basement membranes are stable, continuous and regular structures playing an important role in cell differentiation and cell migration [Timpl, 1989]. For epithelial tissues, basement membranes are the natural borders. Tumour cells have the capacity to transmigrate through the basement membranes into the surrounding mesenchymal stroma as part of the process of invasion [Liotta et al., 1980; Liotta et al., 1983].

Interactions with the basement membrane can be separated into three steps: attachment, matrix dissolution, and migration.

The tumour cell binds to the basement membrane surface mediated by receptors of the integrin [Hynes, 1987] and non-integrin family [Aznavorian et al., 1990]. After attachment, lysis of the basement membrane by proteinases is localized at the point of tumour cell contact. The tumour cell migrates across the basement membrane and stroma through the zone of matrix proteolysis.

The last decade, many investigators have studied basement membranes in a variety of neoplasms [Burtin et al., 1982; Kallioinen et al., 1983; Haglund et al., 1984; Willebrand et al., 1986; Havenith et al., 1988]. Immunostaining with antibodies to intrinsic components e.g. collagen type IV and laminin are useful to gain knowledge of the mechanisms that lead to basement membrane alteration in invasive cancer.

Several authors have reported a partial or complete loss of basement membrane during the process of tumour invasion [Albrechtsen et al., 1981; Siegal et al., 1981; Stenback et al., 1985].

The presence of basement membrane depends on a balance between its production and breakdown. Limited basement membrane composition is probably caused by the production of highly active BM degrading enzymes by tumour cells and by the inability of tumour cells to deposit BM components into an organized basement membrane. This information may be related to the biological behavior of a tumour and a correlation between degree of differentiation, stage, metastasis rate and prognosis is reported for several tumours [Daher et al., 1987; Havenith et al., 1988].

Richards and Furness investigated basement membrane continuity in benign, premalignant and malignant epithelial conditions of the cervix. In this study the number of basement membrane breaks demonstrated by immunohistochemical staining with polyclonal antibodies to laminin, increases with increasingly severe cervical intra-epithelial neoplasia (CIN). Micro-invasive and invasive

cervical carcinomas showed an absent or severely fragmented basement membrane [Richards and Furness, 1990]. This observation doesn't fit in with a study of Stewart and McNicol. They found similar basement membrane defects in situ and in invasive carcinoma of the cervix and in both conditions type IV collagen staining was of limited diagnostic value in the differential diagnosis [Stewart and McNicol, 1992].

Pitt and coworkers observed a significant correlation between the pattern of the basement membrane, indicated by type IV collagen expression, a poor prognosis and an infiltrating growth pattern in 63 cervical carcinomas. They concluded the presence or absence of type IV collagen expression to be a useful prognostic parameter, particularly in adenocarcinoma [Pitt et al., 1992].

3.6.3 Angiogenesis

Angiogenesis is the growth of new blood vessels toward and within a tumour. It has been shown that tumours do not grow beyond a size of 2-3 mm³ unless they are able to attract the growth of new capillaries from the existing vascular network [Folkman, 1990; Bicknell and Harris, 1991].

Angiogenesis starts and develops in response to extracellular signals. A number of soluble molecules, angiogenetic factors, influence endothelial cell growth and differentiation. Direct angiogenetic factors can stimulate endothelial cells to migrate, divide and differentiate in vivo and in vitro. Indirect angiogenetic factors have no effect on endothelial cells in vitro and operate in vivo by mobilizing other cells and factors.

Although endothelial cells are able to secrete their own growth factors, many of the angiogenetic factors, such as vascular endothelial growth factor (VEGF), transforming growth factor α (TGF α), and transforming growth factor β (TGF β) are produced by tumour cells, macrophages and platelets, suggesting that angiogenesis is under both autocrine and paracrine control [Blood and Zetter, 1990; Conn et al., 1990; Dvorak et al., 1991; Weidner, 1992a].

In addition to regulation by soluble growth factors, angiogenesis is also controlled by the extracellular matrix. A major step in the migration of endothelial cells is the degradation of the extracellular matrix by proteases. Endothelial cell-extracellular matrix adhesion and migration are mediated by integrins that mediate the interactions with collagens, laminin and fibronectin [McCormick and Zetter, 1992].

Angiogenesis has an important role in the process of metastases. By increasing vascular area, neovascularisation enhances the opportunity for a tumour cell to gain access to the circulation. In the target organ, tumour cells must promote angiogenesis to grow and form metastases.

Quantitative evidence that the degree of vascularization was related to the probability of metastases was first reported for cutaneous melanoma [Srivastava et al., 1988].

In 1991, Weidner and coworkers studied angiogenesis in breast cancer. They stained endothelial cells immunohistochemically in 49 invasive breast carcinomas. In this study microvessel count correlated with metastatic disease either in axillary lymph nodes and/or at distant sites [Weidner et al.,1991]. A similar founding has been reported in non-small-lung cancer and prostate carcinoma [Macchiarini et al., 1992; Weidner et al., 1993].

Further studies in breast carcinoma showed microvessel density to be an independent prognostic parameter [Weidner et al., 1992b; Toi et al., 1993]. These data support the hypothesis that microvessel density can help in selecting patients with low and high risk for recurrence and may have therapeutic implications.

Chapter 4

Aims of the study

4.1 AIMS OF THE STUDY

Cervical cancer stages IB and IIA is associated with a good prognosis of approximately 80 percent five year survival. When the pelvic lymph nodes are involved, the five year survival rate decreases to 40-60 percent. Many clinical and histopathological parameters appear to be related to nodal involvement and survival.

Of all the prognostic parameters, metastasis to the pelvic lymph nodes is the most predictable and certain risk factor in cervical cancer stages IB and IIA. This risk appears to increase with the number and the highest level of the involved nodes. Despite the prognostic importance of pelvic lymph node metastasis, it does not fully account for recurrent disease and survival. In absolute terms, an equal number of recurrences and deaths occur in the node negative as in the node positive group of patients. Accurate detection of patients at risk for recurrence in both node negative and node positive groups is of the utmost importance in cervical cancer stages IB and IIA. After stratification for pelvic lymph node involvement, tumour size and histological cell type are probably of prognostic importance in the majority of the reviewed studies.

The independent prognostic value of other parameters, such as lymph vascular space involvement and depth of invasion is controversial and a lot of other factors need further investigation to prove their value in predicting clinical outcome.

Despite the great effort made to investigate tumour characteristics, treatment modalities designed on the basis of these parameters have not been shown to improve survival.

To prevent over and undertreatment, it is mandatory to explore new and accurate parameters that predict tumour behavior.

In the last decade, increasing evidence has shown the important role of the stromal compartment in tumour behavior. Basement membrane deposition and angiogenesis have been shown to be of prognostic importance in several human cancers and the exact role of desmoplasia, proliferation of fibroblasts and myofibroblasts, oncogene expression, growth factors and cytokines is still unresolved.

In this thesis the tumour stroma in cervical cancer is analyzed and the prognostic value of several stromal parameters is investigated.

The study is based on the clinical and histopathological profiles of 205 patients with cervical cancer who were admitted to the Department of Obstetrics and Gynecology, University Hospital of Maastricht, The Netherlands, between 1978 and 1993.

In chapter 5 the clinical and therapeutical results are presented of 99 patients with stages IB and IIA cervical cancer treated in the period 1978-1990. The mode of treatment is discussed.

A study on the prognostic significance of basement membrane pattern in stages IB-IV tumours is presented in chapter 6.

Chapter 7 and 8 respectively present the correlation of stroma volume and tumour angiogenesis with pelvic lymph node status, histopathologic parameters as well as disease free survival.

Finally, in chapter 9, the results of these studies are correlated in a multivariate analysis and discussed against the background of recent literature. Future prospects are speculated upon.

Chapter 5

Early stage cervical cancer: aborted versus completed radical hysterectomy

- 5.1 Summary
- 5.2 Introduction
- 5.3 Material and methods
 - 5.3.1 Patients
 - 5.3.2 Surgical procedure
 - 5.3.3 Radiotherapy
 - 5.3.4 Follow-up
 - 5.3.5 Recurrence
 - 5.3.6 Complications
- 5.4 Results
- 5.5 Discussion

5.1 SUMMARY

In a retrospective study, the treatment results of patients with stages IB and IIA cervical cancer were evaluated. In 26 patients radical hysterectomy was discontinued after intraoperative finding of positive lymph nodes. These patients received radiotherapy. In 57 patients lymph nodes were negative and radical hysterectomy was completed. Of these, thirteen patients received adjuvant radiotherapy because of positive surgical margins or parametrial involvement and 44 patients received no adjuvant therapy.

Five-year survival was 61% in patients with positive pelvic lymph nodes and 88% in patients with negative pelvic lymph nodes, comparable with the results mentioned in literature. The complication rate did not differ from similar other reports.

This management shows treatment results comparable with other reports with minimal morbidity.

5.2 INTRODUCTION

The management of patients with early stage cervical cancer, i.e. stages IB and IIA, remains a controversial issue [Morley and Seski, 1978; Potter et al., 1990]. The treatment in most institutions consists of radical hysterectomy with pelvic lymphadenectomy. This is combined with adjuvant radiotherapy when positive lymph nodes or positive surgical margins are found after completing radical surgery.

In these therapeutic regimes, the mode of treatment is not influenced by the intraoperative finding of lymph node involvement. It is questionable, however, if completing the radical hysterectomy after the intraoperative finding of positive lymph nodes adds to the cure rate [Potter et al., 1990].

Moreover, radiotherapy in an area of recent major surgery may influence the complication rate [Barter et al., 1989].

We therefore adjusted the approach in the treatment of patients with cervical cancer stages IB and IIA. In patients with positive lymph nodes as diagnosed by frozen section during surgery the procedure was stopped and the patient subsequently received radiotherapy. In patients without lymph node involvement radical hysterectomy was completed.

The results of this policy are compared with the results known from literature and the rationale is discussed.

Table 5.1: Clinical stage according to the FIGO classification (N=164).

stage	N	%
IA	13	7.9
IB	70	42.7
IIA	29	17.8
IIB	30	18.3
III	15	9.1
IV	7	4.2
total	164	100

5.3 MATERIAL AND METHODS

5.3.1 Patients

From august 1978 to january 1991, 164 patients with cervical cancer were treated in the University Hospital of Maastricht.

The patients were staged according to the FIGO classification (Table 5.1).

In 99 patients cervical carcinoma was diagnosed as stages IB and IIA before treatment was started. Out of these 99 patients, 13 patients underwent primary radiotherapy, since surgery was estimated too great a risk due to obesity or poor medical condition. The remaining 86 patients underwent surgery and are included in this study.

The leading surgeon was the same in all procedures (JdH).

5.3.2 Surgical procedure

The peritoneal cavity was reached by a left paramedian lower abdominal incision extending cranial of the umbilicus.

The procedure was started by a pelvic lymphadenectomy at the left side or at the contralateral side if pathologic lymph nodes were suspected by palpation. Pelvic lymph nodes near the common iliac artery, the external iliac and hypogastric artery and the lymph nodes in the obturator fossa were dissected and separately sent to the pathologist for frozen section investigation. If one of the lymph nodes was found to be positive for cancer, surgery was discontinued. In case of negative lymph nodes the same procedure was performed on the opposite side. Again positive lymph nodes called for discontinuation of the procedure. In case of negative lymph nodes a radical hysterectomy was performed, consisting of removal of the uterus and contiguous parametrial tissue to its most lateral extent,

of the paravaginal tissue and the upper portion of the vagina and the proximal uterosacral ligaments.

5.3.3 Radiotherapy

In patients with positive lymph nodes or positive resection margins radiotherapy was started, consisting of whole pelvis radiation, if necessary combined with intracavitary application in case the uterus was not removed because of intra-operative positive lymph nodes.

5.3.4 Follow-up

After completion of therapy patients were seen at regular intervals every twelve weeks during the first two years and every six months thereafter. Clinical examination and vaginal cuff cytology were performed.

Five year survival rates were calculated for patients followed for five years or more.

5.3.5 Recurrence

Local recurrence was defined as cancer recurring in the uterus or the upper two thirds of the vagina. Pelvic recurrence was defined as cancer in the pelvis or the lower one third of the vagina. Distant metastases were defined as cancer in distant lymph nodes or organs outside the pelvic cavity.

5.3.6 Complications

Two different kinds of complications were discerned.

Complications related to surgery which appeared within 30 days and complications related to full pelvic irradiation.

5.4 RESULTS

A total of 86 patients were included in this study: 64 patients with stage IB and 22 patients with stage IIA (Figure 5.1).

The age at diagnosis reached from 24 to 69 years (median 45 years).

Patients were followed up for a period of 12 to 160 months (median 60 months). No patient was lost to follow up. Histologic examination showed squamous cell carcinoma in 73 (84.9%), adenocarcinoma in 10 (11.6%) and adenosquamous carcinoma in 3 (3.5%) patients.

In 57 patients radical hysterectomy and pelvic lymphadenectomy was completed. In 44 patients pelvic lymph nodes were negative and adjuvant radiotherapy

Figure 5.1: Treatment of 99 patients with cervical cancer stages IB and IIA (University Hospital, Maastricht, The Netherlands).
RT: Radiotherapy, WM: Wertheim-Meigs

was not indicated (group 1). Thirteen patients underwent adjuvant radiotherapy: in five patients surgical margins were positive for cancer, in five other patients cancer invaded to less than 2 millimeters distance from the surgical margin and in three patients there was microscopic involvement of the parametrium (group 2). In 29 out of 86 patients the procedure was discontinued: in 26 patients the lymph nodes were positive for cancer: 18 out of 64 patients (28%) in stage IB and 8 out of 22 patients (36%) in stage IIA (group 3). In one patient the ovary was involved and two patients had macroscopic involvement of the parametrium. These last three patients were excluded from further study.

The survival and pelvic recurrence rates of the three groups of patients (N=83) were compared with the results known from literature.

Sixty-two out of the 83 patients were followed up for five years or more, 36 patients with negative lymph nodes and radical surgery (group 1), 8 patients with negative lymph nodes and radical surgery plus adjuvant radiotherapy (group 2) and 18 patients with positive lymph nodes and aborted radical surgery plus radiotherapy.

Five year survival of the entire group was 81% (50/62). Five year survival was 88% in group 1 and group 2 patients unlike the 61% survival in patients with positive lymph nodes (group 3). Figure 5.2 shows the survival curves for patients with and without lymph node involvement.

Twelve out of 62 patients (19%) had a relapse within five years after diagnosis. All these patients died of disease.

Local and pelvic recurrence and distant metastases as first sign of relapse in relation to nodal involvement and therapy are shown in table 5.2.

The number of complications in patients with negative or positive lymph nodes are shown in table 5.3. Overall there were 22 (27%) lymphocysts and 8 (9.6%)

Table 5.2: Local and pelvic recurrence and distant metastases as first sign of relapse in relation to nodal involvement and therapy in patients with cervical cancer stages IB and IIA followed for five years or more (N=62).

relapse within five years	radical surg group 1(N=36)	radical surg +radiotherapy group 2(N=8)	aborted surg +radiotherapy group 3(N=18)
local recur	1	1	3
pelvic recur	2	0	3
distant metast	1	0	1

Surg: surgery, recur: recurrence, metast: metastasis.

Table 5.3: Complications related to radical surgery and/or full pelvic irradiation in patients with cervical cancer stages IB and IIA (N=83).

	radical surg group 1(N=44)		radical surg +radiotherapy group 2(N=13)		aborted surg +radiotherapy group 3(N=26)	
	N	(%)	N	(%)	N	(%)
lymphocyst	13	(30)	4	(31)	5	(19)
thrombo-embolism	6	(14)	1	(7.7)	1	(3.8)
ileus	2	(4.5)	0		0	
fistula	0		0		2	(7.7)
ureter stricture	0		0		1	(3.8)
mortality	0		0		0	

thrombo-embolic complications. Two patients developed an entero-uterine fistula which was surgically closed. One patient had a stricture of the right ureter which was re-implanted into the bladder. None of these three patients had recurrent disease.

5.5 DISCUSSION

Previous studies on the management of early stage cervical cancer have reached various conclusions. It is generally accepted that treatment for localized cervical

Figure 5.2: Survival curves in the three groups of patients.

cancer should be truly radical but the controversy between operative and radiotherapy is still unresolved [Morley and Seski, 1976].

Advantages of operation are the possibility to preserve the ovaries, a better sexual function and the emotional satisfaction that the tumour has been removed. Furthermore, patients in high-risk groups (lymph node involvement, bulky tumours) can be selected with the possibility to define relevant morphologic criteria and to individualize treatment. This also allows an accurate comparison between the results of treatment in the different centres.

After radical hysterectomy, pelvic radiation has been a standard treatment in patients with positive pelvic lymph nodes or other poor prognostic factors. In this study five patients were treated with postoperative radiotherapy because of positive surgical margins. In another five patients tumour margins were less than 2 mm free of tumour. The management of these patients is controversial. In order to be safe, they were treated with postoperative radiotherapy.

From retrospective studies it has been known that radiotherapy may limit or control pelvic recurrence but not necessarily improve survival when pelvic lymph nodes are involved. Morrow found in a study of 195 women with stage I squamous cell cancer treated with radical surgery and post-operative radiotherapy when lymph nodes were positive, no significant improvement in survival [Morrow, 1980]. Baltzer and coworkers evaluated 199 women with pelvic lymph node metastases and found that post-operative radiotherapy did not improve survival [Baltzer, 1985].

In our series five-year survival rate in patients with negative lymph nodes is 88%. In patients with positive pelvic lymph nodes and aborted hysterectomy five year survival rate is 61%, comparable with the results mentioned in literature.

The incidence of positive pelvic lymph nodes in our series is high. Thirty percent of the surgically treated patients had pelvic lymph node metastasis. The exact reason for this high number of positive pelvic lymph nodes is not sure; an explanation can be that also patients with bulky stage IB and large stage IIA disease were initially treated with radical surgery. The accurate removal of all lymphatic tissue can be another explanation.

In a recent report, Potter and coworkers concluded that no survival advantage or improved local control was demonstrated by completing radical hysterectomy when post-operative radiation therapy has already been decided upon [Potter et al., 1990]. The benefit of this approach to discontinue the surgical procedure in favour of radiation therapy when lymph nodes are involved by tumour is a less extensive surgical procedure with theoretically fewer complications.

In this study lymphocysts were found in a high number of 30% in patients with completed radical surgery compared to 19% when radical surgery was aborted. Kenter observed lymphocysts in 6.7% of the patients surgically treated [Kenter, 1991]. Two reasons probably are an explanation for our high number of lymphocysts. In the early years of this study we closed the visceral peritoneum accurately and nearly every patient was checked for lymphocysts by ultrasound. The total number of patients with severe complications of the urinary and gastro-intestinal tract is 6.0% in our series. The majority of these severe complications were observed in the group of patients with positive pelvic lymph nodes. All these patients had large tumor volumes with multiple lymph node metastasis. The incidence of these severe complications are not different from other similar reports [Hatch et al., 1984; Kjorstad et al., 1983; Stryker et al., 1988].

It can be concluded that the presented management shows treatment results comparable to those reported in literature. It probably has the added advantage that it minimalizes morbidity.

Chapter 6

Basement membranes in cervical cancer: relationship to pelvic lymph node metastasis and prognosis

- 6.1 Summary
- 6.2 Introduction
- 6.3 Material and methods
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 - 6.4.3 Basement membrane pattern and survival
- 6.5 Discussion

6.1 SUMMARY

In this retrospective study, the pattern of basement membrane expressed by type IV collagen immunostaining, was evaluated in 156 patients with cervical cancer FIGO stages IB-IV. Staining patterns were assessed semiquantitatively and divided into three patterns, according to intensity and continuity of staining: thick basement membranes with minimal discontinuity (pattern I), thin basement membranes with moderate discontinuity (pattern II) and fragmentary or absent basement membranes (pattern III).

Correlations between basement membrane pattern, FIGO stage and pelvic lymph node status were tested using Chi Square statistics. Kaplan-Meier estimates of disease free survival were calculated for groups of patients with basement membrane pattern I, II or III and compared using the log-rank test.

A fragmented or absent basement membrane, pattern III, was more frequently observed in advanced cervical cancer stages IIB/IV ($p=0.02$). In patients with early cervical cancer stages IB/IIA this pattern was associated with the presence of pelvic lymph node metastasis ($P<0.0001$) and significantly associated with a decreased survival rate ($p=0.05$). In advanced stage disease no prognostic value was observed.

These data indicate that in patients with early-stage cervical cancer the basement membrane pattern, expressed as the type IV collagen staining pattern, may be a useful marker in predicting lymph node metastasis and survival.

6.2 INTRODUCTION

Basement membranes (BM) are complex extracellular matrix structures, separating epithelium from the underlying stroma. They provide physical support to the epithelial cells and consist of a network of type IV collagen fibrils to which the other constituents, including laminin, entactin, and heparan sulphate proteoglycan, are attached [Martinez-Hernandez and Amenta, 1983; Leblond and Inque, 1989].

In normal tissues, basement membranes are stable, continuous and regular structures with an important role in cell differentiation and cell migration [Timpl, 1989]. Cancer cells have the capacity to destroy the basement membrane [Liotta et al., 1980] and are able to migrate into the surrounding mesenchymal stroma [Liotta et al., 1983]. Using immunohistochemical staining methods, discontinuities and focal absence of basement membranes are reported in several invasive tumours [Barsky et al., 1983; Havenith et al., 1988; Nakamura et al., 1987] and are associated with a high rate of metastasis and a poor prognosis [Willebrand et al., 1986; Havenith et al., 1988b; Daher et al., 1987].

In cervical cancer, the prognosis is largely dependent on the presence or absence of pelvic lymph node metastasis. Five year survival drops by 40-50% in each

stage of disease when pelvic lymph nodes are involved by tumour [van Bommel et al., 1987].

The purpose of this study was to determine in patients with cervical cancer stages IB-IV whether the integrity of the basement membrane, expressed as the type IV collagen staining pattern, is indeed a parameter predicting metastasis and survival.

The hypothesis we wished to test was that patients whose tumours showed discontinuity and focal absence of basement membranes, had a high rate of metastasis and a poor prognosis, compared to those patients in which the tumours showed a thick, continuous basement membrane.

6.3 MATERIAL AND METHODS

6.3.1 Clinical data

From 1978 to 1993, 205 patients with invasive cervical cancer were admitted to the University Hospital of Maastricht, including 67 referrals from four different regional community hospitals. All patients were staged according to the classification of the International Federation of Gynaecology and Obstetrics.

The number of patients assessed as having early cervical cancer was 145; 21 (10.2%) patients stage IA, and 124 (60.5%) stages IB and IIA. Advanced disease (stages IIB, III and IV) was diagnosed in 60 (29.3%) patients. Patient follow up ranged from 6 months to 15 years. (median 6.2 years).

Twenty eight patients were excluded from the study because of incomplete follow-up data (6) or insufficient material for immunohistological studies (22). Patients with early cervical cancer stage IA were also excluded; the remaining 156 patients; 116 patients stages IB/IIA and 40 patients with advanced disease (stages IIB, III and IV) form the basis of this study.

The primary treatment for stages IB/IIA patients was radical surgery according to Wertheim-Meigs. During the surgical procedure, pelvic lymph nodes were examined by frozen sections and if negative the procedure was completed. When pelvic lymph node metastasis was found the surgical procedure was abandoned and full pelvic and intracavitary radiation therapy was started after two weeks. Of the 116 stages IB/IIA patients, 75 patients were treated by complete radical hysterectomy and pelvic lymphadenectomy. Fourteen of these 75 patients received post-operative radiation therapy because of positive surgical margins (n=5), near positive (less than 2 mm) surgical margins (n=6) or microscopical involvement of the parametrium (n=3). In this group of 75 patients, survival without evidence of disease was 88%.

In 27 of the 116 patients the procedure was abandoned because of pelvic lymph node involvement found by frozen section during operation. All of these patients underwent radiation therapy and survival without evidence of disease was 65%.

Figure 6.1: Kaplan-Meier Analysis of Survival of Patients with Cervical Cancer, According to FIGO Stage (N=156)

IB: stage IB; IIA: stage IIA; IIB: stage IIB; III: stage III; IV: stage IV. (Log rank, $X^2=45.1$, $DF=4$, $P<0.0001$)

Fourteen out of the 116 patients received primary radiation therapy because of relative or absolute contraindications for surgery such as age, obesity or poor medical condition. The survival rate without evidence of disease was 65%. A detailed report of the results of this treatment policy was described elsewhere [Bremer et al., 1992].

All patients with advanced disease were treated with primary radiation therapy. Five year survival in stage IIB was 38%, in stage III 22% and in stage IV, no patient survived for at least five years. Figure 6.1 shows the Kaplan Meier survival curves according to stage.

6.3.2 Histopathological analysis

Specimens were routinely processed, paraffin-embedded and stained by standard histochemical techniques. A diagnosis of a squamous cell carcinoma in 139 (89.2%), an adenocarcinoma in 14 (8.9%) and an adenosquamous carcinoma in 3 (1.9%) was made. The basement membranes were stained with mouse monoclonal antibody directed against type IV collagen [Havenith et al., 1987] by standard immunohistochemical procedures using an avidin-biotin-HRP detection system [Ormanns et al., 1985]. Briefly, tissue sections (3m) were deparaffinized and endogenous peroxidase was blocked with 0,6% H_2O_2 in methanol.

Enzymatic digestion was performed with 0,1% pepsin in 0,1N HCL. After rinsing sections were incubated with the monoclonal antibody (diluted 1:500)

for 45 min at room temperature, rinsed in phosphate buffered saline, incubated with a biotinylated rabbit anti-mouse immunoglobulin (DAKO 1:1000) for 30 min, rinsed and incubated with HRP labelled streptavidin-biotin complex (DAKO 1:1000) for 30 min. After final rinsing a diaminobenzidine H_2O_2 complex with 0,01 M imidazole (Merck) was used to visualize the immunoreaction. External and internal controls for specificity and sensitivity of the staining were used. In all sections tested a strong and well-defined staining pattern of the normal epithelium- and vessel wall basement membranes was obtained.

The amount of type IV collagen immunoreactivity at the tumour/stroma interface was scored semi-quantitatively and 3 patterns of basement membranes, based on intensity of staining and continuity of the type IV collagen immunoreactivity were discerned:

- pattern I* : a thick and normal basement membrane with minimal discontinuity; more than 75% of the tumour/stroma interface showed type IV collagen immunoreactivity.
- pattern II* : a thin basement membrane with moderate discontinuity; between 25% and 75% of the tumour/stroma interface showed type IV collagen immunoreactivity.
- pattern III*: a fragmentary or absent basement membrane; less than 25% of the tumour/stroma interface showed type IV collagen immunoreactivity.

6.3.3 Statistical analysis

The objective of this study was to assess the relationship between the pattern of basement membrane, stage, pelvic lymph node metastasis and disease free survival. The correlation between basement membrane pattern and stage or pelvic lymph node metastasis was tested using chi square statistics. Kaplan-Meier Survival curves were calculated and compared using the log-rank test. Data were analyzed with BMDP software, version 1990 (University of California Press, Berkeley) on an IBM personal computer.

6.4 RESULTS

6.4.1 Basement membrane pattern and stage

Table 6.1 shows the relation between the pattern of the basement membrane and the stage. Since no major differences could be observed between stages IB and IIA, and stages IIB, III and IV, statistical analysis was performed after combin-

Table 6.1: Correlation between Basement Membrane Pattern and FIGO Stage in Cervical Cancer (N=156).

Stage of disease	Basement membrane pattern			
	pattern I N	pattern II N	pattern III N	total N
IB	41	15	29	85
IIA	17	1	13	31
early stage (IB+IIA)	58	16	42	116
IIB	6	2	13	21
III	6	0	8	14
IV	2	0	3	5
advanced stage (IIB+III+IV)	14	2	24	40

BM pattern I: thick and minimal discontinuous; BM pattern II: thin and moderate discontinuous; BM pattern III: fragmentary or absent ($X^2=7.42$, $DF=2$, $P=0.02$).

ing stages IB and IIA patients in early cervical cancer, and combining patients in stages IIB, III and IV in advanced cervical cancer.

In patients with early cervical cancer basement membrane pattern I was observed in 50%, pattern II in 14% and pattern III in 36%, whereas in advanced cervical cancer, the basement membrane pattern distribution was 35%, 5% and 60% for pattern I, II and III respectively. The difference was statistically significant. ($X^2=7.42$, $DF=2$, $P=0.02$)

Table 6.2: Correlation between Basement Membrane Pattern and Pelvic Lymph Node Status in Cervical Cancer FIGO Stages IB and IIA (N=102).

Pelvic lymph node status	Basement membrane pattern		
	pattern I	pattern II	pattern III
negative	58%(44/75)	19%(14/75)	23%(17/75)
positive	26%(7/27)	4% (1/27)	70%(19/27)

BM pattern I: thick and minimal discontinuous; BM pattern II: thin and moderate discontinuous; BM pattern III: fragmentary or absent ($X^2=20.1$, $DF=2$, $P<0.0001$).

Figure 6.2: Kaplan-Meier Analysis of Survival of Patients with Cervical Cancer FIGO Stages IB-IV, According to Basement Membrane Pattern (N=156).

I: BM pattern I; thick and minimal discontinuous; II: BM pattern II; thin and moderate discontinuous; III: BM pattern III; fragmentary or absent (Log rank, $X^2=6.75$, $DF=2$, $P=0.03$).

6.4.2 Basement membrane pattern and lymph node metastasis in cervical cancer stages IB and IIA.

Pelvic lymph node status was known in the 102 patients with stages IB and IIA only. In 27 patients pelvic lymph node metastasis were present and, as shown in Table 6.2, associated with a fragmented or absent basement membrane (pattern III). ($X^2=20.1$, $DF=2$, $P<0.0001$)

6.4.3 Basement membrane pattern and survival.

The Kaplan Meier survival curves according to basement membrane pattern are shown in Figure 6.2. Survival was significantly decreased in patients with a fragmented or absent basement membrane (pattern III). Patients whose tumours showed basement membrane patterns I or II had nearly equal survival rates and were combined in the further analysis.

The Kaplan-Meier survival curves of patients whose tumours had a basement membrane pattern I/II and a basement membrane pattern III are presented for early (Figure 6.3) and advanced cervical cancer (Figure 6.4). Only in early cervical cancer a statistically significant correlation between survival and the basement membrane pattern was observed. (Log rank: $X^2=3.8$, $DF=1$, $P=0.05$) In advanced disease, no correlation between basement membrane pattern and survival was observed.

Figure 6.3: Kaplan-Meier Analysis of Survival of Patients with Cervical Cancer FIGO Stages IB and IIA, According to Basement Membrane Pattern (N=116).

I + II: BM pattern I + II; thick and minimal discontinuous or thin and moderate discontinuous;
 III: BM pattern III; fragmentary or absent (Log rank, $X^2=3.8$, $DF=1$, $P=0.05$).

Figure 6.4: Kaplan-Meier Analysis of Survival of Patients with Cervical Cancer FIGO Stages IIB, III and IV, According to Basement Membrane Pattern (N=40).

I + II: BM pattern I + II; thick and minimal discontinuous or thin and moderate discontinuous,
 III: BM pattern III; fragmentary or absent (Log rank, $X^2=0.033$, $DF=1$, $P=0.86$).

No difference in basement membrane pattern was observed between the different histological subtypes.

6.5 DISCUSSION

This study demonstrates that in patients with cervical cancer loss of basement membrane integrity, as expressed by the pattern of type IV collagen staining, is associated with more advanced stages of disease, whereas in patients with an early stage of the disease this loss of integrity is associated with pelvic lymph node metastasis and decreased survival rate. In advanced cervical cancer no correlation between basement membrane pattern and survival was observed.

The basement membrane is the natural border between the epithelial tumour cells and the stromal compartment and prior to invasion and metastasis the tumour cell has to penetrate the basement membrane in order to invade the mesenchymal stroma and subsequently the vascular lumen [Martinez-Hernandez and Amenta, 1983; Timpl, 1989; Liotta et al., 1980; Liotta et al., 1983]. The basement membrane integrity depends on a balance between production and breakdown. Specific and non-specific proteases are acting in the degradation whereas both epithelial and stromal cells contribute to the production of the basement membrane [Tryggvason, 1991; Simon-Assman et al., 1988].

In cancer there is a wide variation in basement membrane quality; the basement membrane may be rather pronounced and thick, the amount of discontinuities, fragmentation is variable and occasionally the basement membrane is even completely absent. In humans, in many malignant tumours a correlation between basement membrane integrity, metastatic potential and survival has been observed [Havenith et al., 1988; Daher et al., 1987].

The correlation between loss of basement integrity and prognosis in patients with stages IB and IIA cervical cancer has been described before and claimed to be important particularly in adenocarcinoma [Pitt et al., 1992].

Our results are partly in agreement with this study, although we were not able to demonstrate a correlation between basement membrane pattern and histologic subtype. In patients with advanced cervical cancer stages IIB - IV, loss of basement membrane integrity although more frequently observed than in patients with early cervical cancer, was not associated with a decrease in survival. This indicates that in these patients other parameters such as extent of the tumour and the high probability of metastasis should be looked for in predicting disease outcome.

In cervical cancer, clinical tumour size, capillary-lymphatic space involvement and depth of tumour invasion into the cervix have shown to be independent prognostic parameters in two GOG studies [Delgado et al., 1989; Delgado et al., 1990]. Surprisingly, in these studies, pelvic lymph node involvement did not substantially alter 3-year disease-free interval. This finding is different from

several other reports in literature. In two large studies using multivariate analysis, pelvic lymph node metastasis showed to be the strongest predictor of outcome and histologic subtype and tumour size were also of independent prognostic significance [Kamura et al., 1992; Fuller et al., 1989]. Probably the data presented in the GOG studies can not be compared to the other studies given the exclusion of patients with para-aortal and/or gross lymph node involvement. The major disadvantage of all these prognostic parameters are twofold. First, the parameters can only be determined on the radical hysterectomy specimen itself, and secondly the strongest parameter, being pelvic lymph node metastasis [van Bommel et al., 1987; Kamura et al., 1992], does not fully account for survival. Approximately, 15 percent of the patients with negative pelvic lymph nodes will get recurrent disease [Thomas and Dembo, 1991]. Identification of new and accurate prognostic parameters are therefore still necessary in order to select patients at risk for recurrent disease.

Both in the present study and in the study by Pitt and coworkers, it is shown that in patients with early cervical cancer stages IB and IIA loss of basement membrane integrity is strongly associated with pelvic lymph node metastasis and hence survival [Pitt et al., 1992]. The pattern of the basement membrane can be determined on a biopsy specimen and could be helpful in determining the probability of pelvic lymph node metastasis. In the appropriate patient this might be valuable in deciding whether to institute primary radiation therapy or choose a surgical approach.

Chapter 7

Tumour stroma fraction: a novel parameter predicting metastasis and survival in cervical cancer stages Ib and IIa

7.1 Summary

7.2 Introduction

7.3 Methods

7.3.1 Patients

7.3.2 Histopathological analysis

7.3.3 Statistical analysis

7.4 Results

7.4.1 Pelvic lymph node involvement and survival

7.4.2 Tumour stroma fraction and survival

7.5 Discussion

7.1 SUMMARY

In all carcinoma a stromal reaction in the tumour is observed, a process known as tumour desmoplasia. Experimental evidence suggests that an abundant tumour stroma has an anti-invasive effect and might be associated with increased survival. This was addressed in a retrospective study in cervical cancer.

In 114 patients with cervical cancer FIGO stages IB and IIA, the relative volume of the tumour stroma, expressed as the stromal fraction of the tumour was determined morphometrically and correlated with patient survival using a Cox Regression Analysis model.

We found the stroma fraction of the tumour ($p=0.004$) and pelvic lymph node metastasis ($p=0.02$) to correlate significantly and independently with survival. The relative mortality risk was increased 50 percent with every 10 percent decrease in the stromal fraction of the tumour in both patients with and in patients without lymph node metastasis. The survival rate was 88 percent in node negative versus 61 in node positive patients.

This study provides support for the hypothesis that an abundant stromal reaction in human cancer has an anti-invasive effect and is associated with increase in survival.

In addition, it is shown that in patients with cervical cancer stages IB and IIA the tumour stroma volume is a novel and strong parameter determining survival probability, irrespective of pelvic lymph node metastasis and offers a possibility to individualize treatment.

7.2 INTRODUCTION

In all carcinoma, tumour stroma is observed in between the malignant epithelial cell proliferation. The extent of this tumour stroma varies greatly. In some tumours, it is hardly detectable whereas in others it is very extensive, a process known as tumour desmoplasia. The source and etiology of the tumour stroma is controversial. Whereas some claim it to be considered a nonspecific fibrotic reaction similar to wound healing [Dvorak et al., 1981] or produced by tumour cells [Al-adnani et al., 1987], others suggest that the tumour stroma should be produced by host stromal cells and its constituents should be mediated by tumour cell-derived growth factors [Barsky and Gopalakrishna, 1987; Furcht, 1986; Barsky et al., 1982].

In experimental studies using melanoma- and lung carcinoma cell-lines it has been shown that inhibition of the desmoplastic response caused increased invasion and metastasis [Barsky and Gopalakrishna, 1987; Nakanishi et al., 1994].

This suggests that the extent of the tumour stroma in cancer has an anti-invasive effect and an inverse relationship might be expected between its intensity and

recurrence of disease and survival in patients with cancer. However, clinical studies showing this effect have not been published yet.

The purpose of this study was to determine in patients with cervical cancer FIGO stages IB and IIA whether the volume of the stroma in the tumour is indeed an important parameter predicting.

The hypothesis to test was that patients with cervical cancer stages IB and IIA and a small amount of tumour stroma were at high risk for recurrence and death due to disease, unlike patients with tumours in which the volume of the stroma was large.

7.3 METHODS

7.3.1 Patients

From 1978 to 1993, a total of 205 patients with invasive cancer of the uterine cervix were admitted to the Department of Gynecology, University Hospital of Maastricht, The Netherlands including 67 referrals from four different regional community hospitals. All patients were staged according to the classification of the International Federation of Gynecology and Obstetrics [FIGO, 1986]. The number of patients assessed as having early cervical cancer was 145; 21 (10.2%) patients stage IA, 124 (60.5%) stage IB and IIA. Advanced disease (stages IIB, III and IV) was diagnosed in 60 (29.3%) patients. This study focuses on the 124 patients with cervical cancer stages IB and IIA only.

The primary treatment for these patients was radical surgery (Wertheim-Meigs procedure) with intraoperative evaluation of the pelvic lymph node status using frozen sections.

If pelvic lymph nodes were involved by cancer, the surgical procedure was abandoned and full pelvic and intracavitary radiation therapy was started two weeks after surgery.

Of the 124 patients 108 patients were initially treated with radical surgery; 75 had negative pelvic lymph nodes and underwent complete radical hysterectomy and pelvic lymphadenectomy. Fourteen of these 75 patients received post-operative radiation therapy because of positive surgical margins in 5, near positive (less than 2 mm) surgical margins in 6 or microscopical involvement of the parametrium in 3.

In 33 of the 108 patients the surgical procedure was abandoned because of pelvic lymph node involvement. All of these patients received radiation therapy. Sixteen out of the 124 patients received primary radiation therapy because of relative or absolute contraindications for surgery such as age, obesity or poor medical condition. Patient follow up ranged from 6 months to 15 years (median 6.2 years). A detailed report of the results of this treatment policy was described elsewhere [Bremer et al., 1992].

Ten patients were excluded since paraffin blocks could not be retrieved or did not allow further histologic studies. The remaining 114 patients form the basis of this study.

7.3.2 Histological analysis

Diagnostic cervical biopsies and representative samples of tumours found in the hysterectomy specimens were routinely processed, paraffin-embedded, and stained by standard histochemical techniques. Histologically the tumour was a squamous cell carcinoma in 94, an adenocarcinoma in 15, and an adenosquamous carcinoma in 5 patients. Tumour grade and lymph vascular space involvement was determined in all tumours.

The epithelial components of the tumour were highlighted by staining for cytokeratin intermediate filaments (Dako-CK, Mouse monoclonal antibody MNF116, Dako, Denmark) by standard immunohistochemical procedures using an avidin-biotin-HRP detection system [Ormanns and Schaffer, 1985]. This staining identifies both the epithelial (cytokeratin positive) and stromal (cytokeratin negative) components of the tumour. The stromal tissue area (expressed as a fraction of the total tissue area) and designated stromal fraction was measured by computerized morphometry in a double blind matter. (Quantimet 570, Leica Cambridge Ltd, U.K.). The number of fields to be measured was determined by computing progressive means. In the center of the tumour at least 10 microscopical fields (100x) were measured and the stromal fraction was calculated. Inter- and intraobserver variation were both less than 5 percent.

In 20 patients of whom both diagnostic cervical biopsy specimens and radical hysterectomy specimens were available the stromal fraction of both specimens was measured.

The variation between the stromal fractions in all specimens was less than 5 percent. Therefore, we concluded that the tumour stroma fraction as determined in a biopsy specimen was representative for the stromal fraction of the tumour in general. This enabled us to use the stromal fraction as determined in a diagnostic cervical biopsy specimen in patients of whom no hysterectomy specimen was available, either because of positive lymph nodes or primary radiation therapy.

7.3.3 Statistical analysis

The objective of this study was to assess the relationship between the stromal fraction of the tumour and disease free survival. Disease free survival was calculated from the date of diagnosis. Kaplan-Meier estimates of disease free survival were calculated for groups of patients with negative, positive and unknown pelvic lymph nodes and for different stroma categories (0.0-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.9). First, these groups were compared using the log-rank

test. Then, the importance of several factors, including pelvic lymph node metastasis, Figo stage, histological grade and cell type, lymph vascular space involvement, parametrial invasion, surgical margins and tumour stroma fraction in predicting disease free survival was estimated using a Cox proportional hazards regression model with backward elimination [Altman, 1991]. The likelihood ratio test was applied to compute the P-values.

The goodness of fit, of the Cox model to the data, was verified in several ways: there was no significant interaction and linearity was checked using dummies, whereas the calculated survival curves were in agreement with Kaplan-Meier curves computed for the different stromal fraction categories. Data were analyzed with BMDP2L software, version 1990 (University of California Press, Berkeley) on an IBM personal computer.

7.4 RESULTS

7.4.1 Pelvic lymph node involvement and survival

Kaplan-Meier disease free survival curves for 114 patients with cervical cancer stages IB or IIA are shown in figure 7.1. Overall survival probability without evidence of disease was 76 percent. In the 73 patients without lymph node involvement survival probability was 88 percent compared to 61 percent in the 27 patients with pelvic lymph node metastasis (Logrank; $X^2=13.6$, $DF=2$, $P=0.0011$). Fourteen of the 73 patients with negative pelvic lymph node were treated with adjuvant radiotherapy for the reasons mentioned before. In this group of patients, survival probability was similar compared to the other patients with negative lymph nodes. Survival probability in the 14 patients with unknown pelvic lymph node involvement, treated primarily with radiation therapy, was identical to those in patients with positive pelvic lymph nodes.

7.4.2 Tumour stroma fraction and survival

In all 114 patients, of whom 21 patients died due to disease, with positive, negative or unknown pelvic lymph node involvement the stroma fraction of the tumour was determined and presented in figure 7.2. This was true in patients with and in patients without pathological involvement of the pelvic lymph nodes. The importance of the tumour stroma fraction is stressed even more in the group of patients where no information on pelvic lymph node involvement was available: only patients with a relatively low tumour stroma fraction died due to recurrent disease. Figure 7.3 shows the Kaplan-Meier Survival curves for patients classified in four different tumour stroma fraction categories. (Logrank; $X^2=18.7$, $DF=3$, $P=0.0003$)

Figure 7.1: Kaplan-Meier Analysis of Survival of Women with Early Cervical Cancer Stages IB and IIA, According to Pelvic Lymph Node Involvement. (N=114)
I: negative pelvic lymph nodes, II : positive pelvic lymph nodes, III: unknown pelvic lymph nodes. (Log Rank $X^2=13.6$, $P=0.001$)

Figure 7.2: Tumour stroma fraction in 114 Women with Early Cervical Cancer Stages IB and IIA, According to Pelvic Lymph Node Involvement.
■ survivors + nonsurvivors

Figure 7.3: Kaplan-Meier Analysis of Survival of 114 Women with Early Cervical Cancer Stages IB and IIA Classified into Four Categories of Tumour stroma fraction. Tumour stroma fraction categories 0.0-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.9

Figure 7.4: Survival Curves Calculated by Cox Regression Analysis for Women with Early Cervical Cancer Stages IB and IIA and Negative Pelvic Lymph Nodes, According to Tumour stroma fraction. Tumour stroma fractions 0.80, 0.60, 0.40, 0.20, 0.10.

Figure 7.5: Survival Curves Calculated by Cox Regression Analysis for Women with Early Cervical Cancer Stages IB and IIA and Positive/Unknown Pelvic Lymph Nodes, According to Tumour stroma fraction.
Tumour stroma fractions 0.80, 0.60, 0.40, 0.20, 0.10.

Table 7.1: Significant Prognostic Factors Selected by Cox Regression Analysis in Patients with Early Cervical Cancer Stages IB and IIA.

Variable	Coeff	SE	P value
pelvic lymph node status	-1.1122	0.5075	0.02
0=positive/unknown 1=negative			
tumour stromal fraction	-4.1124	1.5688	0.004

Pelvic lymph node status: positive N=27, unknown N=14, negative N=73. Covariance between the regression coefficients was -0.242. (Coeff: coefficient, SE: standard error)

Table 7.2: Relative Risk (RR) and 95 Percent Confidence Intervals (CI) Derived by Cox Regression Analysis for Death due to Early Cervical Cancer Stages IB and IIA in Women with Negative or Positive/Unknown Pelvic Lymph Node Metastasis, According to Tumour stroma fraction.

tumour stroma fraction	pelvic lymph node status			
	negative		positive/unknown	
	RR	(CI)	RR	(CI)
0.80	1.0		3.0	(1.1 - 8.2)
0.70	1.5	(1.1 - 2.1)	4.6	(1.8- 11.8)
0.60	2.3	(1.2 - 4.2)	6.9	(2.5 - 18.8)
0.50	3.4	(1.4 - 8.6)	10.4	(3.4 - 32.4)
0.40	5.2	(1.5 - 17.7)	15.7	(4.2 - 59.3)
0.30	7.8	(1.7 - 36.4)	23.7	(5.0 - 112.7)
0.20	11.8	(1.9 - 74.7)	35.8	(5.8 - 219.2)
0.10	17.8	(2.1 - 153.4)	54.0	(6.7 - 432.5)

RR: Relative Risk to die due to disease, CI: 95 percent confidence interval.

The prognostic value of pelvic lymph node metastasis, FIGO stage, histological grade and cell type, lymph vascular space involvement, parametrial invasion, surgical margins and tumour stroma fraction was investigated in a multivariate analysis using a Cox proportional hazards regression model. Only pelvic lymph node involvement ($p=0.02$) and stromal fraction of the tumour ($p=0.004$) were statistically significant and independent parameters in predicting survival probability (table 7.1). All other parameters were not significant and showed p -values above 0.4.

The Cox regression model was used to compute for several stromal fractions the relative risk to die due to disease (table 7.2). Irrespective of the amount of tumour stroma, patients with positive or unknown pelvic lymph node metastasis had a 3.0 fold increased risk to die due to cervical cancer than patients with negative pelvic lymph nodes. In addition the tumour stroma fraction proved also to be a significant and important parameter predicting survival independent of pelvic lymph node involvement. As shown in table 7.2 a decrease in the amount of stroma in the tumour indicated by a 0.10 decrease in the tumour stroma fraction results in a 50 percent increase in mortality risk, irrespective of pelvic lymph node involvement. The Cox regression model was also used to calculate survival curves for patients with cervical cancer stages IB and IIA, according to different tumour stroma fractions for patients without pelvic lymph node metastasis

(figure 7.4) and for patients with or with unknown lymph node metastasis (figure 7.5). These figures stress the importance of the stroma fraction of the tumour as a prognostic parameter in patients with cervical cancer stages IB and IIA even more and demonstrates that the prognostic importance of the stroma fraction of the tumour at least in some patients overshadows the impact of pelvic lymph node metastasis on survival probability.

7.5 DISCUSSION

This study shows a significant correlation between the volume of the tumour stroma, expressed as the stroma fraction of the tumour and survival in patients with cervical cancer stages IB and IIA. Patients with a low tumour stroma fraction had an increased risk to die due to recurrent disease, unlike patients with a high tumour stroma fraction, e.g. an abundant stroma reaction within the tumour. This was true in patients with and in patients without pathological involvement of the pelvic lymph nodes and even in patients in whom no information on the involvement of pelvic lymph nodes was available.

Increasing evidence has emerged supporting the active role of the stromal compartment in regulating tumour behavior [Bosman et al., 1993]. The stroma epithelial interface in a malignant tumour is a site of dynamic interactions which are decisive in processes such as invasion and metastasis. These processes, most of which have been shown to have biological and clinical implications, are among others dissolution of intercellular and cell-matrix interactions with down-regulation of the expression of cellular adhesion molecules, integrins and cadherins [Albeda, 1993], basement membrane degradation [Martinez-Hernandez and Amenta, 1983] and tumour-induced angiogenesis [Weidner et al., 1991]. Production of proliferation and differentiation inducing growth factors, i.e. basic-Fibroblast Growth Factor and Transforming Growth Factor- β will result in increased production and deposition of collagen fibers, angiogenesis with neovascularization and transformation of fibroblasts into myofibroblasts.

On the other hand degradation of the stromal matrix components will also occur and is mediated through metallo-proteinases [Liotta et al., 1991]. The volume of the stromal component of the tumour is a representation of the net results of all these processes and it is conceivable that the presence of an extensive stromal response is associated with an anti-invasive effect. The studies already mentioned using melanoma- and lung carcinoma cell-lines gave experimental evidence whereas the present study gives clinical evidence supporting this hypothesis [Barsky and Gopalakrishna, 1987; Nakanishi et al., 1994].

In patients with cervical cancer stages IB and IIA prognosis as indicated by five year survival is largely dependent on the presence or absence of pelvic lymph node metastases. Without involvement of lymph nodes the survival rate is 70-90 percent dropping to 40-60 percent when pelvic lymph node metastases are

present [Morley and Seski, 1976; Baltzer et al., 1982; Delgado et al., 1990; van Bommel et al., 1987]. This percentage has not changed in the past two decades and still, about 20 percent of patients suffering from early cervical cancer will die of recurrent disease. At present, in most institutions the treatment consists of radical hysterectomy combined with pelvic lymphadenectomy.

This treatment is followed by adjuvant radiotherapy when positive pelvic lymph nodes are found. Thomas and Dembo discussed the role of adjuvant pelvic radiotherapy in early stage cervical cancer; 15 out of 100 patients with early cervical cancer are likely to have lymph node involvement and 45 percent of these patients will be cured. The remaining 85 patients will have no pelvic lymph node metastasis and 90 percent of them will be cured. In both groups however 8 patients will develop recurrence of disease and will probably die [Thomas and Dembo, 1991]. Up to now no strong parameters have been available predicting survival probability in these patients. Many studies have focussed on potential parameters, mainly histological, and tried to correlate it with survival. These include tumour size, lymph vascular space involvement, tumour cell differentiation, oncogene expression, basement membrane deposition and inflammatory infiltrate [van Bommel et al., 1987; Kamura et al., 1992; Pitt et al., 1992; Hilders et al., 1993; Oka et al., 1994] and although related to some extent to prognosis clinical impact of these factors is rather limited.

The correlation observed in this study between the volume of the tumour stroma and survival in patients with cancer of the uterine cervix stages IB and IIA irrespective of pelvic lymph node involvement, is a novel and promising parameter predicting prognosis. It is easy to determine by simple morphometric techniques in both biopsy and surgical specimens and, if confirmed in a prospective study, it offers a possibility to individualize treatment which could include adjuvant chemo, radio, or immunotherapy.

Chapter 8

Tumour angiogenesis: an independent prognostic parameter in cervical cancer stages Ib and IIa

- 8.1 Summary
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 - 8.4.3 Microvessel density and survival
- 8.5 Discussion

8.1 SUMMARY

The purpose of this study was to investigate whether the intensity of tumour angiogenesis, expressed as micro vessel density, is a parameter predicting the probability of lymph node metastasis and survival in patients with cervical cancer stages IB and IIA.

In a retrospective study of 114 patients with cervical cancer stages IB and IIA, microvessel density, lymph node status and several other prognostic parameters were correlated to disease free survival using a multivariate analysis according to Cox Proportional Hazards Model.

There was a significant difference in mean microvessel density between tumours with and without pelvic lymph node metastasis ($p=0.002$). Both microvessel density (vessels per millimeters squared stroma) ($P=0.05$) and pelvic lymph node metastasis ($P=0.007$) correlated significantly and independently with disease free survival.

This study demonstrates that microvessel density is an independent prognostic parameter for disease free survival in patients with cervical cancer stages IB and IIA.

8.2 INTRODUCTION

The prognosis of patients with cervical cancer stages IB and IIA is thought to be largely dependent on the presence or absence of pelvic lymph node metastasis. The five year survival rate is 70-90 percent in patients without pelvic lymph node involvement and decreases to 40-60 percent when pelvic lymph nodes are involved by tumour [Burghardt et al., 1987; Martimbeau et al., 1982]. Hence, patients with positive pelvic lymph nodes are considered to be at risk for recurrence and are treated more aggressively [van Bommel et al., 1987].

On the other hand, node status does not fully account for the clinical outcome among patients with this disease. In fact, 10-15 percent of the patients without pelvic lymph node involvement will have tumour recurrence and approximately 50 percent of the patients with pelvic lymph node involvement are cured after primary radiotherapy [Thomas and Dembo, 1991]. Until now, no adequate parameters predicting survival have been available for these patients. Other more accurate prognostic parameters correlating with outcome allow not only an improved understanding of the biological behavior of the tumour but could also help to define a subgroup of patients at risk for recurrence which makes it possible to individualize treatment.

Angiogenesis is the growth of new blood vessels toward and within a tumour and it has been shown that tumours do not grow beyond a size of $2\text{-}3\text{ mm}^3$ unless they are able to recruit the growth of new capillaries from the existing vascular network [Folkman, 1990].

It is unclear whether angiogenesis regulates tumour behaviour or is only an indicator for the growth and metastatic potential of a tumour [Weidner, 1992; Bicknell and Harris, 1991].

Clinical studies have shown that the intensity of angiogenesis, expressed as microvessel density, predicts the probability of metastasis and survival in breast and prostate carcinoma [Weidner et al., 1991; Toi et al., 1993; Weidner et al., 1992; Fregene et al., 1993; Weidner et al., 1993].

The purpose of this study was to determine in patients with early cervical cancer whether the intensity of tumour angiogenesis, expressed as micro vessel density, is indeed an important parameter predicting lymph node metastasis and survival. The hypothesis we wished to test is that patients with early cervical cancer and low microvessel density have a low metastatic rate and a good prognosis, unlike patients with tumours that have a higher angiogenic state.

8.3 MATERIAL AND METHODS

8.3.1 Patients

From 1978 to 1993, a total of 205 patients with invasive cancer of the uterine cervix was admitted to the Department of Gynecology, University Hospital of Maastricht, The Netherlands including 67 referrals from four different regional community hospitals. All patients were staged according to the classification of the International Federation of Gynecology and Obstetrics [FIGO, 1986]. The number of patients assessed as having early cervical cancer was 145; 21 (10.2%) patients stage IA, 124 (60.5%) stages IB and IIA. Advanced disease (stages IIB, III and IV) was diagnosed in 60 (29.3%) patients. This study focuses on the 124 patients with cervical cancer stages IB and IIA only.

The primary treatment for these patients was radical surgery (Wertheim-Meigs procedure) with intraoperative evaluation of the pelvic lymph node status using frozen sections. If pelvic lymph nodes were involved by cancer, the surgical procedure was abandoned and full pelvic and intracavitary radiation therapy was started two weeks after surgery. Of the 124 patients 108 patients were initially treated with radical surgery; 75 patients had negative pelvic lymph nodes and underwent complete radical hysterectomy with bilateral pelvic lymphadenectomy.

Fourteen of these 75 patients received post-operative radiation therapy because of positive surgical margins in 5, near positive (less than 2 mm) surgical margins in 6 or microscopical involvement of the parametrium in 3 patients.

In 33 of the 108 patients the surgical procedure was abandoned because of pelvic lymph node involvement. These patients received radiation therapy. Sixteen out of the 124 patients were radiated primarily because of relative or absolute contraindications for surgery such as age, obesity or poor medical condition.

Patient follow up ranged from 6 months to 15 years. (median 6.2 years). A detailed report of the results of this treatment policy was described elsewhere [Bremer et al., 1992].

Ten patients were excluded since paraffin blocks could not be retrieved or did not allow further immunohistologic studies. The remaining 114 patients form the basis of this study.

8.3.2 Histological analysis

Diagnostic cervical biopsies and representative samples of tumours found in the hysterectomy specimens were routinely processed, paraffin-embedded, and stained by standard histochemical techniques. Ninety four tumours were a squamous cell carcinoma, fifteen tumours an adenocarcinoma and five tumours an adenosquamous carcinoma. Tumour grade and lymph vascular space involvement was determined in all tumours. The bloodvessels were highlighted by staining the endothelial cells using Ulex europaeus I lectin by a standard immunoperoxidase technique [Holthoffer et al., 1982; Ormanns and Schaffer, 1985]. The quality of the staining was judged by using bloodvessels in adjacent benign tissue as internal controls. Microvessels were quantitated according to Weidner and coworkers [Weidner et al., 1991].

Briefly, the stained slides were examined at low magnification (x40 and x100) to identify the areas with the highest number of vessels. The stromal tissue area was measured by computerized morphometry (Quantimet 570, Leica Cambridge Ltd, U.K.) and within this area all vessels were counted (x250). Any brown-staining endothelial cell or endothelial cell aggregate, clearly separated from adjacent microvessels, tumourcells or other connective tissue elements, and with or without a lumen, was considered a countable microvessel. The number of fields to be measured was determined by computing progressive means. At least 8-10 microscopic fields were measured and the mean microvessel density was calculated and expressed as vessels per millimeters squared stroma (vv/mm²) for subsequent analysis.

In patients of whom both diagnostic cervical biopsy and hysterectomy specimens were available the variation between the microvessel density in the tumour measured in the biopsy specimen or the hysterectomy specimen was less than 10 percent. Therefore we concluded that the microvessel density determined in a biopsy specimen was representative for the stromal fraction of the whole tumour. This enabled us to include in this study all the patients of whom no hysterectomy specimen was available, either because of positive lymph nodes or primary radiation therapy.

8.3.3 Statistical analysis

The objective of this study was to assess the relationship between the microvessel density of the tumour, pelvic lymph node metastasis and disease free survival. Microvessel density was compared of patients with and patients without pelvic lymph node involvement, using the Wilcoxon-Mann-Whitney test. Disease free survival was calculated from the date of diagnosis. Kaplan-Meier estimates of disease free survival were calculated for groups of patients with negative, positive and unknown pelvic lymph nodes. First, these groups were compared using the log-rank test.

Next, the importance of several factors, including pelvic lymph node metastasis, FIGO stage, histological grade and celltype, lymph vascular space involvement, parametrial invasion, surgical margins and the microvessel density of the tumour in predicting disease free survival was estimated in a multivariate analysis. A Cox proportional hazards regression model with backward elimination was used [Altman, 1991]. The likelihood ratio test was applied to compute the P-values. The goodness of fit of the Cox model to the data, was verified in several ways: there was no significant interaction and linearity was checked using dummies. Data were analyzed with BMDP2L software, version 1990 (University of California Press, Berkeley) on an IBM personal computer.

8.4 RESULTS

8.4.1 Pelvic lymph node involvement and survival

Kaplan-Meier disease free survival curves for the 114 patients with early cervical cancer (FIGO IB or IIA) are shown in figure 8.1. Overall survival probability without evidence of disease was 76 percent. In the 73 patients without lymph node involvement survival probability was 88 percent compared to 61 percent in the 27 patients with pelvic lymph node metastasis. (Logrank; $X^2=13.6$, $DF=2$, $P=0.001$). Survival probability in the 14 patients with unknown pelvic lymph node involvement, treated primarily with radiation therapy, was identical to that in patients with positive pelvic lymph nodes.

8.4.2 Microvessel density and pelvic lymph node metastasis.

The mean microvessel density of all tumours was 170 vessels/mm² stroma (SD=124.5, range 10-594). Tumours without pelvic lymph node metastasis had a mean microvessel density of 144 vessels/mm² stroma (SD=93.9, range 10-430) while tumours with positive pelvic lymph nodes had a higher mean microvessel density of 254 vessels/mm² stroma (SD=159.4, range 54-594).

Figure 8.1: Kaplan-Meier Analysis of Survival of 114 Women with Early Cervical Cancer Stages IB and IIA, According to Pelvic Lymph Node Involvement. I: negative pelvic lymph nodes (N=73), II: positive pelvic lymph nodes (N=27), III: unknown pelvic lymph nodes (N=14). Log Rank $X^2=13.6$, DF=2, P=0.001

Figure 8.2: Kaplan-Meier Analysis of Survival of 114 Women with Early Cervical Cancer Stages IB and IIA Classified into Three Categories of Microvessel Density. Log Rank $X^2=13.4$, DF=2, P=0.001

The difference between the two groups was statistically significant (Wilcoxon-Mann-Whitney test; $p=0.002$).

8.4.3 Microvessel density and survival.

Figure 8.2 shows the Kaplan-Meier survival curves for patients with a microvessel density of less than 200 vv/mm², between 200-400 vv/mm² and above 400 vv/mm². (Logrank; $X^2 = 13.4$, $DF=2$, $P=0.001$)

The prognostic value of pelvic lymph node metastasis, FIGO stage, histological grade and cell type, lymph vascular space involvement, parametrial invasion,

Table 8.1: Cox Regression Model in Patients with Cervical Cancer Stages IB and IIA.

Variable	Coeff	SE	P-value
pelvic lymph node status	-1.3228	0.5080	0.007
0=positive/unknown			
1=negative			
microvessel density	+0.002886	0.001426	0.05

Pelvic lymph node status: positive N=27, unknown N=14, negative N=73. Covariance between the regression coefficients was +0.00022291. (Coeff: coefficient, SE: standard error).

Table 8.2: Relative Risk and 95 Percent Confidence Intervals (CI) Derived by Cox Regression Analysis for Death due to Early Cervical Cancer Stages IB and IIA in Women with Negative or Positive/Unknown Pelvic Lymph Node Metastasis, According to Microvessel density.

microvessel density (vv/mm ² stroma)	pelvic lymph node status			
	negative (N=73)		positive/unknown (N=27) (N=14)	
	RR	(CI)	RR	(CI)
100	1.0		3.8	(1.4 - 10.2)
200	1.3	(1.0 - 1.8)	5.0	(1.9 - 12.9)
300	1.8	(1.0 - 3.1)	6.7	(2.5 - 17.8)
400	2.4	(1.0 - 5.4)	8.9	(3.0 - 26.4)
500	3.1	(1.0 - 9.4)	11.9	(3.4 - 41.4)

RR: relative risk to die due to disease, CI: 95% confidence interval.

Figure 8.3: Survival Curves Calculated by Cox Regression Analysis for Women with Early Cervical Cancer Stages IB and IIA and Negative Pelvic Lymph Nodes, According to Microvessel density.
Microvessel density: 100 vv/mm² stroma (I), 200 vv/mm² stroma (II), 300 vv/mm² stroma (III), 400 vv/mm² stroma (IV), 500 vv/mm² stroma (V)

Figure 8.4: Survival Curves Calculated by Cox Regression Analysis for Women with Early Cervical Cancer Stages IB and IIA and Positive or Unknown Pelvic Lymph Nodes, According to microvessel density.
Microvessel density: 100 vv/mm² stroma (I), 200 vv/mm² stroma (II), 300 vv/mm² stroma (III), 400 vv/mm² stroma (IV), 500 vv/mm² stroma (V)

surgical margins and microvessel density was investigated in a multivariate analysis using a Cox proportional hazards regression model. Only pelvic lymph node involvement ($p=0.007$) and microvessel density ($p=0.05$) showed to be statistically significant and independent parameters predicting survival probability (Table 8.1). All other parameters were not significant in predicting survival and had p -values above 0.25.

The Cox regression model was used to compute the relative risk for several grades of microvessel density. (Table 8.2).

Irrespective of microvessel density, patients with positive or unknown pelvic lymph node involvement had a 3.8 fold increased risk to die due to cervical cancer compared with patients with negative pelvic lymph nodes. In addition, microvessel density itself proved to be a significant parameter predicting survival. As shown in Table 8.1, an increase of 100 vessels per millimeters squared stroma results in a 33 percent increase in mortality risk, irrespective of pelvic lymph node involvement.

The Cox regression model was also used to calculate survival curves for patients with early cervical cancer, according to different grades of microvessel density for patients without pelvic lymph node metastasis and for patients with or with unknown lymph node metastasis respectively (Figures 8.3 and 8.4). These figures stress the importance of microvessel density as a prognostic parameter in patients with early cervical cancer.

8.5 DISCUSSION

This study shows a correlation between tumour angiogenesis, expressed as microvessel density within the tumour stroma and pelvic lymph node metastasis in patients with early cervical cancer. The mean microvessel density in metastasized tumours (254 vv/mm^2 stroma) is significantly higher ($P=0.002$) than in tumours with negative pelvic lymph nodes (144 vv/mm^2 stroma). In a multivariate analysis, a significant correlation was also found between microvessel density and disease free survival. Patients with a high tumour microvessel density have an increased risk to die due to recurrent disease, as opposed to patients with a low tumour microvessel density, irrespective to pelvic lymph node metastasis.

Angiogenesis, the formation of new bloodvessels, is permissive for expansion of a tumour [Folkman et al., 1989]. In the prevascular phase, tumour cells cannot expand the tumour beyond a few cubic millimeters, restricted by the limits of oxygen and nutrient diffusion. After the onset of angiogenetic activity, tumours expand rapidly both locally and by metastasis.

Angiogenesis is a complex process that involves not only endothelial cell proliferation but also digestion of the extracellular matrix surrounding capillaries, endothelial cell migration and differentiation into functioning capil-

laries. Abundant evidence supports the concept that tumours can induce angiogenesis through a variety of angiogenetic molecules, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), released from the tumour cells or from cells recruited to the area (macrophages) [Blood and Zetter, 1990; Liotta and Stracke, 1988].

In addition, next to an essential requirement for local tumour progression, angiogenesis can facilitate metastatic spread in several ways. It has been shown that tumour cells rarely shed into the circulation in the prevascular phase of a tumour and cannot form a metastatic deposit until they are vascularized [Folkman, 1990]. Moreover, the process of metastasis may be enhanced by the leaky and fragmented basement membranes of the new capillaries [Nagy et al., 1988]. In early cervical cancer, accurate parameters predicting outcome are necessary to identify the patients at risk for recurrence and death. In a prospective study of the GOG, three independent prognostic factors, including tumour size, capillary-lymphatic space invasion and depth of tumour invasion could be identified by multivariate analysis. In this study, the extent of angiogenesis was not reported and surprisingly, pelvic lymph node metastasis was not of significance predicting 3-year disease free interval [Delgado et al., 1990].

In contrast, the results reported in our study indicate that pelvic lymph node metastasis and the extent of angiogenesis correlate independently with disease free survival in cervical cancer stages IB and IIA.

Hence, if confirmed in a prospective study, quantitative analysis of tumour angiogenesis could be useful in predicting the prognosis in these patients and it offers the possibility to individualize treatment. It may also be helpful to select patients for future antiangiogenetic therapy [Bicknell, 1994; Harris et al., 1994].

Chapter 9

Histological parameters predicting prognosis in cervical cancer stages Ib and IIa: the importance of the tumour cell-stroma interactions

- 9.1 Summary
- 9.2 Introduction
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 - 9.3.2 Histological analysis
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 - 9.4.1 Histological parameters associated with pelvic lymph node metastasis.
 - 9.4.2 Histological parameters associated with disease free survival.
- 9.5 Discussion

9.1 SUMMARY

The purpose of this study was to investigate the predictive value of clinical and histopathologic parameters for pelvic lymph node involvement and disease free survival in patients with cervical cancer stages IB and IIA.

In a retrospective study of 100 patients with cervical cancer stages IB and IIA, a comprehensive set of histopathological parameters were analyzed retrospectively and correlated to pelvic lymph node involvement and disease free survival using stepwise logistic regression analysis with backward elimination and a multivariate analysis according to Cox Proportional Hazards Model.

Basement membrane pattern, microvessel density and lymphocytic infiltration in between the tumour cells were found to predict independently the involvement of the pelvic lymph nodes.

Of all parameters, only tumour stroma fraction and microvessel density showed to have an independent influence upon disease free survival.

This study demonstrates that basement membrane pattern, microvessel density and lymphocytic infiltration in between the tumour cells are independent predictors of pelvic lymph node involvement. Microvessel density and tumour stroma fraction are independent prognostic parameters for disease free survival in patients with cervical cancer stages IB and IIA.

9.2 INTRODUCTION

Cervical cancer is an important and life-threatening disease in women. Since the introduction of screening programs, the majority of patients are diagnosed in the early stages of disease with, overall, good therapeutic results. In stages IB and IIA five year survival ranges from 70 - 90 percent, indicating that approximately 20 percent of the patients will have recurrent disease after initial therapy [van Bommel et al., 1987]. In order to identify these patients, who may benefit from aggressive adjuvant therapy, identification of parameters present at initial presentation and indicative of the prognosis is a necessity.

Unfortunately the majority of the risk factors known to be associated with an adverse prognosis, the most important of which is thought to be the presence of pelvic lymph node involvement, are rather weak predictors of outcome and are usually derived from retrospective studies using univariate analysis only. Prospective studies using multivariate analysis are scarce and those reported doubted the importance of pelvic lymph node involvement as an independent adverse prognostic factor [Thomas and Dembo, 1991; Delgado et al., 1990].

Of the other prognostic factors identified which include among others histologic subtype and tumour diameter [Kamura et al., 1992], tumour size, capillary-lymphatic space invasion and depth of tumour invasion [Delgado et al., 1990], only

a few could be related to intrinsic biological properties of the tumour known to be associated with aggressive tumour behavior.

In the last decade, it has become clear that tumour cell-stromal interactions play a pivotal role in tumour progression and metastasis [Bosman et al., 1993]. The stromal-epithelial interface in a malignant tumour is a site of dynamic interactions. Several processes such as the production and degradation of the basement membrane, the growth of fibroblasts and deposition of collagens in the tumour stroma, as well as the process of angiogenesis are all mediated through either tumour cells or the neighbouring stromal cells [Liotta et al., 1980; Delinassios et al., 1983; Rastinejad et al., 1989; Dvorak et al., 1991]. Studies investigating the histologic parameters associated with tumour cell-stroma interactions have shown that some of them yield useful information concerning prognosis [Willebrand et al., 1986; Daher et al., 1987; Weidner et al., 1991; Brawer et al., 1994].

In the present study, in 100 patients with cervical cancer stages IB and IIA who underwent radical surgery, a comprehensive set of histopathological parameters, emphasizing those associated with the above mentioned tumour cell - stroma interactions, were analyzed retrospectively, using multivariate analysis. The association of these factors with pelvic lymph node involvement and with disease-free survival was studied.

9.3 MATERIAL AND METHODS

9.3.1 Patients

From 1978 to 1993, a total of 205 patients with invasive cancer of the uterine cervix were admitted to the Department of Gynecology, University Hospital, Maastricht, The Netherlands including 67 referrals from four different regional community hospitals. All patients were staged according to the classification of the International Federation of Gynecology and Obstetrics [FIGO, 1986]. The number of patients assessed as having early cervical cancer was 145; 21 (10.2%) patients stage IA, 124 (60.5%) stages IB and IIA. Advanced disease (stages IIB, III and IV) was diagnosed in 60 (29.3%) patients. This study focuses on the 124 patients with cervical cancer stages IB and IIA only.

The primary treatment for these patients was radical surgery (Wertheim-Meigs procedure) with intraoperative evaluation of the pelvic lymph node status using frozen sections. If pelvic lymph nodes were involved by cancer, the surgical procedure was abandoned and full pelvic and intracavitary radiation therapy was started two weeks after surgery. Of the 124 patients 108 patients were initially treated with radical surgery; 75 had negative pelvic lymph nodes and underwent complete radical hysterectomy and pelvic lymphadenectomy. Fourteen of these 75 patients received post-operative radiation therapy because of positive surgical

margins in 5, near positive (less than 2 mm) surgical margins in 6 or microscopical involvement of the parametrium in 3.

In 33 of the 108 patients the surgical procedure was abandoned because of pelvic lymph node involvement. All of these patients received radiation therapy. Sixteen out of the 124 patients received primary radiation therapy because of relative or absolute contraindications for surgery such as age, obesity or poor medical condition. Patient follow up ranged from 6 months to 15 years. (median 6.2 years). A detailed report of the results of this treatment policy was described elsewhere [Bremer et al., 1992].

The sixteen patients receiving primary radiation therapy and eight patients of whom only insufficient material could be retrieved were excluded from further histologic studies. The remaining 100 patients form the basis of this study.

9.3.2 Histological analysis

Diagnostic cervical biopsies and representative samples of tumours found in the hysterectomy specimens were routinely processed, paraffin-embedded, and stained by standard histochemical techniques. All assessments were based on the examination of sections through the primary tumour and were performed without knowledge of pelvic lymph node status or patient outcome.

The following parameters were studied:

Stage: Patients were staged clinically according to the classification of the International Federation of Gynecology and Obstetrics [FIGO, 1986].

Pelvic lymph node metastasis: Pelvic lymph nodes (PLN) were evaluated and classified as positive or negative nodes.

Cell type: Tumours were classified as squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma according to the WHO classification.

Tumour grade: Tumours were graded as well, moderately or poorly differentiated using a modification of Broders' classification [Broders, 1926].

Lymph vascular space invasion: Lymph and blood vessels were highlighted by staining the endothelial cells using *Ulex europaeus* I lectin by a standard immunoperoxidase technique [Holthoffer et al., 1982]. Lymph vascular space invasion was recorded when tumour cells were identified microscopically within or adherent to endothelium lined spaces.

Basement membrane pattern: Briefly, tumour specimens were stained immunohistochemically using a mouse monoclonal antibody directed against type IV collagen [Havenith et al., 1987]. Basement membranes were classified as minimal or moderate discontinuous (pattern I/II) or fragmentary or absent (pattern III). (Chapter 6)

Tumour stroma volume: The epithelial components of the tumour were highlighted by staining for cytokeratin intermediate filaments (Dako-CK, Mouse monoclonal antibody MNF116, Dako, Denmark) by standard immunohistochemi-

cal procedures using an avidin-biotin-HRP detection system [Ormanns and Schaffer, 1985]. This staining identifies both the epithelial (cytokeratin positive) and stromal (cytokeratin negative) components of the tumour. The stromal tissue area (expressed as a fraction of the total tissue area) and designated stromal fraction was measured by computerized morphometry in a double blind matter. (Quantimet 570, Leica Cambridge Ltd, U.K.). The tumour stroma fraction was divided into 4 categories; < 0.2 , $0.2 - 0.4$, $0.4 - 0.6$, > 0.6 . (Chapter 7)

Angiogenesis: The presence of bloodvessels or single endothelial cells were considered to be indicative of the amount of angiogenesis. Briefly, microvessels were highlighted by staining the endothelial cells using Ulex europaeus I lectin by a standard immunoperoxidase technique, quantitated according to Weidner and coworkers [Weidner et al., 1991] and expressed as microvessel density. (vessels per millimeter squared (vv/mm^2), grouped into 3 categories; < 200 vv/mm^2 , $200 - 400$ vv/mm^2 , and > 400 vv/mm^2 stroma). (Chapter 8)

Lymphocytic infiltration in the tumour stroma: This was analyzed semiquantitatively as either present or absent by two independent investigators blinded to the clinical information. It was regarded as present when a lymphocytic infiltrate was apparent in the stroma or at the invasive margin of the tumour.

Lymphocytic infiltration in between the tumour cells: This was analyzed semiquantitatively as either present or absent by two independent investigators blinded to the clinical information. It was regarded as present when a prominent lymphocytic infiltrate was apparent in between the tumour cells.

9.3.3 Statistical analysis

The first objective of this study was to evaluate the association of the several clinical, histopathological and stromal parameters with the presence or absence of pelvic lymph node involvement. Initially, in univariate analysis the Chi square test was used to calculate this association for each parameter and subsequently these were tested in a multivariate analysis using stepwise logistic regression analysis with backward elimination.

The second objective was to assess the relationship between the studied parameters and disease free survival. Disease free survival was calculated from the date of diagnosis. Kaplan-Meier estimates of disease free survival were calculated for groups of patients classified for each variable. The groups were compared using the log-rank test.

A stepwise procedure with backward elimination according to Cox's proportional hazards model was used to identify and simultaneously evaluate the independent prognostic parameters associated with disease free survival. The likelihood ratio test was applied to compute the P-values.

Data were analyzed with BMDP2L software, version 1990 (University of California Press, Berkeley) on an IBM personal computer.

9.4 RESULTS

Out of 100 patients entering the study 76 were classified as FIGO stage IB and 24 as FIGO stage IIA. In 27 of the 100 patients pelvic lymph node metastasis were detected during surgery by frozen section and the surgical procedure was abandoned. Histologically the tumour could be classified as a squamous cell carcinoma in 82, an adenocarcinoma in 13, and an adenosquamous carcinoma in 5 patients. Of these tumours 7 were well, 33 were moderately and 60 were poorly differentiated according to Broders. In 32 patients lymph vascular space involvement was noted.

Of the parameters indicative of the tumour cell-stroma interaction basement membranes were mild to moderate discontinuous in 64 and fragmentary or absent in 36 patients. The tumour stroma fraction in the different tumours studied was in 21 patients less than 0.2, in 24 patients between 0.2 - 0.4, in 39 patients between 0.4 - 0.6, and in 16 patients over 0.6. Microvessel density in the tumour stroma was in 70 patients less than 200 vv/mm², in 24 between 200 and 400 vv/mm², and in 6 patients over 400 vv/mm². The inflammatory response to the tumour indicated by the presence of lymphocytes in the tumour stroma was considered to be prominent in 66 whereas the prominence of lymphocytes in the epithelial component of the tumour was observed in 20 of the 100 patients.

9.4.1 Histological parameters associated with pelvic lymph node metastasis.

Table 9.1 shows the relationship between the 9 parameters studied and the absence or presence of pelvic lymph node metastasis. Statistical analysis was performed by a univariate analysis (Chi square test) and a multivariate analysis (stepwise logistic regression). Although basement membrane integrity, tumour stroma fraction, microvessel density and a prominent lymphocytic infiltrate in the stroma were statistically significant in univariate analysis. Multivariate analysis demonstrated that only basement membrane integrity ($p < 0.001$), microvessel density ($p < 0.001$), and albeit weakly the lymphocytic infiltrate in between the tumour cells ($p < 0.05$) were independent predictors of the absence or presence of pelvic lymph node metastasis.

It can be concluded that the risk of nodal involvement was predominantly associated with both the microvessel count and the characteristics of the basement membranes. Its risk was 13 percent at a microvessel count of less than 200 vv/mm² and increased up to 60 percent at a microvessel count over 200 vv/mm². Fifty three percent of the patients with a fragmentary or absent basement membrane around the tumour nests had pelvic lymph node involvement compared to 13 percent when the basement membrane were only mild to moderate discontinuous. That the presence or absence of a prominent lymphocytic infiltrate in the epithelial component of the tumour cells was indeed an

Table 9.1: Histological parameters associated with pelvic lymph node metastasis. (Uni- and multivariate analysis).

Prognostic Parameter	Frequency positive PLN		Univariate analysis P-value	Multivariate analysis P-value
Stage				
IB	20/76	(26%)		
IIA	7/24	(29%)	0.99	0.38
Cell type				
scc	23/82	(28%)		
adeno(squamous)	4/18	(22%)	0.83	0.95
Tumour grade				
grade 1	0/7	(0%)		
grade 2	7/33	(21%)		
grade 3	20/60	(33%)	0.11	0.21
LVS involvement				
absent	20/68	(29%)		
present	7/32	(22%)	0.58	0.45
Lymphoc. infiltr. tumour cells				
absent	25/80	(31%)		
present	2/20	(10%)	0.10	0.01
BM pattern				
mild/moderate discontinuous	8/64	(13%)		
fragmentary or absent	19/36	(53%)	0.00004	<0.0001
Tumour stroma fraction				
0.6-0.9	0/16	(0%)		
0.4-0.6	8/39	(21%)		
0.2-0.4	6/24	(25%)		
0.0-0.2	12/21	(57%)	0.00077	0.11
Microvessel density				
< 200 vv/mm ²	9/70	(13%)		
200-400 vv/mm ²	14/24	(58%)		
> 400 vv/mm ²	4/6	(67%)	<0.00001	0.0016
Lymphoc. infiltr. tumour stroma				
absent	15/34	(44%)		
present	12/66	(18%)	0.011	0.33

PLN: Pelvic lymph nodes; SCC: squamous cell carcinoma; Adeno(squamous): Adenocarcinoma or Adenosquamous cell carcinoma; BM pattern: Basement membrane pattern.

Table 9.2: Histological parameters associated with disease free survival. (Univariate and Multivariate analysis).

Prognostic Parameter	N	DFS	Univariate analysis p-value	Multivariate analysis p-value
Stage				
IB	76	82%		
IIA	24	78%	p=0.48	p=0.31
Cell type				
scc	82	81%		
adeno(squamous)	18	80%	p=0.98	p=0.61
Tumour grade				
grade 1	7	100%		
grade 2	33	77%		
grade 3	60	79%	p=0.47	p=0.52
PLN metastasis				
absent	73	88%		
present	27	61%	p=0.0008	p=0.45
LVS involvement				
absent	68	85%		
present	32	78%	p=0.58	p=0.33
Lymphoc. infiltr. tumour cells				
absent	80	78%		
present	20	88%	p=0.52	p=0.91
BM pattern				
mild/moderate discontinuous fragmentary or	64	87%		
absent	36	66%	p=0.0072	p=0.38
Tumour stroma fraction				
0.6-0.9	16	100%		
0.4-0.6	39	85%		
0.2-0.4	24	79%		
0.0-0.2	21	55%	p=0.0020	p=0.0105
Microvessel density				
< 200 vv/mm ²	70	90%		
200-400 vv/mm ²	24	69%		
> 400 vv/mm ²	6	34%	p=0.0001	p=0.0083
Lymphoc. infiltr. tumour stroma				
absent	34	65%		
present	66	90%	p=0.0049	p=0.19

PLN: Pelvic lymph nodes; SCC: squamous cell carcinoma; Adeno(squamous): Adenocarcinoma or Adenosquamous cell carcinoma; BM pattern: Basement membrane pattern; DFS: Disease free survival.

Table 9.3: Significant Prognostic Parameter Selected by Cox Regression Analysis in Patients with Early Cervical Cancer Stage IB and IIA.

Parameter	Coeff	SE	P value
microvessel density	0.0044	0.0016	0.0083
tumour stromal fraction	-3.9571	1.7247	0.0105

Covariance between the regression coefficients was 0.00063706
(Coeff: coefficient; SE: standard error)

independent additional factor is demonstrated by the following. When a fragmentary or absent basement membrane pattern was present and associated with a prominent lymphocytic infiltrate in between the tumour cells only 18% of the patients had pelvic lymph node metastasis compared to 68% when the lymphocytic infiltrate was absent.

9.4.2 Histological parameters associated with disease-free survival.

Table 9.2 shows the relationship between the 10 parameters studied and disease-free survival. Statistical analysis was performed using univariate analysis (log-rank test) and multivariate analysis (Cox proportional hazards model with backward elimination the coefficients, standard errors and covariance of which are shown in table 9.3).

Although pelvic lymph node metastasis, basement membrane integrity, tumour stroma fraction, microvessel density and lymphocytic infiltration in the tumour stroma turned out to be statistically significant in univariate analysis, multivariate analysis showed microvessel density ($p < 0.01$) and tumour stroma fraction ($p < 0.05$) to be independent risk factors associated with disease-free survival only.

The risk to die due to recurrent disease increased in patients with an increase in microvessel density as well as with a decrease of the tumour stroma fraction.

9.5 DISCUSSION

In this retrospective study of 100 patients with cervical cancer FIGO stages IB and IIA, it is shown that the microvessel density in the tumour stroma and the relative volume of the tumour stroma, expressed as the tumour stroma fraction are the only independent and powerful predictors of disease-free survival, as determined by a multivariate analysis using Cox regression. Surprisingly, the presence or absence of pelvic lymph node metastasis did not add significantly

to the prediction of disease-free survival, despite its highly significant association with disease-free survival as determined by a univariate analysis using a log-rank test.

Several clinical studies have been published in which histological parameters were evaluated for their predictive value of disease free survival [Burghardt et al., 1987; Lee et al., 1989; Fuller et al., 1989; Delgado et al., 1990; Kamura et al., 1992; Zaino et al., 1992]. In the majority of these studies, however, statistical inferences were studied by univariate analysis only. In a prospective study by the Gynecological Oncology Group (GOG) using multivariate analysis, it was demonstrated that clinical tumour size, lymph vascular space involvement and depth of invasion were independent prognostic factors predicting 3-year disease free interval whereas pelvic lymph node involvement was not [Delgado et al., 1990]. This rather surprising outcome was in contrast with the general opinion at the time and with many other studies the majority of them which used univariate analysis only [Fuller et al., 1989; Kamura et al., 1992].

The present study stresses the importance of multivariate analysis in studying parameters which could be interrelated. Although many parameters are statistically significant in a univariate analysis, multivariate analysis showed the majority of them and more specifically pelvic lymph node involvement to be an interrelated and dependent factor on both microvessel density as well as tumour stroma fraction.

Analysis of the data concerning the parameters predicting the presence or absence of pelvic lymph node involvement and presented in Table 9.1, clearly demonstrates why pelvic lymph node metastasis is closely related to the microvessel density. Next to the basement membrane pattern the latter is the strongest predictor of the presence or absence of pelvic lymph node metastasis.

Although one should be very cautious in interpreting data and trying to comment on causal connections it is tempting to speculate on the important and strong predictors for both disease-free survival and pelvic lymph node involvement which have become apparent in this study. All of them are histological parameters associated with the tumour cell-stroma interactions.

Loss of basement membrane integrity is an important step in the process of metastasis. Normal basement membranes do not contain openings large enough to allow the penetration of invading tumour cells [Tryggvason, 1989], and dissolution of the basement membrane at the site of tumour cell penetration results from the activity of proteolytic enzymes produced by tumour cells and/or macrophages and fibroblasts [Liotta, 1984]. In vitro, tumour cell collagenase activity has been correlated with increased metastatic potential [Nakalima et al., 1987] and proteinase inhibitors can block the invasion process [Pauli et al., 1981]. The clinical relevance of basement membrane integrity both in lymph node metastasis and in survival has been stressed in many studies on a wide variety of human cancers [Havenith et al., 1988; Daher et al., 1987].

In cervical cancer stages IB and IIA, Pitt and coworkers first reported the relation between basement membrane pattern and nodal involvement [Pitt et al., 1992], and this is confirmed by us both in the present as well as in a previous study (Chapter 6).

The importance of angiogenesis in the metastatic process is well recognized. Several proteinases, growth factors and cytokines, such as Basic Fibroblast Growth Factor are involved in this process, some may be produced directly or indirectly by tumour cells. Abundant evidence supports the concept that tumours can induce angiogenesis through a variety of angiogenetic molecules, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), released from the tumour cells or from cells recruited to the area (macrophages) [Blood and Zetter, 1990; Liotta and Stracke, 1988].

In addition, next to an essential requirement for local tumour progression, angiogenesis can facilitate metastatic spread in several ways. It has been shown that tumour cells rarely shed into the circulation in the prevascular phase of a tumour and cannot form a metastatic deposit until they are vascularized [Folkman, 1990].

Moreover, the process of metastasis may be enhanced by the leaky and fragmented basement membranes of the new capillaries [Nagy et al., 1988].

A correlation between microvessel density, metastasis and survival has been described for other human malignancies [Weidner et al., 1991; Brawer et al., 1994].

The present study as well as a previous study confirm the importance of angiogenesis both in lymph node metastasis as well as in disease-free survival in cervical cancer (Chapter 8).

In tumours who do have a high angiogenetic capacity both the chance of gaining access to the circulation and growing out as a systemic metastasis are more likely to occur and should not necessarily be accompanied by a pelvic lymph node metastasis.

Tumour stroma fraction is a not widely studied parameter neither in cervical cancer nor in any other human malignancy. In this study it is shown to be correlated with disease free survival (Chapter 7). A low tumour stroma fraction could be interpreted as a tumour simply consisting of relatively more malignant epithelial cancer cells than a tumour with a high tumour stroma fraction; and therefore relatively high tumour load which could be an explanation of the poor prognosis observed. On the other hand, it could also be indicative of a tumour which is not capable of inducing a stromal response and therefore would be prone to metastasize systemically. It can be assumed that the tumour stroma presents a natural barrier to the migration of tumour cells and it could be modified through the release of cytokines such as Transforming Growth Factor- β either by the tumour or by neighbouring cells in the tumour stroma. This TGF- β could induce differentiation in fibroblasts leading to the production of collagen and hence inhibition of the metastatic process. In experimental studies

using melanoma- and lung carcinoma cell-lines it has been shown that inhibition of the desmoplastic response caused increased invasion and metastasis [Barsky and Gopalakrishna, 1987; Nakanishi et al., 1994].

These studies gave experimental evidence for the hypothesis that an extensive stromal response has a putative anti-invasive effect. The present study gives clinical evidence supporting this hypothesis.

The presence of T lymphocytes in cervical cancers has already been reported in other studies [Dietl et al., 1990; Hilders et al., 1993]. Hilders and coworkers reported a marked increase in the presence of infiltrating immune cells in between the tumour cells and in the stroma of cervical cancers compared to normal cervical tissue. Especially T lymphocytes and macrophages were present in between the tumour cells infiltrating and they concluded that this cellular immune response might play an important role in the host defense against the progression of the cervical carcinoma. In this study, we observed an independent albeit weak correlation with pelvic lymph nodes involvement. Future studies should provide the rationale for using immunotherapy in cervical cancer.

It can be concluded that interactions between the tumour cell and the tumour stroma are important in determining the biological behaviour of the tumour. In this study we have demonstrated that some of the histological parameters indicative of these interactions are important and independent parameters in predicting pelvic lymph node involvement and, even more important disease-free survival in patients with early stage cervical cancer FIGO stages IB and IIA.

Chapter 10

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Chapter 11

Summary and conclusions

11.1 Summary

This thesis deals with therapeutic results and prognostic parameters in cancer of the uterine cervix.

In Chapter 1 introductory remarks on epidemiology, etiology, classification, pathways of spread, pathology and treatment of cervical cancer are made. In the Netherlands the incidence and mortality rate are respectively 9.5 and 3.3 per 100,000 women. Many risk factors for cervical cancer have been investigated extensively, however, relatively little is known about the exact cause. Infection with human papillomavirus (HPV) is associated with the development of cervical cancer and probably with a rapid progression of the tumour.

The regular modalities for primary treatment are surgery, radiotherapy and a combination of both. Radiotherapy can be used in all stages of disease whereas surgery is limited to patients with stage I and IIA. Chemo and immunotherapy are used in a palliative or experimental setting only.

In Chapter 2 the literature regarding clinical and histopathological factors related to prognosis is reviewed. In cervical cancer, identification of prognostic factors is necessary in order to select patients at risk for recurrent disease. Many studies concerning this subject have been published, however, conclusions are mainly based on retrospective studies and single factor analysis only. Pelvic lymph node metastasis has been recognized for a long time to be the strongest predictor of recurrent disease and death. In addition, tumour size, lymph vascular space invasion, histological cell type and grade and depth of stromal invasion have all been shown to be associated to some extent with survival. Most of these variables, however, are probably interrelated with pelvic lymph node metastasis and the relative prognostic importance is difficult to determine without multivariate analysis. Only a few studies using multivariate analysis have been published.

Consequently, little is known on the mechanisms leading to metastasis and recurrent disease. In clinical management it still has to be established whether aggressive additional therapy benefits in patients at risk for recurrent disease.

Chapter 3 reviews several factors initiating and promoting the development of normal cells to cancer cells. Special reference to the development of cervical cancer and interactions between cancer cells and the surrounding tumour stroma is made. The tumour stroma can be divided into the basement membrane forming the first barrier for invading tumour cells and the interstitial connective tissue. This connective tissue supports and nourishes the tumour cells. Furthermore, the growth of new blood vessels toward and within a tumour is mandatory for the process of tumour expansion. Moreover, it has been proposed that the tumour stroma forms a barrier around the tumour cells inhibiting invasion and metastasis. It is concluded that the tumour stroma plays an important role in tumour invasion and metastasis.

The aim of this thesis is outlined in Chapter 4. The studies described in this thesis are based on the clinical and histopathological profiles of 205 patients with cervical cancer who were admitted to the University Hospital Maastricht between 1978 and 1993. Some aspects of the tumour stroma in cervical cancer are analyzed and the prognostic value of several stromal parameters is investigated and described in the remaining chapters.

In Chapter 5 the therapy and survival of patients with Figo stage IB and IIA treated in the period between 1978 and 1991 are described. In the University Hospital of Maastricht, early stage cervical cancer patients are initially treated with radical surgery. This procedure according to Wertheim-Meigs is abandoned when pelvic lymph node involvement is present. Ninety-nine patients with cervical cancer stage IB and IIA are included in this study. Of these, 86 patients were initially treated surgically and 13 patients with primary radiotherapy. Five year disease free survival rate of the entire group of patients was 81%. Five year disease free survival rate for patients with negative pelvic lymph nodes was 88% compared to 61% when pelvic lymph node involvement was present. Severe complications of the urinary and gastrointestinal tract were found in 6%. It is concluded that the presented therapeutic strategy shows survival and complication rates comparable to those reported in the literature.

Basement membranes (BM) are complex extracellular matrix structures, separating epithelium from the underlying stroma.

In Chapter 6 basement membrane deposition, expressed as type IV collagen immunostaining at the interface of tumour cells and stroma was studied in 156 patients with cervical cancer stage IB-IV. Staining patterns were assessed semi-quantitatively and divided into three patterns, according to intensity and continuity of staining: thick basement membranes with minimal discontinuity (pattern I), thin basement membranes with moderate discontinuity (pattern II) and fragmentary or absent basement membranes (pattern III).

A fragmentary or absent basement membrane, pattern III, was more frequently observed in advanced cervical cancer stage IIB-IV ($p=0.02$). In patients with early cervical cancer stage IB-IIA this pattern was associated with the presence of pelvic lymph node metastasis ($p<0.0001$) and associated significantly with a decreased disease free survival rate ($p=0.05$). In advanced stage disease basement membrane pattern did not correlate to prognosis.

It is concluded that in patients with early stage cervical cancer the basement membrane pattern, expressed as the type IV collagen staining pattern, may be a useful marker in predicting lymph node metastasis and disease free survival.

In all types of cancer, tumour stroma is observed in between the malignant epithelial cell proliferation. Experimental evidence suggests that an abundant tumour stroma has an anti-invasive effect. In Chapter 7 a novel prognostic parameter is introduced. The relative volume of the tumour stroma, expressed as the tumour stroma fraction was determined morphometrically and correlated with disease free survival in 114 patients with cervical cancer stage IB and IIA using multivariate analysis. The tumour stroma fraction showed to be a significant and independent prognostic parameter in patients with early cervical cancer ($p=0.004$). The relative mortality risk increased 50 percent with every 10 percent decrease of the tumour stroma fraction. This study supports the hypothesis that an abundant stroma reaction in human cancer has an anti-invasive effect.

In addition, in patients with cervical cancer stage IB and IIA the tumour stroma fraction proved to be a novel and strong parameter determining survival probability.

Angiogenesis is the growth of new blood vessels toward and within a tumour. In Chapter 8 tumour angiogenesis, expressed as microvessel density (vessels per millimeters squared stroma), was investigated in 114 patients with cervical cancer stage IB and IIA and correlated to pelvic lymph node metastasis and disease free survival using multivariate analysis. There was a significant difference in mean microvessel density between tumours with and without pelvic lymph node metastasis ($p=0.002$). In addition, both microvessel density ($P=0.05$) and pelvic lymph node metastasis ($P=0.007$) correlated significantly and independently with disease free survival.

It is concluded that microvessel density is an independent prognostic parameter for disease free survival in patients with cervical cancer stage IB and IIA.

Identification of clinical and histological parameters present at initial presentation of patients with early cervical cancer indicating prognosis is of the utmost importance. Unfortunately, the majority of the risk factors known to be associated with an adverse prognosis are rather weak predictors of outcome and the majority of them are derived from studies using univariate analysis only.

In Chapter 9 clinical and histopathological parameters were correlated to pelvic lymph node metastasis and disease free survival using multivariate analysis. Basement membrane pattern, microvessel density and lymphocytic infiltration in between the tumour cells were found to predict independently the involvement of the pelvic lymph nodes. Of all parameters, only tumour stroma fraction and microvessel density showed to have an independent influence upon disease free survival whereas surprisingly pelvic lymph node involvement was not.

11.2 Conclusions

In order to select patients with cervical cancer at risk for recurrent disease after initial therapy additional parameters predicting prognosis are essential.

To be useful clinically, a prognostic parameter should provide information on the course of the disease and defines a small subset of patients accounting for the majority of relapses.

The investigations described in this thesis revealed a correlation between some histopathological features of the tumour and both pelvic lymph node involvement and disease free survival. A fragmentary or absent basement membrane, a high angiogenic rate and the absence of lymphocytic infiltration in between the tumour cells are all independent predictors of pelvic lymph node involvement. Although one should be cautious in translating these data to the biological phenomenon of metastasis, these results nevertheless support the hypothesis that tumour cells may gain more access to the circulation after the tumour has eroded the basement membrane, the natural barrier between the tumour cells and the stromal compartment.

With regard to the amount of angiogenesis, a tumour with high angiogenic activity has a better supply of nutrients and therefore its ability to grow. In addition, the change of entering lymphatic or blood vessels is increased.

Both the destruction of the basement membrane and the ability to reach the circulation are important steps in the process of metastasis.

The role of lymphocytes in between the tumour cells in this process is unclear. A strong cellular reaction usually indicates a host defense against the tumour and this is in agreement with the independent correlation between the absence of lymphocytes in between the tumour cells and pelvic lymph node involvement. In this study disease free survival was independently correlated with tumour stroma fraction and microvessel density. A tumour with a low tumour stroma fraction has a relatively high tumour cell load in contrast to tumours with a high tumour stroma fraction. In addition, an abundant stromal reaction has an anti-invasive effect. This tumour cell load and the low stromal defense response can be an explanation for the poor prognosis of patients with a low tumour stroma fraction.

Although many research has been focussed on tumour cell characteristics, interactions between tumour cells and the host are important in understanding biological behaviour of cancer. The results presented in this thesis stress this importance.

Understanding the biological phenomena associated with lymph node metastasis and survival in patients with cervical cancer could improve patient management. This thesis reveals some novel parameters which could be helpful in identifying a subset of patients who could benefit from additional aggressive therapy such as chemotherapy and immunotherapy.

These parameters are promising for this goal and warrant further investigations. Its clinical usefulness remains to be determined initially by studying them in another independent study group and subsequently in a prospective clinical trial.

Chapter 12

Samenvatting en conclusies

12.1 Samenvatting

Dit proefschrift handelt over baarmoederhalskanker.

Hoofdstuk 1 geeft een algemene inleiding over baarmoederhalskanker. Achtereenvolgens worden in het kort de epidemiologie, de mogelijke oorzaken, de indeling, de wijze van uitzaaiing, de pathologie en de behandeling besproken. In Nederland doen zich jaarlijks 9,5 nieuwe gevallen per 100.000 vrouwen voor en sterven 3,3 patiënten per 100.000 vrouwen aan deze ziekte.

Een groot aantal factoren die mogelijk een oorzakelijk verband met baarmoederhalskanker hebben zijn onderzocht, echter een eenduidige oorzaak is niet bekend. Een infectie met het humane papillomavirus wordt sterk met baarmoederhalskanker in verband gebracht en met name met een snelle progressie van de ziekte.

Patiënten met baarmoederhalskanker kunnen zowel primair chirurgisch, primair radiotherapeutisch als met een combinatie van beide methoden worden behandeld.

Bestraling kan in alle stadia van de ziekte worden toegepast terwijl alleen patiënten in stadium I of IIA chirurgisch worden behandeld. Behandeling met celremmende middelen en met stoffen die de afweer versterken wordt alleen toegepast indien genezing niet meer mogelijk is of als experimentele geneeskunde.

Hoofdstuk 2 geeft een overzicht van de literatuur betreffende klinische en histopathologische parameters die mogelijk een voorspellende waarde hebben ten aanzien van de prognose van een patiënt met baarmoederhalskanker. De identificatie van deze parameters is van belang om patiënten met een verhoogde kans op tumorrecidief vroegtijdig op te sporen. Vele studies zijn beschreven, echter de meeste onderzoeken zijn retrospectief en maken gebruik van univariate analyses.

Metastasering van tumor naar de lymfeklieren in het kleine bekken is een prognostische parameter met een goede voorspellende waarde. Daarnaast zijn tumorgrootte, lymfe en bloedvat ingroei, celtype, gradering en diepte ingroei in

het stroma van de baarmoederhals van prognostisch belang. Een aantal van deze parameters zijn echter ook geassocieerd met lymfeklier metastasering zodat zonder multivariaat analyse hun eigen prognostische waarde moeilijk is vast te stellen. Slechts een gering aantal studies maken gebruik van multivariaat analyse.

Om de prognose te verbeteren zal daarnaast moeten worden vastgesteld of patiënten met een verhoogde kans op tumorrecidief baat hebben bij een agressieve adjuvante behandeling.

In **Hoofdstuk 3** worden verschillende factoren beschreven die bijdragen aan de transformatie van normale cellen tot tumorcellen en die de uitbreiding van een tumor stimuleren.

Speciale aandacht wordt besteed aan de ontwikkeling van baarmoederhalskanker en de interacties tussen tumorcellen en omliggend tumorstroma. Het tumorstroma kan worden onderverdeeld in de basale membraan, de eerste barrière voor invasieve tumorcellen, en het interstitiële bindweefsel.

Dit bindweefsel geeft steun en voeding aan de tumorcellen. Tevens is voor progressie van tumor de groei van nieuwe bloedvaten in de tumor noodzakelijk. Daarnaast wordt verondersteld dat het tumorstroma een barrière tegen invasie en metastasering door tumorcellen vormt. Al deze gegevens tonen aan dat het tumorstroma een belangrijke rol speelt bij de invasie en metastasering van tumorcellen.

De doelstellingen van het onderzoek worden geformuleerd in **Hoofdstuk 4**. De studies beschreven in dit proefschrift zijn verricht met zowel de klinische gegevens als het tumorweefsel van 205 patiënten met baarmoederhalskanker die vanaf 1978 tot 1993 werden verwezen naar het Academisch Ziekenhuis Maastricht. Een aantal aspecten van het tumorstroma in baarmoederhalskanker werden bestudeerd en de voorspellende waarde ten aanzien van de prognose werd onderzocht en beschreven in de volgende hoofdstukken.

Hoofdstuk 5 beschrijft de therapieresultaten van patiënten met baarmoederhalskanker FIGO stadium IB en IIA die vanaf 1978 tot 1991 werden behandeld. In het Academisch Ziekenhuis Maastricht worden patiënten met baarmoederhalskanker FIGO stadium IB en IIA primair chirurgisch behandeld. De operatie volgens Wertheim-Meigs wordt gestopt wanneer tijdens de procedure lymfeklier metastasering in de vriescoupes wordt gevonden.

Negenennegentig patiënten zijn in de studie opgenomen: 86 patiënten werden primair chirurgisch en 13 patiënten werden primair met bestraling behandeld. De vijfjaars ziektevrije overleving bedroeg 81% voor de gehele patiëntengroep, onderverdeeld in 88% voor de patiëntengroep met negatieve lymfeklieren en 61% voor de patiëntengroep met lymfekliermetastasering. In 6% van de patiënten traden complicaties van urinewegen en darmen op.

De gepresenteerde behandelingswijze vertoont vergelijkbare resultaten met de onderzoeken beschreven in de literatuur.

Basale membranen (BM) zijn complexe structuren gelegen in de extracellulaire matrix die epitheel en onderliggend stroma van elkaar scheiden.

In **Hoofdstuk 6** wordt de BM depositie op het grensvlak van tumor en stroma in 156 carcinomen van de baarmoederhals FIGO stadium IB-IV onderzocht. Met antilichamen tegen collageen type IV werden de BM immunohistochemisch gevisualiseerd. De immunoreactiviteit werd semiquantitatief gescoord en verdeeld in drie typen gebaseerd op intensiteit en continuïteit van kleuring: type I; een dikke basale membraan met minimale discontinuïteit, type II; een dunne basale membraan met matige discontinuïteit en type III; een gefragmenteerde of afwezige basale membraan.

Een gefragmenteerde of afwezige basale membraan (type III) werd frequenter gezien in het gevorderde carcinoom van de baarmoederhals (stadium IB-IV) ($p=0,02$).

Basale membraan type III was in het vroege carcinoom van de baarmoederhals (stadium IB-IIA) significant gecorreleerd met zowel lymfekliermetastasering in het kleine bekken ($p<0,0001$) als een kortere ziektevrije overleving ($p=0,05$). In het gevorderde carcinoom bestond er geen correlatie met ziektevrije overleving. We concluderen dat in het vroege stadium van baarmoederhals kanker het basale membraan type, aangetoond met antilichamen tegen collageen type IV, een bruikbare parameter kan zijn ter voorspelling van lymfekliermetastasering en ziektevrije overleving.

Tumorstroma kan in alle type carcinomen tussen de epiteliale cel proliferaties worden gevonden. Onderzoekgegevens suggereren dat een sterke stromale reactie een invasie remmend effect heeft. In **Hoofdstuk 7** wordt een nieuwe prognostische parameter geïntroduceerd.

Het relatieve tumorstromavolume (de tumor stroma fractie) werd morfometrisch bepaald bij 114 patiënten met baarmoederhals kanker stadium IB en IIA en in een multivariaat analyse gecorreleerd met ziektevrije overleving.

De tumor stroma fractie was een significante en onafhankelijke prognostische parameter in het vroege carcinoom van de baarmoederhals ($p=0,004$). Bij elke vermindering van de tumor stroma fractie met 10% steeg de relatieve sterftekans met 50%.

Deze studie ondersteunt de hypothese dat een sterke stromale reactie een invasie remmend effect heeft. Tevens bewijst de tumor stroma fractie een nieuwe en sterke prognostische parameter te zijn in het carcinoom van de baarmoederhals stadium IB en IIA.

Vatnieuwvorming (angiogenese) betreft de groei van nieuwe bloedvaten naar en in een tumor. In **Hoofdstuk 8** wordt tumorangiogenese, gedefinieerd als

microvatdichtheid (vaten per vierkante millimeter stroma oppervlak) onderzocht in 114 carcinomen van de baarmoederhals stadium IB en IIA. De resultaten worden in een multivariaat analyse gecorreleerd met lymfeklier metastasering en ziektevrije overleving.

Er werd een significant verschil in gemiddelde microvatdichtheid tussen carcinomen met en zonder lymfeklier metastasering gevonden ($p=0,002$). Tevens waren zowel microvatdichtheid ($p=0,05$) als lymfekliermetastasering ($p=0,007$) significant en onafhankelijk gecorreleerd met ziektevrije overleving.

Derhalve kan microvatdichtheid worden beschouwd als een onafhankelijke prognostische parameter in baarmoederhalskanker stadium IB en IIA.

Het is van groot belang om klinische en histopathologische parameters te identificeren die in een vroeg stadium van baarmoederhalskanker prognostische betekenis hebben. Helaas hebben veel van deze parameters slechts een geringe voorspellende waarde ten aanzien van de prognose en veelal zijn zij bestudeerd in een univariaat analyse.

In **Hoofdstuk 9** worden klinische en histopathologische parameters gecorreleerd met lymfekliermetastasering in het kleine bekken en ziektevrije overleving.

Van alle parameters voorspelden alleen basale membraan type, microvatdichtheid en infiltratie van lymfocyten tussen de tumorcellen het optreden van lymfekliermetastasering. Twee parameters (tumor stroma fractie en microvatdichtheid) waren onafhankelijk gecorreleerd met ziekte vrije overleving en verrassenderwijs toonde lymfekliermetastasering hier geen correlatie mee.

12.2 Conclusies

Patiënten met baarmoederhalskanker en een verhoogde kans op tumorrecidief na initiële behandeling kunnen met de hulp van prognostische parameters geselecteerd worden. Om deze parameters klinisch te kunnen gebruiken moet het ziektebeloop in hoge mate worden voorspeld zodat de patiënten met een zeer grote kans op tumorrecidief worden geïdentificeerd.

De resultaten beschreven in dit proefschrift tonen een correlatie tussen een aantal histopathologische tumorkenmerken en lymfekliermetastasering in het kleine bekken. Bovendien wordt een correlatie gevonden tussen een aantal histopathologische tumorkenmerken en ziekte vrije overleving.

Een gefragmenteerde of afwezige basale membraan, een hoge mate van angiogenese en de afwezigheid van lymfocyten tussen tumorcellen voorspellen allen in hoge mate het optreden van lymfeklier metastasering. Hoewel men voorzichtig moet zijn met de interpretatie van deze gegevens ondersteunen de resultaten de hypothese dat tumorcellen sneller de bloedbaan bereiken als de barrière tussen epitheel en stroma (de basale membraan) is vernietigd. Daarnaast wordt een tumor met veel vatnieuwvorming goed voorzien van voedingsstoffen

en krijgt daarmee een grote groeipotentie. Tevens wordt de kans op ingroei in lymfe en bloedvaten vergroot.

Zowel de vernietiging van de basale membraan als de wijze waarop de lymfe en bloedbaan bereikt kan worden zijn belangrijke stappen in het proces dat leidt tot metastasering.

De rol die de infiltratie van lymfocyten in dit proces spelen is niet geheel duidelijk. Een heftige cellulaire immuunreactie weerspiegelt meestal een sterke mate van afweer en dit komt overeen met het onderzoeksgegeven dat de afwezigheid van lymfocyten en lymfekliermetastasering met elkaar samen hangen.

Ziektevrige overleving is in deze studie onafhankelijk gecorreleerd met tumorstromafractie en microvatdichtheid.

Een carcinoom met een lage tumorstromafractie bevat een relatief grote hoeveelheid tumorcellen in vergelijking met een carcinoom met hoge tumorstromafractie. Daarnaast heeft een heftige stromale reactie een invasie remmend effect. Beide factoren kunnen een verklaring zijn voor de slechtere prognose van patiënten met carcinomen met een lage tumorstromafractie.

Naast het vele onderzoek dat verricht is naar tumorcelkenmerken is ook de interactie tussen tumorcellen en gastheer van belang voor de kennis over het biologisch gedrag van een tumor. De resultaten van dit onderzoek benadrukken nogmaals het belang van deze interactie. Kennis van de biologische processen die van belang zijn voor metastasering en prognose kunnen het klinisch beleid beïnvloeden.

Dit proefschrift toont een aantal nieuwe parameters die behulpzaam kunnen zijn bij het selecteren van patiënten met een verhoogde kans op tumorrecidief. Wellicht hebben deze patiënten baat bij een agressievere behandeling zoals chemo of immunotherapie. Deze prognostische parameters zijn veelbelovend en rechtvaardigen verder onderzoek. De klinische waarde zal in een onafhankelijke onderzoeksgroep nogmaals moeten worden onderzocht en vervolgens in een prospectieve studie.

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