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Aan mijn ouders

Contents

Chapter 1

General introduction

1.1 Epidemiology

Endometrial cancer is the most common malignancy of the female genital tract in Western countries, with a stable incidence in the Netherlands over the last decade of approximately 16 per 100,000 women per year. Endometrial cancer is the fourth most common invasive tumour in Dutch women, and accounts for about 5% of all tumours. In 2000 there were 1457 new cases of endometrial cancer in the Netherlands, with the highest incidence among women aged 55 -75 years. 316 women died of the disease that year.[1] Factors that increase the exposure of the uterus to unopposed estrogens, either exogenous or endogenous, are associated with increased risk of endometrial carcinoma.[2] Early menarche, late menopause, and no pregnancies are classically associated with the development of endometrial cancer. Obesity is another well-recognized risk factor. Elevated levels of estrogens, due to increased peripheral conversion of androstenedione, may be the underlying mechanism for this risk. Endometrial cancer is also known to be associated with several other medical conditions, such as diabetes and hypertension,[3] but it might be that these conditions are simply markers of obesity. Little is known about genetic influence on the risk of endometrial cancer. An increase in the risk associated with family history of endometrial cancer has been observed, especially in women aged under 50 years,[4,5] but less than 1% of all endometrial cancers were found to be attributable to familial, and hence potentially genetic, factors.[5] Women with mutations in either the MLH1 or MSH2 gene, responsible for hereditary non-polyposis colorectal cancer (HNPCC), are also at increased risk of developing endometrial cancer at younger age. Identification of HNPCC, or Lynch syndrome, is based on the Amsterdam criteria, stating that at least three relatives should have histological verified colorectal cancer; one of them should be a first degree relative to the other two. At least two successive generations should be affected, and in one of the relatives colorectal cancer should be diagnosed before age 50.[6] HNPCC is subclassified based on the presence or absence of extracolonic malignancies: Lynch syndrome II is accompanied by a high risk for carcinoma of the endometrium.[7] Women with the Lynch II syndrome, have a risk of approximately 20% to develop endometrial cancer before age 50, and this risk increases to 60% by age 70.[8]

1.2 Pathology

The most common histological type of endometrial carcinoma is the endometrioid type (EEC), which accounts for approximately 75% of all cases.[9-11] Other histological subtypes, also referred to as non-endometrioid endometrial carcinomas (NEEC), include serous papillary carcinoma (5-10%), clear cell carcinoma (1-5%), mucinous carcinoma (1%) and carcinosarcomas also known as malignant mixed Müllerian tumours (MMMT) (1-5%).[11] This latter type is composed of malignant epithelial and stromal components, and because of this biphasic appearance, the origin of MMMTs has long been debated. Based on molecular genetic analysis, the current opinion is that these neoplasm's should be considered as metaplastic carcinomas.[12] Clinical data support the view that MMMTs should be considered high-risk carcinomas.[11,13] Pre-malignant lesions often precede EEC and NEEC. Endometrial intraepithelial carcinoma (EIC) is associated with NEEC, and hyperplasia can precede EEC. EIC is composed of malignant cells overlying a basement membrane and appears to represent malignant transformation of atrophic surface endometrium. It is found in the adjacent endometrium of nearly 90% of serous carcinomas.[14] Endometrial hyperplasia consists of an increase in the density or abnormalities in the configuration or distribution of glands.[15] Lesions lacking nuclear atypia are designated hyperplasia and those displaying atypia are designated atypical hyperplasia. Further subclassification of these two forms of hyperplasia is based on the degree of architectural abnormalities. Proliferations displaying marked architectural abnormalities are designated complex (atypical) hyperplasia and lesions with lesser degrees of architectural abnormalities are designated simple (atypical) hyperplasia. Progression of simple hyperplasia to endometrioid carcinoma is rare (< 2%), whereas progression to carcinoma occurs in 30% of the patients with complex atypical hyperplasia.[16]

Endometrial tumours are staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system.[17] This system closely resembles the TNM classification system, a system used for many solid malignancies[18] (Table 1). Until the 1980s FIGO used a clinical staging system.[19] In 1988 FIGO designated endometrial cancer a surgically staged malignancy, partly as a response to publications detailing the inaccuracy of clinical staging and the importance of extra uterine spread.[20] Although mandated through this staging system, lymphadenectomy of the pelvis and para-aortic areas remains controversial. Many individuals with endometrial cancer are obese or elderly, with other medical problems, and according to the FIGO committee on Gynecologic Oncology clinical judgement is required to determine if additional surgery is warranted. In the Netherlands, a lymphadenectomy is usually not a routine component of surgical staging in stage I endometrial cancer. Several studies confirmed the improved prognostic power of the FIGO 1988 surgical staging system, compared with the former clinical staging system.[21,22] Due to the early presenting symptom of vaginal bleeding, most patients (75-80%) are diagnosed at an early stage, i.e. FIGO stage I.[23] The histological grade is an important prognostic factor in endometrial carcinoma. The most widely used grad-

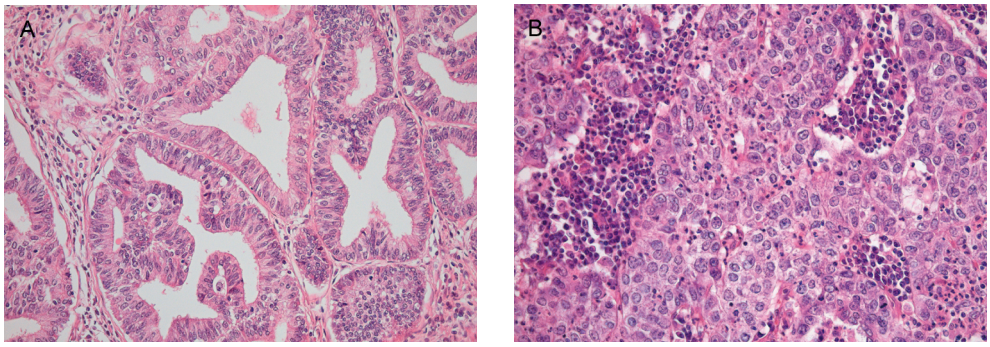

Figure 1. Endometrioid endometrial carcinoma. (A) Grade 1. (B) Grade 3.

Table 1. Pathological FIGO staging system (1988) and TNM classification (2002) for endometrial carcinoma[17,18]

TNM* Category		FIGO Stage
Tis	Carcinoma in situ (preinvasive carcinoma)	
T1a	Tumour limited to endometrium	**IA**
T1b	Invasion to < ½ myometrium	**IB**
T1c	Invasion to ≥ ½ myometrium	**IC**
T2a	Endocervical glandular involvement only	**IIA**
T2b	Cervical stromal invasion	**IIB**
T3a	Tumour involves serosa and/or adnexa and/or positive peritoneal cytology	**IIIA**
T3b	Vaginal involvement	**IIIB**
N1	Metastases to pelvic and/or para-aortic lymph nodes	**IIIC**
T4	Tumour invades bladder and/or bowel mucosa	**IVA**
M1	Distant metastases	**IVB**

*TNM: T – Primary tumour, N – Regional lymph nodes, M – Distant metastases

ing system for endometrial carcinoma is the system recommended by FIGO in 1988. It is primarily based on the extent of nonsquamous solid growth and secondarily on nuclear atypia. Grade 1 tumours have 5% or less areas of solid growth, grade 2, 6% to 50%, and grade 3 more than 50% solid growth (Figure 1). In case of marked nuclear atypia, a grade 1 or 2 is raised by one grade. Marked nuclear atypia is defined as cells with markedly enlarged, pleomorphic nuclei, displaying irregular coarse chromatin and prominent, eosinophilic nucleoli.[11,24] Most tumours are found to be grade 1 or 2. Only 10-30% of all endometrial carcinomas are classified as grade 3, although this percentage is higher in advanced stage tumours.[25] FIGO grading has been shown to have significant prognostic value.[26-28] However,

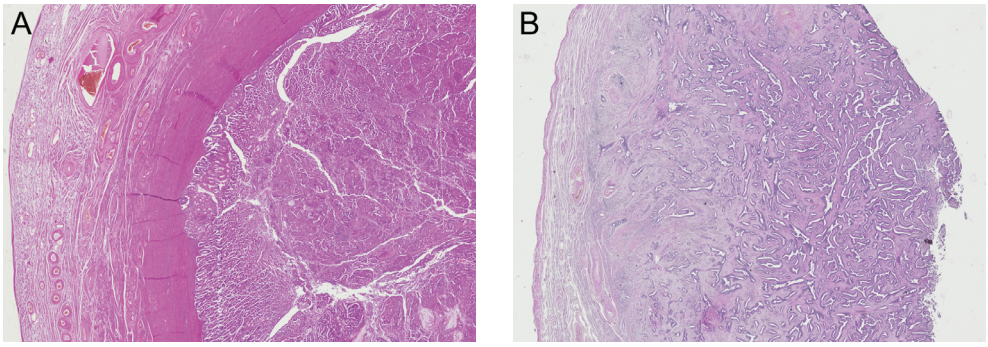

Figure 2. Pattern of myometrial invasion. (A) Pushing border. (B) Infiltrating pattern.

several previous studies have questioned the reproducibility of this grading system. Reported percentages of interobserver agreement vary between 63% and 81% with corresponding κ-values of 0.49-0.65.[29-32] Causes of this lack of reproducibility are the difficulty of distinguishing between squamous and nonsquamous solid growth, especially in case of immature squamous metaplasia, the determination of the percentage of nonsquamous solid growth (more or less than 5%) which is relatively arbitrary, and the subjectivity of the determination of the degree of nuclear atypia, illustrated by the 35% agreement (κ = 0.22) for nuclear grading reported by Lax *et al*.[29] Several alternative grading systems for endometrial carcinoma have been proposed to improve the reproducibility. Lax *et al* [29] proposed a binary grading system based upon the proportion of solid growth (50% or less versus more than 50%), the pattern of myometrial invasion (infiltrating versus pushing border) and the presence of tumour cell necrosis. Taylor *et al* [32] divided tumours into low-grade and high-grade based solely on the proportion of solid growth (more or less than 20%). However, these alternative grading systems need to be tested on large numbers of endometrial carcinomas, in order to evaluate their improved reproducibility and their prognostic power. The depth of myometrial invasion is, besides the histological type, FIGO stage, and histological grade, another pathological feature with major prognostic value in endometrial carcinoma. Deep invasion into the outer part of the myometrial width, defined as either the outer 50% or outer 33%, increases the risk of vessel invasion, since the major vessels of the myometrium are located in the subserosal areas. Deep myometrial invasion has been reported to be a strong adverse prognostic factor.[27,33-35] However, in daily practice it is often difficult to determine the depth of infiltration precisely, since it depends on the way the macroscopic specimen has been processed, which may be subject to sampling errors. Several investigators evaluated the value of the pattern of myometrial invasion. Two distinct infiltration patterns have been identified. First, a diffusely infiltrative growth pattern characterized by irregularly distributed glands, masses, cords, or nests of tumour cells infiltrating the myometrium haphazardly (infiltrating pattern), and second, an expansive growth pattern in which the invasive tumour has a lobulated appearance with pushing borders (push-

ing border) [29,36] (Figure 2). The pattern of invasion has been found to be an independent prognostic factor: the infiltrating pattern was indicative of poor clinical outcome.[36,37] This might be due to the fact that in the infiltrative type, the tumour actually grows into the myometrium, whereas in case of an expansive growth pattern, the tumour pushes the myometrium away, rather than infiltrating it, without reaching the vessels in the outer part of the myometrium.

1.3 Prognostic factors

Patients with endometrial carcinoma in general have a good prognosis, since most patients present with early-stage disease. For stage I endometrial cancer 5-year survival rates are 80-90%[26,34,38,39] and for stage II tumours 60-80%.[39,40] A wide range of 5-year survival rates have been reported for patients with stage III tumours. In most studies rates between 30% and 50% were found,[25,41] but survival rates up to 80% have also been reported in selected groups of patients,[42,43] illustrating the diversity of tumours grouped under stage III endometrial carcinoma. The 5-year survival rate for stage IV disease is approximately 5%.[39]
Over the last two decades, numerous clinical-pathological factors have been found to have prognostic power for survival in patients with endometrial carcinoma. Major prognostic factors include age, FIGO stage, depth of myometrial invasion, histological subtype, lymph vascular space invasion and histological grade.[21,22,26,27,33-35,44-46] Both high oestrogen and high progesterone receptor levels have been found to be associated with improved survival.[47-50] As discussed in §1.4, several molecular abnormalities have been reported to have prognostic implications, though conclusions are contradictory and the clinical relevance of these observations has yet to be elucidated.

1.4 Molecular biology

In 1983 Bokhman was the first to describe two different types of endometrial carcinoma.[51] Type I tumours are oestrogen-related, often preceded by a hyperplasic condition, and are typically low-grade endometrioid carcinomas of the endometrium. They usually develop in an oestrogen rich environment (obesity, pre- and peri-menopausal state), and in general have a good prognosis. On the other hand, type II tumours are unrelated to oestrogen, mostly developing in atrophic endometrium, presumably preceded by endometrial intraepithelial carcinoma, and are more often serous papillary and clear cell carcinomas, i.e. non-endometrioid endometrial carcinomas. They affect older women, and usually have a poor clinical outcome[11] (Table 2). The genetic abnormalities involved in the carcinogenesis of endometrial

cancer appear to be different for type I and type II carcinomas. Essentially, every cancer is the uncontrolled growth that results from a series of mutations in important genes that control cell cycle, cell death or cellular proliferation. Mutations in three types of genes are believed to be responsible for the development of most cancers: oncogenes, tumour suppressor genes, and mismatch repair genes.[52] The protein products of oncogenes, such as K-*ras* and HER-2/neu, participate in growth pathways in normal cells. A mutation in such a gene causes a change in the normal cellular protein and can induce unrestrained proliferation. Products from tumour suppressor genes, such as p53 and PTEN, inhibit cell proliferation. Loss or inactivation of these genes removes this normal constraint on cellular growth. In addition, p53 responds to DNA damage either by forcing these cells to go into apoptosis, or by causing cell cycle arrest, thus promoting DNA repair. Mismatch repair genes, such as MLH1 and MSH2 are responsible for detecting and correcting lesions in DNA, which have occurred during normal replication or were caused by environmental agents. A marker for defects in mismatch repair genes is microsatellite instability (MI). Microsatellites are short, highly polymorphic tracts of simple repeating units that are widely dispersed throughout the genome and are usually non-

Table 2. Predominant features of endometrial carcinoma type I and II

Characteristic	Type I	Type II
Estrogen related	yes	no
Hyperplasia	present	absent
Histological grade	1 or 2	3
Histological subtype	EEC	NEEC
Depth of myometrial invasion	superficial	deep
Stage	low	high
Prognosis	good	poor

EEC: endometrioid carcinoma; NEEC: non-endometrioid carcinoma

Table 3. Comparison of major genetic alterations between type I and type II endometrial carcinoma

Genetic alteration	Type I	Type II
p53 mutations	10-20%	90%
Her-2/neu overexpression	10-15%	20-25%
PTEN inactivation	35-50%	5-10%
K-*ras* mutations	15-30%	0-5%
Microsatellite instability	20-30%	0-5%

coding.[53] MI refers to alterations in the lengths of these repetitive sequences in tumour DNA as compared to DNA obtained from normal tissue of the same person. In type II endometrial carcinomas, p53 mutations seem to be the most important molecular abnormality, reported in up to 90% of the cases, both in the invasive and in the intraepithelial components, suggesting this to be an early event in carcinogenesis[54,55] (Table 3). HER-2/neu overexpression has been observed in 10-25% of endometrial carcinomas, more frequently in NEEC than in EEC.[56-59] In type I carcinoma (EEC) no specific molecular abnormality has been found to be responsible for the majority of tumours. This type of endometrial carcinoma seems to encompass a heterogeneous group of tumours, in which different combinations of several molecular abnormalities have been observed. In 10-20% of EEC p53 mutations have been found, mostly in grade 3 lesions.[54,60,61] These mutations have not been found in endometrial hyperplasia, implying that p53 mutations are late events in the development of EEC, related to dedifferentiation of endometrioid carcinomas.[62] PTEN mutations have been identified in 35-50% of EEC.[61,63-65] Since these have also been found in approximately 25% of atypical hyperplasias, this suggests that PTEN mutations occur early in carcinogenesis. K-*ras* mutations are found in 15-30% of EEC and hardly ever in serous papillary or clear cell carcinoma.[61,62,65,66] Furthermore, K-*ras* mutations have been found in 16% of atypical and simple hyperplasias,[62] suggesting that this mutation is also an early event in a subset of EEC. MI phenotype is present in most endometrial carcinomas in patients with the HNPCC syndrome.[67] In addition, 20-30% of sporadic EECs are MI positive.[65,67-69] MI has been observed in atypical hyperplasia,[69] but not in hyperplasia lacking nuclear atypia,[70] suggesting it to be a late event in endometrioid carcinogenesis.
The prognostic significance of these molecular changes has been investigated in several studies. Overexpression of p53 has been reported to be independently related to decreased survival in endometrial cancer,[56,57,71] although it is not yet clear whether this prognostic significance persists when only EEC are analysed. HER-2/neu overexpression is associated with decreased survival.[56-59] However, in most multivariate analyses, including p53 mutation or DNA ploidy, its prognostic significance is lost.[57,58] Ploidy describes the DNA content of the cell. Normal cells are diploid, tumour cells often miss DNA or contain extra DNA (aneuploidy). Aneuploidy is observed in 15-30% of endometrial carcinomas. Since DNA ploidy reflects the overall degree of genetic damage, it is often found to be a stronger adverse prognostic factor than other more specific genetic changes.[58,72,73] Studies on the prognostic significance of PTEN show conflicting results. Some authors have reported that loss of PTEN was a poor prognostic factor,[74] whereas others noted that PTEN mutation was associated with improved survival.[75,76] Similarly, the prognostic significance of K-*ras* mutations has not been clarified yet. Some investigators reported it to be an adverse prognostic factor,[77] whereas others reported prognostic implications only in postmenopausal women,[78] or no prognostic significance at all.[79] Contrary to observations in colon cancer, where MI is reported to be associated with favourable outcome,[80] in a study on endometrial cancer an adverse outcome has been observed.[81] However, most studies do not find any prognostic significance of MI in endome-

trial carcinoma.[82-84] Assessment of molecular abnormalities can provide information about the carcinogenesis of endometrial carcinoma, it can provide prognostic information, and it holds the promise for improved means of treatment of endometrial cancer. So far, identification of molecular abnormalities has not yet been implemented in daily clinical practice, because their prognostic power is not superior to other, traditional factors, such as age, FIGO stage, and tumour grade. However, further research into this area is worthwhile, because knowledge about molecular abnormalities may aid to a more refined identification of high-risk and low-risk subpopulations and may provide the foundation for molecularly directed therapies.

1.5 Treatment

The cornerstone of treatment for most patients with endometrial carcinoma is surgery. In 1878, Freund was the first to perform a successful total abdominal hysterectomy for this disease.[85] By the 1920s the role of irradiation in the management of endometrial carcinoma was well recognised, either as an adjunct to surgical treatment or as an alternative to operation. The Stockholm method, an intracavitary technique, reported by Heyman in 1947 [86] generated further interest. With improved surgical outcomes in the 1940s, more patients had surgical treatment. For the next decades the combination of intracavitary irradiation and surgery was the treatment of choice.[9] By the late 1960s, concerns rose about the complications from this irradiation technique, and treatment changed to preoperative external beam irradiation.[87,88] When it was recognized that many patients who had preoperative irradiation received unnecessary radiation, as pathological evaluation postoperatively demonstrated low-risk features, another approach to treatment was developed by the 1970s: surgery with adjuvant radiotherapy tailored to prognostic factors.[20,89] Three large, prospective, randomised trials have been conducted to evaluate the effectiveness of postoperative pelvic radiotherapy in stage I endometrial cancer. In the study by Aalders *et al*,[35] 540 women with clinical (FIGO 1971) stage I endometrial carcinoma, who had undergone a total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH-BSO) and postoperative vaginal radiotherapy (RT) were randomly assigned to additional pelvic RT or observation. This trial included patients with both high and low risk of recurrence, however, the majority of patients had low-risk features. Although pelvic RT reduced vaginal and pelvic recurrence rates (2% in the RT versus 7% in the control group), overall survival was not improved (5-year survival of 89% versus 90%). In the multicentre Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial,[26] patients with stage I endometrial carcinoma, either grade 1 or 2 with deep (50% or more) myometrial invasion (stage IC), or grade 2 or 3 with superficial (less than 50%) myometrial invasion (stage IB), were randomly assigned after TAH-BSO to pelvic RT or observation. In the RT group the 5-year locoregional recurrence rate was 4% versus 14% in

the observation group ($p < 0.001$). Again, this difference in locoregional recurrence did not translate into a survival benefit: 5-year overall survival rates were 85% in the control and 81% in the RT group (not significant). Risk factors for recurrence were found to be deep myometrial invasion (50% or more), histological grade 3 and age 60 years or over. The Gynecological Oncology Group (GOG) trial included 392 patients with a surgical stage IB-IIB (occult), node negative endometrial carcinoma, who were randomised to receive no additional treatment (NAT) or pelvic radiotherapy (RT) after TAH-BSO and selective lymphadenectomy.[90] The 2-year locoregional recurrence rates were 1.6% and 7.4%, for the RT and the NAT group, respectively. In this study there was a slight, though not significant, survival benefit: 4-year overall survival rates were 92% and 86%, for the RT and the NAT group, respectively. Presently, the standard surgical treatment in the Netherlands for stage I-III endometrial cancers is a TAH-BSO. Postoperative radiotherapy is indicated in all stage II or III carcinomas, and in stage I carcinomas when 2 of 3 risk factors are present (histological grade 3, 50% or more myometrial invasion, and age 60 years or over).[26] The role of a staging lymphadenectomy in stage I endometrial carcinoma is controversial. Some authors suggest that it might lead to a better outcome, and that radiotherapy could be omitted when lymph node metastases are absent.[91-95] However, these small, retrospective studies include highly selected, mostly young patient groups. Furthermore, other studies did not find a therapeutic benefit,[96] and so far it has not been established that the procedure yields additional prognostic information to the information obtained at the time of hysterectomy,[20,97] while it increases the risk of complications.[97] The results of the ongoing randomised MRC-ASTEC study may clarify in the future whether or not a staging lymphadenectomy should be a routine component of surgical staging in stage I endometrial cancer. Chemotherapy and hormonal treatment are used in the treatment of recurrent or metastatic endometrial carcinoma. Response rates with progestins range from 18% to 34%, with a median duration of several months.[98,99] Highest response rates are achieved in patients with progesterone receptor positive tumours.[100,101] In a meta-analysis of 6 randomised trials, including 3544 patients with endometrial carcinoma, no survival benefit was found for adjuvant progestational treatment.[102] With combined chemotherapy treatment (doxorubicin or paclitaxel and cisplatin), response rates of 31-67% are reported, but toxicity rates (mainly haematological) are substantial, and the median duration of these responses is limited to 7-11 months.[103-106] In the adjuvant setting, no benefit of doxorubicin was found.[107]

1.6 Aims and outline of this thesis

The prognosis for patients with endometrial carcinoma is known to be dependent on several clinical and pathological factors. A strong prognostic factor is the histological grade. However, the reproducibility of the most commonly used three-tiered FIGO grading system has been

questioned in several studies. With the use of prognostic factors, subgroups of patients with distinct outcome perspectives, requiring different therapies, can be identified. Incorporation of molecular biologic information could further differentiate between low-risk and high-risk subgroups of patients and could also provide the foundation for further scientific research on molecularly directed therapies. The purpose of this thesis was to investigate the significance of prognostic factors in endometrial carcinoma, especially the value of the FIGO grading system. Furthermore, the incidence and significance of molecular abnormalities and their potential role as prognostic factors for clinical practice was evaluated.

The patient data and histology specimens of two patient cohorts were analysed. First, a cohort of 253 patients with endometrial carcinoma FIGO stages I to III, who were treated in the Leiden University Medical Center (LUMC) between 1984 and 1993 was retrospectively analysed. In 233 cases (89%), tumour samples could be obtained for immunohistochemical analyses. Secondly, data and histology specimens from the multicentre Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial were analysed. Between 1990 and 1997, this trial included 714 evaluable patients with a FIGO stage I endometrial carcinoma. Histology specimens for review were obtained in 80% of the cases.
In **chapter 2** the long-term outcome and prognostic factors for the 253 patients of the LUMC study is analysed and described. Median follow-up of this retrospective analysis was over 10 years. The prognostic value of FIGO stage, histological grade, age, myometrial invasion (depth and pattern), and histological subtype was analysed, focussing on the value of histological grade.
In 2000, the outcome of the randomised PORTEC trial was published. The objectives of this Dutch multicentre trial were to compare locoregional control and overall survival of patients with a stage I endometrial carcinoma, treated with postoperative pelvic radiotherapy or surgery alone. In **chapter 3** the long-term outcome of the 714 patients included in this trial, and the results with central pathology review are presented.
Chapter 4 describes the results of a histological grading study of 800 endometrial carcinomas. The slides of 231 patients (91%) from the LUMC study, and of 569 patients (80%) from the PORTEC trial could be obtained for this study. These 800 cases were reviewed and graded independently by two pathologists. The prognostic significance and the interobserver variability of two grading systems were compared: the traditionally used three-tiered FIGO grading system and a two-tiered grading system, based on the amount of solid growth, the pattern of myometrial invasion and the presence of tumour cell necrosis.
In **chapter 5** the expression of E-cadherin, α- and β-catenin in 225 endometrial carcinomas is evaluated. The correlation between E-cadherin and the catenins, and their correlation with several histological and clinical parameters was analysed, as well as their prognostic value.
In **chapter 6** it is investigated whether an abnormality in the Wnt/β-catenin signalling pathway, leading to nuclear β-catenin expression, is a molecular feature of type I endometrial

carcinoma. β-catenin expression was investigated in 233 endometrial carcinomas. The correlations with several immunohistochemical, histological and clinical parameters, such as the proliferation rate (Ki-67), oestrogen and progesterone receptor expression, and survival were analysed. In **chapter 7** a summary is given, and the results and their implications for clinical practice are discussed.

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Chapter 2

Long-term outcome in endometrial carcinoma favours a two- instead of a three-tiered grading system

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Abstract

Purpose: Endometrial carcinoma is the most common malignancy of the female genital tract. Recognized prognostic factors include FIGO stage, histological grade, depth of myometrial invasion, and age. Although determination of these factors may seem clear and reproducible, the histological grade has recently been the subject of debate. A retrospective analysis of long-term outcome and prognostic factors in endometrial carcinoma was conducted, focusing on the prognostic value of tumour grade.

Materials and Methods: The study included 253 patients with endometrial carcinoma stages I to III, who were treated between 1984 and 1993. The histological slides were reviewed and the prognostic value of stage, age, myometrial invasion (depth and pattern), tumour grade, and histological subtype was analysed. The end point was cancer-specific death; the median follow-up time was 11.7 years.

Results: The actuarial 5-year and 10-year cancer-specific survival rates (CSS) were 85% and 82%, respectively. The 5-year vaginal or pelvic recurrence and distant relapse rates were 7% and 15%. In multivariate analysis, stage, pattern of myometrial invasion, tumour grade, and age were independent prognostic factors. At pathology review, a shift from grade 2 to grade 1 was seen in 112 of the original 144 grade 2 lesions (78%). There was no difference in CSS between grade 1 and grade 2 (94% vs. 90% for original grade and 92% vs. 94% for grade after review), whereas grade 3 was found to be a significant adverse prognostic factor ($p < 0.001$).

Conclusions: The independent prognostic factors for patients with endometrial cancer were stage, pattern of myometrial invasion, tumour grade, and age. Systematic grading led to a considerable shift from grade 2 to grade 1. However, there was no difference in prognostic significance between grade 1 and 2, whereas grade 3 was a major adverse prognostic factor. A two-tiered grading system, instead of the currently used three-tiered system seems preferable, because it has a better correlation with clinical outcome and is expected to have less interobserver variability.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract in Western countries, with an incidence in The Netherlands of approximately 16 per 100,000 women per year.[1] Over the last two decades, numerous clinical-pathological factors have been found to predict survival in patients with endometrial carcinoma. Recognized prognostic factors include stage, tumour grade, myometrial invasion, and age.[2-5] Although the determination of these factors may seem clear and reproducible, the tumour grade has recently become a subject of debate.[6,7] Currently, the most widely used grading system for endometrial carcinoma is the system recommended by the International Federation of Gynecology and Obstetrics (FIGO) in 1988. It is primarily based on the extent of nonsquamous solid growth and secondarily on nuclear atypia.[8] Recently, the reproducibility of this three-tiered grading system was shown to be poor, with a high interobserver variability ($\kappa = 0.63$).[6] This was especially due to the difficulty in consistent nuclear grading ($\kappa = 0.22$).[7] A two-tiered system was proposed, which divides tumours into poorly differentiated and well-differentiated tumours. This system showed greater inter- and intraobserver reproducibility and had a better correlation with clinical outcome.[7] The depth of myometrial invasion has been proven to be of prognostic significance.[2-5] However, the determination of the depth of invasion is not always easy and depends on the way the macroscopic specimen has been processed.

Our analysis was done to determine the long-term results of treatment for endometrial cancer and to analyse risk factors for relapse, including the depth as well as the pattern of myometrial invasion and focusing on the prognostic value of tumour grade. This retrospective study included all patients who were treated in our institution with curative intent for endometrial carcinoma during a 10-year period. With the long follow-up time (median 11.7 years), we were able to illuminate many aspects of the clinical course of this tumour.

Materials and Methods

Patients and treatment

From January 1984 to December 1993, 253 patients with endometrial carcinoma (FIGO stages I-III) were referred to the Department of Radiotherapy at the Leiden University Medical Center (LUMC). During that period, all patients with an endometrial carcinoma had an indication for post-operative radiotherapy, except those with a stage I, well-differentiated adenocarcinoma (grade 1) of the endometrium, with less than 50% myometrial invasion. These were not included in this study.

Surgery had been performed in the LUMC or in a regional hospital, and consisted of a total abdominal hysterectomy and bilateral salpingo-oophorectomy in most cases (91%). Fourteen

Table 1. Postoperative radiotherapy

Radiotherapy modality	Number of patients (%)	Median dose (Gy)	SD	Range
External beam and brachytherapy	212 (84)	40	1.3	40 - 50
		15*	0.7	15 - 20
External RT alone	38 (15)	50	6.9	40 - 64
Brachytherapy alone	3 (1)	50*	11.5	33 - 55

SD: standard deviation * Low dose rate (60-80 cGy/hr)

patients (5%) underwent a radical hysterectomy, and the remaining 4% underwent a vaginal hysterectomy. Additionally, peritoneal fluid was sent for cytological evaluation in 21% of cases. A lymphadenectomy was performed in 4% of cases; pelvic and periaortic nodes were sampled in 5% of all cases. For most patients (84%), postoperative radiotherapy consisted of whole pelvic external irradiation followed by vaginal brachytherapy. Thirty-eight patients (15%) received external irradiation only, and three patients (1%) were treated by brachytherapy only. Pelvic irradiation was delivered using a four-field box technique, to a total dose of 40-50 Gy, in 2 Gy daily fractions. The target volume included the proximal half of the vagina, the previous site of the uterus and adnexa, as well as the internal and external iliac nodes. The dose was specified at the isocenter of the fields. Dose homogeneity requirements were according to the criteria of the International Commission on Radiation Units and Measurements. Brachytherapy (low-dose-rate, LDR) was delivered using either colpostats or a vaginal cylinder, to a dose of 15-20 Gy. The dose was specified at 5 mm from the vaginal surface. Further details about the radiotherapy are shown in Table 1.

Four patients received adjuvant systemic therapy, because of advanced disease (FIGO stage III). Therapy consisted of progestional agents for two patients, and two patients received adjuvant chemotherapy (three courses of cyclophosphamide, hexamethylmelamine, adriamycin, and cisplatinum, CHAP).

Pathology review

The histopathological slides of 246 patients (97%) were reviewed by one pathologist (V.S.), especially with respect to myometrial invasion and grade, according to the FIGO 1988 grading criteria.[8] Patient and tumour characteristics are shown in Table 2. Malignant mixed Müllerian tumours (carcinosarcomas) and papillary serous carcinomas were not graded, but were considered as grade 3 in the multivariate analysis. In case of malignant cells in the

adnexa, tumours were classified as stage III endometrial carcinoma, when histology and grade were identical to the primary lesion (n = 22). All other cases (n = 4) were considered to be synchronous ovarian carcinomas. Other stage III tumours were classified as such because of positive peritoneal cytology (n = 8), pelvic lymph node involvement (n = 3), or vaginal involvement (n = 1).

Data collection and statistics

Follow-up data were collected from the patient charts, the cancer registry, and the patients' general practitioners. All patients have been followed for a minimum of six years (median follow-up duration 11.7 years, range 6.3 – 16.8 years) or until death.

Survival, local control, and late side effect rates were calculated using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test. In instances of ordered variables, the trend test was used. Multivariate analysis of prognostic factors was performed using the Cox proportional hazards model. All factors with a p-value < 0.10 in univariate analysis were included in the multivariate analysis. The original tumour grade was left out of the main multivariate analysis, and was analysed in a separate multivariate analysis, in which it replaced the tumour grade after review. The endpoint for the survival analysis was cancer-specific death, calculated from initial treatment with censoring at date of last contact or death due to other causes than endometrial carcinoma.

Table 2. Patient and tumour characteristics

Characteristic	Number of patients (%)
Age (years)	
< 60	77 (30)
≥ 60	176 (70)
mean	65.1
range	40 - 89
Menopausal status	
pre-menopausal	15 (6)
post-menopausal	238 (94)
Histological diagnosis	
endometrioid adenocarcinoma	241 (95)
papillary serous carcinoma	8 (3)
malignant mixed Müllerian tumours	4 (2)
FIGO stage	
I	185 (73)
II	34 (13.5)
III	34 (13.5)
Histological grade*	
1	164 (67)
2	19 (8)
3†	63 (25)
Myometrial invasion*	
< 50%	91 (37)
≥ 50%	155 (63)

* myometrial invasion and grade assigned at pathology review

† malignant mixed Müllerian tumours and papillary serous lesions were all considered grade 3

Results

Outcome

Overall (OS), disease-free (DFS), and cancer-specific (CSS) actuarial survival rates at five years were 77%, 75%, and 85%, and 10-year survival rates were 64%, 63%, and 82%, respectively (Figure 1). At the time of this analysis (January 2001) 105 patients had died. Two patients (2%) died of locally recurrent endometrial cancer, 45 (43%) died of metastatic disease, and 44 (42%) of intercurrent disease. One patient (1%) died of treatment complications, and in 13 cases (12%) the cause of death was unknown.

Relapses occurred in 48 patients (20%). In most cases (n = 40), distant metastases were the first site of relapse. Vaginal, pelvic, and periaortic lymph node recurrences were seen as first site of relapse in five, two, and one cases, respectively. Vaginal or pelvic lymph node recurrences were diagnosed synchronously with a distant relapse in seven and nine cases, respectively. Distant metastases developed after the diagnosis of a local or regional recurrence in three and one cases, respectively. In this analysis we refer to vaginal or pelvic recurrences as locoregional recurrences. The 5-year rates of locoregional recurrences and distant metastases were 7% and 15%, respectively. Various clinical and pathological factors were analysed for their value in predicting clinical outcome, as defined by CSS. The results of univariate and multivariate analyses are shown in Table 3. Because of their small number (n = 12), malignant mixed Müllerian tumours and papillary serous carcinomas were grouped together in this analysis. There was no significant difference in CSS between patients with these two histological subtypes (data not shown). It can be seen in Table 3 that stage, tumour grade, pattern of myometrial invasion, and histological subtype all had prognostic significance for CSS in univariate analysis. A subanalysis of stage IIIA tumours revealed no significant difference in survival between the patients with a stage IIIA tumour because of adnexal involvement and those with IIIA due to positive peritoneal cytology, with 5-year CSS rates of 58% and 63%, respectively (p = 0.63). In multivariate analysis, histological subtype did not reach prognostic significance. Stage, tumour grade, pattern of myometrial invasion, and age appeared to be independent prognostic factors. We have calculated survival after the occurrence of a relapse and analysed several factors for their prognostic significance. Two-year overall survival rates after locoregional and distant relapse were 57% and 12%, mean survival time was 2.4 years and 5 months, respectively (p = 0.01, Figure 2). We analysed several pretreatment variables, namely, age, stage, tumour grade, myometrial invasion and histological subtype, and in addition the interval between initial diagnosis and the occurrence of a relapse (less than two years vs. two years or more). None of these factors was found to predict survival after a locoregional recurrence. In case of distant metastases, an interval of more than two years between the initial diagnosis and the relapse resulted in a significantly better 2-year survival rate compared with an interval of less than two years, 22% vs. 4%, p = 0.04 (median survival five and four months, respectively).

Table 3. Univariate and multivariate analysis of risk factors related to cancer-specific survival

Variable	*n* (%)	5 yr. CSS %	UV *p*-value	MV HR (95% CI)	*p*-value
Stage					
I	185 (73)	91		1	
II	34 (13.5)	79		3.4 (1.6-7.3)	
III	34 (13.5)	58	< 0.001	3.7 (1.8-7.6)	< 0.001
Tumour grade: after review					
1	164 (67)	92		1	
2	19 (8)	94		0.4 (0.1-3.3)	
3	63 (25)	63	< 0.001	3.6 (1.8-7.1)	< 0.001
Tumour grade: original					
1	53 (21)	94		1	
2	144 (57)	90		1.8 (0.7-5.1)	
3	56 (22)	63	< 0.001	4.6 (1.7-12.7)	0.004
Myometrial invasion: depth					
< 50%	91 (37)	89		1	
≥ 50%	155 (63)	82	0.07	1.0 (0.5-2.0)	0.99
Myometrial invasion: pattern					
pushing border	91 (39)	90		1	
infiltrating pattern	141 (61)	80	0.01	2.2 (1.1-4.4)	0.03
Histology					
endometrioid adenocarcinoma	241 (95)	87		1	
other	12 (5)	57	< 0.001	1.2 (0.8-1.6)	0.36
Age (years)					
< 60	77 (30)	89		1	
≥ 60	176 (69)	83	0.07	2.4 (1.1-5.1)	0.02

UV: univariate analysis; MV: multivariate analysis; HR: hazard ratio

Complications

Complications were recorded and graded using the EORTC/RTOG system.[9] Due to the retrospective nature of the study, mild complications (grade 1) could not be assessed. Late side effects of radiotherapy, defined as those occurring more than one month after completion of irradiation, were seen in 17 patients. Twelve patients had radiation proctitis. Other complications included cystitis (n = 2), small bowel obstruction (n = 2), and vaginal ulcer (n = 1).

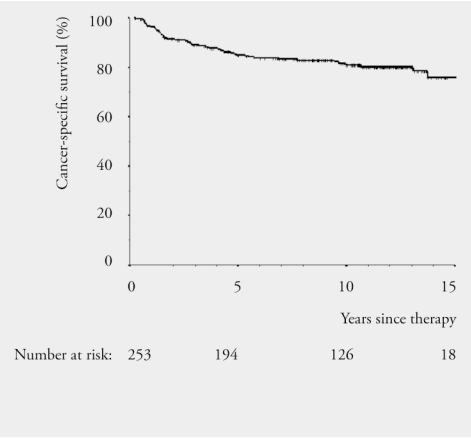

Figure 1. Cancer-specific survival.

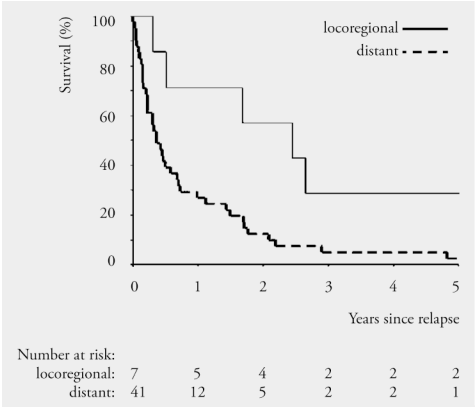

Figure 2. Survival after relapse according to first site of relapse (p = 0.01).

Using the actuarial method, the complication rate at five years was 6.6%. Severe late complications (≥ grade 3) were seen in four patients: one patient developed a radiation proctitis, because of which she required several blood transfusions; one patient had persistent haematuria (for which coagulation was required); and one patient developed a vaginal ulcer. One patient with a small bowel obstruction died due to heart failure after the required operation. The 5-year actuarial severe late complication rate was 1.7%.

Other malignancies

Second (and in three cases even third) primary tumours were diagnosed in 28 patients (11%). Seven patients had a second gynaecological tumour, simultaneously with their endometrial carcinoma, but of distinct pathology. In 21 patients a second (or third) primary tumour was diagnosed, which consisted mostly of breast (n = 6) and colorectal cancers (n = 7). In addition, 11 patients had been treated for other cancers before the diagnosis of their endometrial cancers (five breast cancers, one cancer of the colon, five other sites).

Pathology review

The histopathological slides of 246 patients (97%) were reviewed. The diagnosis of endometrial carcinoma was confirmed in all cases. Seven cases (3%) were assigned a different depth of myometrial invasion, 118 (47%) were assigned a different tumour grade, as compared to the original grade at the time of diagnosis, and in nine cases (4%) both grade and myometrial invasion were scored differently by the reviewing pathologist. One-hundred twenty-two of all 144 cases originally assigned grade 2 (78%) shifted to grade 1 at review. Ten cases (6%) shifted from grade 2 to grade 3. The reviewer assigned a different grade to original grades 1 and 3

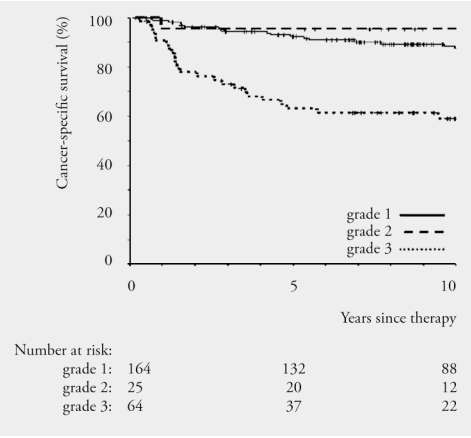

Figure 3. Cancer-specific survival according to tumour grade ($p < 0.001$).

in five cases. These different assignments consisted of a shift from grade 1 to grade 2 (one case), a shift from grade 1 to grade 3 (one case), a shift from grade 3 to grade 2 (one cases), and a shift from grade 3 to grade 1 (one case). It can be seen in Table 3 that the considerable shift from grade 2 to grade 1 did not influence the CSS rates. Patients with grade 1 and grade 2 tumours had equal survival rates, whereas patients with a grade 3 tumour had a significantly worse outcome. Figure 3 shows the CSS by revised tumour grade and illustrates the fact that the diagnosis of grade 3 is associated with an unfavourable prognosis, whereas grades 1 and 2 both predict a favourable outcome ($p < 0.001$). At pathology review, we identified distinct infiltration patterns. Whereas some tumours infiltrated in a diffuse way, with nests of tumour cells infiltrating the myometrium haphazardly, others showed an expansive growth pattern, with pushing borders and a continuous line between tumour and unaffected myometrium. A pushing border was found in 91 cases, an infiltrating pattern in 141 cases. In 14 cases there was no invasion or the pattern of invasion could not be assessed. The pattern of myometrial invasion was found to be an independent prognostic variable, with a survival benefit for patients with a tumour with a pushing border as opposed to patients with infiltrating tumours. There was no significant correlation between the pattern of myometrial invasion and the tumour grade.

Discussion

Survival rates for patients with endometrial carcinoma are highly dependent on the extent of disease. We found 5-year cancer-specific survival (CSS) rates of 91% and 79% for patients with stage I and stage II tumours, respectively. These numbers are in accordance with survival rates reported in previous studies.[2,4,10,11] In this study, survival for patients with advanced primary disease (FIGO stage III) dropped significantly to a 5-year CSS rate of 58%. A wide variation in 5-year survival rates is reported for patients with stage III tumours. Most studies report rates between 30% and 50%,[12-14] but survival rates up to 80% have also been reported in certain groups of patients,[15,16] illustrating the diversity of tumours grouped under stage III endometrial carcinoma. Although we found a difference in 5-year CSS in favour of patients

with a stage IIIA tumour based on positive peritoneal cytology, as compared to those with a stage IIIA tumour based on adnexal involvement (63% vs. 58%), this difference did not reach statistical significance. This might be due to the small numbers of stage IIIA patients in this study (22 and 8, respectively). Because most patients present with early-stage disease, the prognosis for patients with endometrial carcinoma is generally good. Nonetheless, in some patients treatment fails. The chances of survival for these patients are severely impaired. For patients with distant metastases, reported median duration of survival is 7-8 months.[13,17] Survival after a vaginal or pelvic relapse is also impaired, with 2-year survival rates of 20-65%.[18] We found that survival for patients with a locoregional recurrence was significantly better than for those with a distant relapse, with 2-year survival rates of 57% and 12%, respectively. However, mean survival of 2.4 years after a locoregional recurrence remains poor.
We have analysed several variables for their prognostic value for clinical outcome after the occurrence of a relapse. The interval between initial treatment and relapse was found to be a significant prognostic factor. Survival for patients with a distant relapse diagnosed less than two years after initial treatment was significantly worse than for those with longer intervals. However, the difference in mean survival, four months vs. five months, respectively, seems to be of little practical relevance. Prognosis after a locoregional relapse did not depend on the interval between initial treatment and relapse, contrary to data published by Morgan *et al* [19] None of the analysed pretreatment variables - age, stage, tumour grade, myometrial invasion and histological subtype - proved to be of any prognostic significance.
Because salvage probabilities after a relapse are modest, and survival is impaired, the prevention of such a relapse is an important therapeutic goal. Locoregional control rates have improved significantly since the introduction of postoperative radiotherapy. This was proven by two large, prospective, randomized trials, evaluating the effectiveness of postoperative pelvic radiotherapy in patients with stage I endometrial carcinoma.[2,4] Locoregional relapse rates were 2% after postoperative vaginal brachytherapy and additional pelvic radiotherapy, compared with 7% after brachytherapy only,[4] and 4% vs. 14% after postoperative pelvic radiotherapy as compared to no adjuvant irradiation.[2] Distant relapse and survival rates were not affected by adjuvant radiotherapy: 7% and 8% distant relapses, and 85% and 81% 5-year overall survival rates, for patients with surgery only and patients with postoperative radiotherapy, respectively.[2]
We found a comparable locoregional relapse rate, but a higher 5-year distant relapse rate (7% and 15%, respectively), reflecting the use of radiotherapy in all patients and the inclusion of more advanced stages in this study, unlike the studies mentioned above.
The considerable difference in survival after a distant relapse as compared to survival after a locoregional relapse suggests a difference in tumour biology. Determination of molecular markers, e.g., proliferation or apoptotic markers or hormone receptors, could help to predict the occurrence of a relapse and might even enable us to differentiate between patients at risk of a locoregional vs. a distant relapse. Accurate identification of patients at increased risk of

either of the two relapse patterns would be an important step towards a more individualized treatment, where different subsets of patients with different risk estimations receive different adjuvant treatment regimens.

Contrary to previously published data, the depth of myometrial invasion was not found to be a significant prognostic factor in our study, whereas the pattern of invasion had clear prognostic value. At pathology review, we identified two distinct patterns of invasion, an infiltrating pattern and a pattern with a pushing border.[7] We found a significant survival benefit, both in univariate and in multivariate analysis, for patients with tumours invading with a pushing border as opposed to patients with infiltrating tumours. There was no significant correlation between the pattern of myometrial invasion and the tumour grade. Other independent prognostic factors for a worse clinical outcome were older age, advanced stage of disease, and poor histological grade. Although all these factors have been found to have significant prognostic value in earlier studies,[2-5] the tumour grade requires further elaboration.

We have reviewed and graded all tumours using the standard three-tiered system as introduced by FIGO in 1988.[8] The considerable shift of the tumour grade at revision is probably due to the relative subjectivity of the determination of nuclear atypia, the presence of which causes a grade to be raised by one grade, most frequently from grade 1 to grade 2. Furthermore, at time of review, slides were consistently scored according to the FIGO 1988 grading criteria, whereas these criteria had not yet been implemented in all hospitals at the time of the patients' treatment (between 1984 and 1993). The considerable shift from grade 2 to grade 1 is in accordance with data from the Postoperative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial reported by Creutzberg *et al*.[2] In their study, the initial pathology showed 21%, 68%, and 11% grade 1, 2, and 3 tumours, respectively, as opposed to 60%, 32%, and 8% grade 1, 2, and 3 tumours at review.

Recently, the practical clinical utility and reproducibility of the moderate grade of differentiation (grade 2) have been the subject of debate.[6,7] In our study, patients with a grade 1 or grade 2 tumour had equal CSS rates, whereas those with a grade 3 tumour had a significantly worse outcome ($p < 0.001$). This was the case both for the grade as assigned by the original pathologist and for the grade assigned at review, despite the considerable shift from grade 2 to grade 1, which explains why the revised grade had no better prognostic value than the original. The same phenomena were observed in the PORTEC trial.[2] In that trial, the revised grade had no better prognostic power than the original grade and there was hardly any difference in prognostic significance between grade 1 and grade 2, whereas grade 3 was a major adverse prognostic factor. It can be concluded that the intermediate grade does not have sufficient discriminating power to be of clinical value. A grading system with two grades, discriminating between high-risk and low-risk cases, as proposed by Lax *et al* [7] seems to be preferable. Identifying low-risk patients would prevent overtreatment. At our pathology review, 34 cases were diagnosed to have grade 1, stage I tumours with less than 50% myometrial invasion, implying that these cases lost their indication for radiotherapy, or in other words never had

such an indication.

Late side effects found in this study appear to be comparable to those previously reported,[3,11,20,21] although a proper comparison is difficult because most reported incidences are absolute numbers, rather than actuarial rates. We found 5-year overall and severe complication rates of 6.6% and 1.7%, respectively. These low rates might be due to the retrospective nature of this study, as only severe complications were rigorously assessed. Creutzberg *et al* [2] found an overall late complication rate of 25% in their prospective study. Although most of these side effects were mild (grade 1), the impact on daily life for most patients cannot be ignored.

In conclusion, new prognostic factors are needed to identify those patients at increased risk of relapse, leading to a worse clinical outcome. We have confirmed the strong prognostic value of several easily definable adverse prognostic factors for CSS: advanced stage, older age, and poor histological grade. Since distinguishing a grade 2 tumour from a grade 1 has no additional prognostic value, the two-tiered grading system as proposed by Lax *et al* [7] seems preferable to the traditional three-tiered FIGO grading system. We are currently analysing and comparing the reproducibility and the prognostic value of these two grading systems in a large group of cases from the current study and from the prospective PORTEC trial.[2]

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Chapter 3

Postoperative radiotherapy for stage-1 endometrial carcinoma: long-term outcome of the randomised PORTEC trial with central pathology review

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Int J Radiat Oncol Biol Phys (accepted for publication)

Abstract

Background: In 2000, the results of the multicentre Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial were published. This trial included 714 stage I endometrial carcinoma patients randomly assigned to postoperative pelvic radiotherapy (RT) or no further treatment, excluding those with stage IC, grade 3 or stage IB, grade 1 lesions. Radiotherapy significantly decreased the risk of locoregional recurrence (4% vs. 14%), without affecting overall survival. In this report the long-term outcome and results with central pathology review are presented.

Methods: The slides of 569 patients (80%) could be obtained for pathology review. Median follow-up for patients alive was 97 months. Analysis was done according to the intention-to-treat principle. The primary study endpoints were locoregional recurrence and death.

Results: Ten-year locoregional relapse rates were 5% (RT) and 14% controls, $p < 0.0001$, and 10-year overall survival was 66% and 73%, respectively ($p = 0.09$). Endometrial cancer related death rates were 11% (RT) and 9% (control, $p = 0.47$). Pathology review showed a substantial shift from grade 2 to grade 1, but no significant difference for grade 3. When cases diagnosed at review as grade 1 with superficial myometrial invasion were excluded from the analysis, the results remained essentially the same, with 10-year locoregional recurrence rates of 5% (RT) and 17% (controls, $p < 0.0001$).

Conclusions: In view of the significant locoregional control benefit, radiotherapy remains indicated in stage I endometrial carcinoma patients with high-risk features for locoregional relapse.

Introduction

In 2000, the results of the multicentre Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial were published.[1] Patients were eligible for this trial if they had a stage I endometrial carcinoma, either grade 1 or 2 with deep (50% or more) myometrial invasion (stage IC), or grade 2 or 3 with superficial (less than 50%) myometrial invasion (stage IB). Eligibility was based on the grade assigned by the regional pathologist. After total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO) patients were randomised to receive pelvic radiotherapy or no further treatment. Of a total of 715 randomised patients, 714 could be evaluated; 354 were assigned to radiotherapy (RT) and 360 to no further treatment. The study groups were well balanced for characteristics such as age, concurrent morbidity, histological grade, and myometrial invasion.[1] The risk of locoregional recurrence decreased significantly with RT (5-year locoregional recurrence rates of 14% and 4%, respectively), without affecting overall survival (5-year overall survival rates of 85% and 81% for the RT group and the control group). However, the analysis had been done with the grade assigned by the regional pathologist and a substantial shift from grade 2 to grade 1 was found at pathology review.[1,2] Due to this shift, some tumours were diagnosed as grade 1 tumours with superficial myometrial invasion (stage IB), and would not have met the inclusion criteria for the study. The present analysis was done to determine whether exclusion of these favourable cases would alter the trial outcome, and to present long-term results.

Materials and Methods

Pathology review

The slides of 569 patients (80%) could be obtained for pathology review. Slides were graded independently by two pathologists (V.S. and H.B.), according to the FIGO 1988 grading criteria,[3] blinded to patient outcome. For cases that were graded differently a consensus grade was obtained during a joint evaluation session.[2] Cases without pathology review were included in the analyses as a separate category ND (not done).

Statistics

Overall survival, and locoregional (vaginal or pelvic, or both) recurrence rates were calculated using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test. Median follow-up for patients alive was 97 months. Analysis was done according to the intention-to-treat principle. The primary study endpoints were locoregional recurrence and death. Multivariate analysis of prognostic factors was performed using the Cox proportional hazards model. All *p*-values are based on two-sided tests.

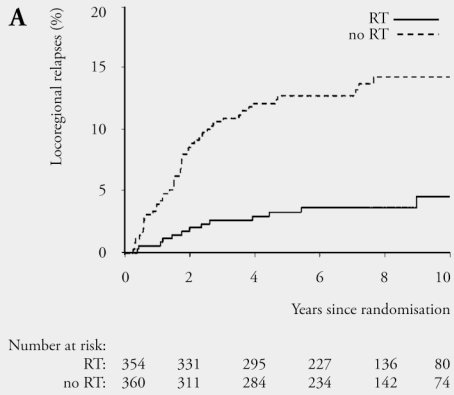
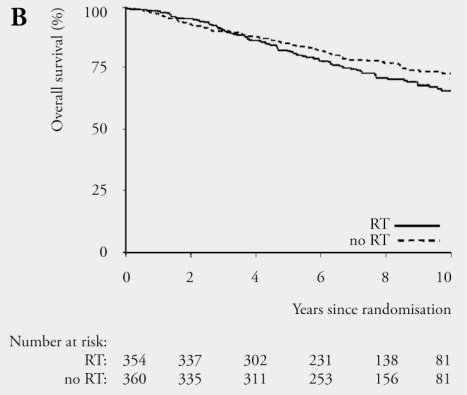


Figure 1. (A) Probability of locoregional (vaginal or pelvic) relapse for patients assigned to radiotherapy or no further treatment ($p < 0.001$).



(B) Probability of survival for patients assigned to radiotherapy or no further treatment ($p = 0.09$).

Results

Locoregional recurrences were diagnosed in 13 patients assigned to radiotherapy and in 47 patients assigned to observation. The 10-year actuarial locoregional relapse rates were 5% in the RT group and 14% in the control group ($p < 0.001$, Figure 1A). There was no significant survival difference between the treatment arms, with 10-year actuarial overall survival rates of 66% (RT) and 73% (controls) ($p = 0.09$, Figure 1B). Most patients died of intercurrent diseases. Endometrial cancer related death rates were similar: 11% in the RT group and 9% in the control group at 10 years ($p = 0.47$). Five-year survival after any relapse was 13% in the RT group and 48% in the control group ($p < 0.001$). Most locoregional relapses were isolated vaginal recurrences (73%). In the control group, salvage therapy for a vaginal recurrence is effective with an actuarial 5-year survival rate of 70% compared with 38% in the RT group.

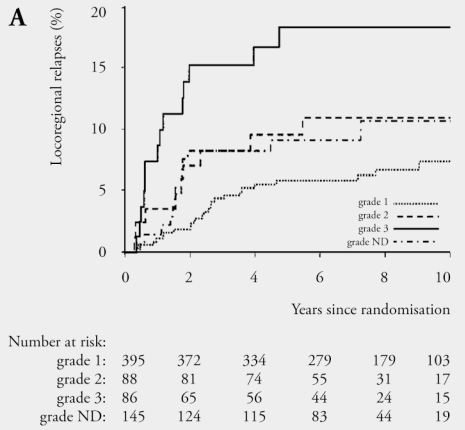
Ten-year locoregional recurrence rates were 7%, 11% and 18% for revised grades 1, 2 and 3 ($p = 0.005$, Figure 2A), and 10-year rates of death due to endometrial cancer were 5%, 12% and 31%, respectively ($p < 0.0001$). In multivariate analyses, postoperative radiotherapy was found to be an independent prognostic factor for locoregional recurrence (Hazard ratio (HR) 3.8; $p < 0.0001$), but not for death due to endometrial cancer (Table 1). For patients aged below 60, 60-70 years and 70 years or over, 10-year locoregional recurrence rates were 4%, 11% and 13% ($p = 0.007$, Figure 2B), and 10-year rates of death due to endometrial cancer were 5%, 9% and 14%, ($p < 0.0001$). Ten-year locoregional recurrence rates according to the depth of myometri-

al invasion (less than 50% vs. 50% or more) were 6% and 12%, respectively (p = 0.07, Figure 2C), and 10-year rates of death due to endometrial cancer were 8% and 11% for tumours with superficial (less than 50%) and deep (50% or more) myometrial invasion, respectively (p = 0.47). Patients with at least 2 of 3 risk factors (age 60 years and over, histological grade 3 and 50% or more myometrial invasion) were found to have an increased risk of locoregional relapse, and thus to have the highest absolute benefit of pelvic RT. The 10-year locoregional relapse rates in this "high-risk" category were 4.6% in the RT group and 23.1% in the control group. At pathology review a substantial shift from grade 2 to grade 1 was observed.[2] Originally, 21%, 68% and 11% of tumours were assigned grade 1, 2 and 3, respectively, while after pathology review, this changed to 69%, 16% and 15%. Due to this shift, 134 tumours were diagnosed as grade 1 tumours with superficial myometrial invasion (stage IB), and would not have met the inclusion criteria for the study. After exclusion of these 134 cases from the analyses, outcome remained essentially the same, with 10-year recurrence rates of 5% for the RT group and 17% for the control group (p < 0.0001), and 10-year overall survival rates of 65% and 70%, respectively (p = 0.23).

Table 1. Cox-regression analyses

		Locoregional relapse			**Death due to EC**		
	***n* (%)**	Events	HR (95% CI)	***p*-value**	Events	HR (95% CI)	***p*-value**
All patients	714 (100)	60			65		
No radiotherapy	360 (50)	47	3.8 (2.0-7.0)	< 0.0001	30	0.7 (0.4-1.2)	0.17
Age ≥ 60	514 (72)	53	3.4 (1.5-7.5)	0.0005	56	2.6 (1.3-5.3)	0.003
Invasion ≥ 50%	420 (59)	42	1.9 (1.1-3.3)	0.03	41	1.7 (1.0-2.9)	0.048
Grade				0.005			< 0.0001
Grade 2*	88 (12)	9	2.0 (0.9-4.2)	0.08	9	2.1 (0.9-4.6)	0.07
Grade 3*	86 (12)	14	3.5 (1.8-6.8)	0.0003	25	9.3 (5.0-17.4)	< 0.0001
Grade ND*	145 (20)	13	2.0 (1.0-3.9)	0.049	11	1.9 (0.9-4.0)	0.09
Exclusion of grade 1 with < 50% invasion							
All patients	580 (100)	55			62		
No radiotherapy	286 (46)	43	3.8 (2.0-7.2)	< 0.0001	29	0.7 (0.4-1.2)	0.25
Age ≥ 60	435 (75)	49	3.5 (1.5-8.2)	0.0009	54	2.7 (1.3-5.7)	0.003
Invasion ≥ 50%	420 (72)	42	1.9 (0.9-3.8)	0.07	41	1.5 (0.8-2.8)	0.18
Grade				0.02			< 0.0001
Grade 2*	88 (15)	9	2.0 (0.9-4.5)	0.10	9	1.9 (0.8-4.3)	0.14
Grade 3*	86 (15)	14	3.5 (1.6-7.5)	0.002	25	8.1 (4.0-16.7)	< 0.0001
Grade ND*	145 (25)	13	2.0 (0.9-4.2)	0.07	11	1.7 (0.8-3.8)	0.18

EC: Endometrial Carcinoma; HR: Hazard Ratio; * versus grade 1



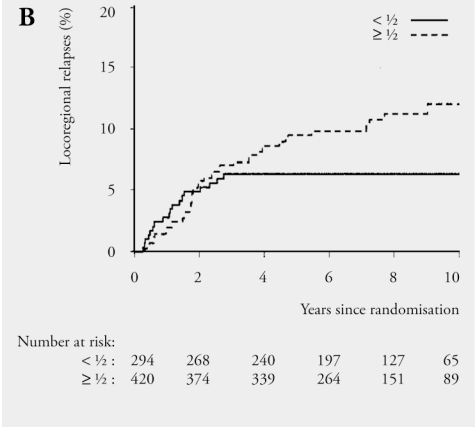


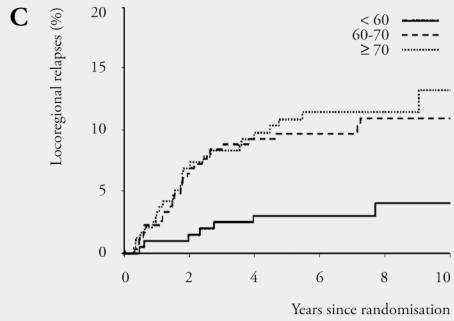
Figure 2. Probability of locoregional (vaginal or pelvic) relapse (A) According to revised grade (p = 0.005).

(B) According to age group (p = 0.007).

Discussion

Postoperative radiotherapy for FIGO stage I endometrial carcinoma has been a subject of controversy due to the low relapse rate and the lack of randomized trials. In 2000, the results of the PORTEC study were published, showing pelvic RT to provide a highly significant improvement of local control, but without a survival advantage. Furthermore, RT was found to be a very effective salvage treatment for vaginal relapse in patients not previously irradiated. Pelvic RT was associated with a small (3%) risk of grade 3 complications, but with a substantial (22%) risk of mild, mainly gastrointestinal side effects, of which 50% were transient.[4] It was concluded that the use of pelvic RT should be limited to those patients at sufficiently high risk of locoregional relapse (15% or over) to warrant the risk of treatment associated morbidity in order to maximize initial local control and relapse-free survival. Patients with 2 of the 3 major risk factors grade 3, age 60 or over, and outer 50% myometrial invasion, were found to be at increased risk of locoregional relapse and to have the highest absolute benefit of pelvic RT (23% versus 4.6% at 10 years).

In the PORTEC trial, low risk patients were identified for whom the indication for postoperative radiotherapy could be omitted. These results have influenced the guidelines for treatment of endometrial cancer worldwide. However, the main criticism has been the fact that the results were based on the grade assigned by the regional pathologist, while a significant shift from grade 2 to grade 1 had been observed at pathology review. Therefore, the current analysis was conducted, with a median follow-up of 97 months, based on the grade



(C) According to depth of myometrial invasion (p = 0.07).

diagnosed at pathology review. The actuarial 10-year locoregional relapse rates were 5% in the RT group and 14% in the control group ($p < 0.0001$). There was no significant survival difference between the treatment arms, with 10-year overall survival rates of 66% (RT) and 73% (controls, $p = 0.09$). Recently, the results of the randomized Gynecologic Oncology Group (GOG-99) trial have been published,[5] which included 392 evaluable patients with FIGO stage IB, IC or II (occult disease) of any histological grade. After TAH-BSO and lymphadenectomy the patients were randomly assigned to pelvic RT or no additional treatment (NAT). A high-intermediate risk group (HIR) was identified based on age and three other risk factors for recurrence: (1) histological grade 2 or 3; (2) presence of lymph vascular invasion; and (3) outer-third myometrial invasion. The HIR subgroup was defined as: (1) 70 years and 1 risk factors; (2) 50-70 years and 2 risk factors; or (3) < 50 years and 3 risk factors. RT provided a hazard reduction for any relapse of 54%, which resulted in a 58% hazard reduction and a 14% absolute benefit for the HIR group (4-year relapse 13% after RT, versus 27% for NAT), results similar to the PORTEC trial. For comparison we applied the GOG high-risk definition to the PORTEC data, which yielded similar results: 10-year locoregional relapse for the high-risk group 7.5% after RT versus 21.7% in the control group (details not shown). Apart from the presence of lymph vascular invasion, which in the PORTEC data was found to be a risk factor for distant recurrence rather than for locoregional recurrence,[6] the prognostic factors identified in both trials were similar. The high-risk criteria, however, were chosen differently. Primary endpoint of the PORTEC trial was locoregional relapse, whereas definition of the high-risk group in the GOG study was based on the risk of developing any relapse. Both in the PORTEC trial[1] and in a previous study[7] we found that outcome for patients with grade 1 or 2 tumours was similar, whereas those with a grade 3 tumour had a significantly worse outcome, indicating that, from a clinical point of view, the discrimination between grade 1 and grade 2 is not very useful. Moreover, it was shown that the reproducibility of identifying grade 1 and 2 tumours was poor.[2] The PORTEC results show that the risk of locoregional recurrence is almost similar for patients aged 60-70 and for those 70 years and over, in contrast to patients younger than 60 years who have a significantly more favourable outcome (Figure 2B), and that the strongest cut-off for age would be 60 years. Main message from both the PORTEC and the GOG analyses is that these criteria accurately identify those

patients with stage I endometrial carcinoma at high risk for relapse, which would benefit from radiotherapy.

In conclusion, previous analyses from the PORTEC trial using the grade assigned by the original pathologist showed postoperative pelvic radiotherapy for stage I endometrial carcinoma to significantly decrease the risk of locoregional recurrence, but without a survival advantage. The current analysis with long-term follow-up and central pathology review confirms these results, which also persisted when grade 1 carcinomas with superficial myometrial invasion were excluded. In view of the significant locoregional control benefit, radiotherapy remains indicated in stage I endometrial carcinoma patients with high-risk features for locoregional relapse.

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Chapter 4

Prognostic significance and interobserver variability of histological grading systems for endometrial carcinoma

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Abstract

Purpose: The most widely used histological grading system for endometrial carcinoma is the three-tiered International Federation of Gynecology and Obstetrics (FIGO) system. Although FIGO grading has significant prognostic value, the reproducibility of grade 2 is limited. Recently, a binary grading system was proposed based on the amount of solid growth, the pattern of myometrial invasion, and the presence of tumour cell necrosis. The authors analysed and compared the prognostic significance and the interobserver variability of both grading systems and of the three criteria for the binary grading system.

Materials and methods: Eight hundred patients with stage I-III endometrioid endometrial carcinoma were reviewed and graded independently by two pathologists according to the three-tiered FIGO grading system and the novel binary grading system.

Results: The interobserver agreement for both systems was moderate, with 70% and 73% agreement rates for the FIGO (κ = 0.41) and binary (κ = 0.39) grading systems, respectively. When converting the FIGO grading system into an artificial, two-tiered grading system (grade 3 vs. grades 1-2), the agreement was much better (agreement rate, 85%; κ = 0.58). Of the three criteria for the binary grading system, amount of solid growth (50% or less vs. more than 50%) had the greatest reproducibility (agreement rate, 80%; κ = 0.50). Both the two-tiered FIGO grading system and the binary grading system were significant predictors of local recurrence, distant recurrence, and cancer-specific survival (hazard ratios [HRs]: 1.7, 2.5, and 2.6, respectively, for FIGO and 2.1, 4.1, and 3.8, respectively, for the binary grading system). The amount of solid growth also was a strong prognostic factor for these three endpoints (HRs: 2.4, 3.9, and 3.8, respectively).

Conclusions: Both the binary grading system and the FIGO grading system had strong prognostic significance. Their reproducibility, however, was limited. A simple architectural binary grading system that divided tumours into low-grade lesions and high-grade lesions based on the proportion of solid growth (less than 50% vs. 50% or more) had superior prognostic power and greater reproducibility.

Introduction

The most widely used grading system for endometrial carcinoma is the three-tiered International Federation of Gynecology and Obstetrics (FIGO) system. This histological grading system is based on both architectural (proportion of solid growth) and cytonuclear criteria.[1] It has been shown that FIGO grading has significant prognostic value.[2-5] However, in a previous retrospective study of 253 patients with stage I-III endometrial carcinoma,[5] we found that patients with grade 1 or 2 tumours had equal cancer-specific survival rates (92% and 94% 5-year survival, respectively), whereas patients with grade 3 tumours had a significantly worse outcome (63% 5-year survival), indicating that, from a clinical point of view, the discrimination between grade 1 and grade 2 is not very useful. Moreover, it was shown that the reproducibility of identifying grade 1 and 2 tumours was poor. Initial pathologic examination identified 21%, 57%, and 22% grade 1, 2, and 3 tumours, respectively, compared with 67%, 8%, and 25% grade 1, 2, and 3 tumours, respectively, at review. A similar shift from grade 2 to grade 1 was reported in a large, Dutch, multicenter, prospective, randomised study, known as the Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial,[3] which included 714 patients with stage I endometrial carcinoma. Lax *et al* [6] have proposed a new binary grading system that discriminates between high-grade and low-grade tumours based on the proportion of solid growth (50% or less vs. more than 50%), the pattern of myometrial invasion (infiltrating vs. pushing border), and the presence of tumour cell necrosis. Those authors found that their binary system had superior interobserver agreement compared with the traditional, three-tiered FIGO grading system. Furthermore, it proved to have prognostic significance for survival.

We conducted a review of 800 patients with endometrioid adenocarcinoma of the endometrium. All slides were reviewed independently by two pathologists and were graded according to both the three-tiered FIGO grading system and the novel binary grading system proposed by Lax *et al*.[6] The objectives of the current study were to analyse and compare the prognostic significance, as well as the interobserver variability, of both grading systems and, separately, to analyse and compare the three criteria for the binary grading system.

Materials and methods

Patients

A total of 800 patients with endometrioid endometrial carcinoma from two different studies were analysed. The histopathological slides of hysterectomy specimens from 231 patients (91%) from a retrospective study[5] of 253 patients with stage I-III endometrial carcinoma who underwent surgery and received radiotherapy at the Leiden University Medical Center were

Table 1. Patient, tumour, and treatment characteristics

Characteristic	Number of patients (%)
Age (years)	
< 60	203 (25)
≥ 60	597 (75)
mean	66.3
range	40-91
Radiotherapy	
yes	506 (63)
no	294 (37)
FIGO stage	
I	740 (92)
II	32 (4)
III	28 (4)
Myometrial invasion	
< 50%	309 (39)
≥ 50%	491 (61)
Total	800

FIGO: International Federation of Gynecology and Obstetrics

obtained. Follow-up data were collected from the patient charts, from the cancer registry, and from the patients' general practitioners. All patients were followed for a minimum of six years or until death (median follow-up, 9.8 years). In addition, histopathological slides of hysterectomy specimens from 569 of 714 patients (80%) who were included in the PORTEC trial[3] were obtained. That trial included patients with FIGO stage I endometrial carcinoma who were randomised to receive pelvic radiotherapy or no further treatment after undergoing a total abdominal hysterectomy and bilateral salpingo-oophorectomy. Of the 569 patients who were included in the current study, 279 patients received postoperative radiotherapy, and 290 patients received no further treatment. The median follow-up was 6.5 years. Patient, tumour, and treatment characteristics are shown in Table 1. The histological diagnosis of endometrial carcinoma was confirmed in all patients.

Grading systems

The 800 slides were graded independently by two pathologists (V.S. and H.B., who were blinded to the patient outcomes) using two different systems: the three-tiered FIGO grading system and the recently proposed binary grading system. The FIGO grading system is based primarily on the proportion of nonsquamous solid growth. Grade 1 tumours have 5% or less areas of solid growth, grade 2 tumours have 6-50% areas of solid growth, and grade 3 tumours have more than 50% areas of solid growth. In patients with marked *notable* nuclear atypia, a grade 1 or 2 is raised by one grade. Notable nuclear atypia is defined as the presence of cells with markedly enlarged, pleomorphic nuclei that display irregular, coarse chromatin and prominent, eosinophilic nucleoli.[7] The FIGO grading system was analysed not only as a three-tiered grading system but also when converted into an artificial, two-tiered grading system (grade 3 vs. grades 1-2). The binary grading system classifies a tumour as "high-grade" if at least two of three architectural features are present: 1) more than 50% solid growth, without distinction between squamous and nonsquamous differentiation; 2) a diffusely infiltrative

growth pattern characterized by irregularly distributed glands, masses, cords, or nests of tumour cells infiltrating the myometrium haphazardly, in contrast to an expansive growth pattern in which the invasive tumour has a lobulated appearance with pushing borders; and 3) tumour cell necrosis, defined as areas of necrotic tumour immediately adjacent to viable tumour, whereas necrotic debris within masses of squamous epithelium is not qualified as tumour cell necrosis.[6] The pattern of myometrial invasion was judged as infiltrative if glands or tumour cells haphazardly infiltrated the myometrium, by which there was no longer a more or less smooth border between tumour and myometrium, even when this happened in only a small part of the tumour. In addition to the analyses of the FIGO and binary grading systems, the three criteria for the binary grading system also were analysed separately for their prognostic significance and interobserver variability. For tumours that were graded differently by the two pathologists, a consensus grade was obtained during a joint evaluation session. These consensus grades were used for the recurrence and survival analyses.

Statistics

Analysis of the interobserver variability was based on the percentage of agreement and was assessed by the statistic. The κ-values, as measurements of agreement, are interpreted as follows: 0.00-0.39, *poor*; 0.40-0.75, *fair to good*; and 0.76-1.00, *excellent*.[8] In the outcome analyses, only stage I tumours (92%) were included, because of the relatively small number of advanced-stage tumours (8%). Recurrence and survival rates were calculated using the Kaplan-Meier method, and differences between curves were assessed with the log-rank test. In instances of ordered variables, the trend test was used. Multivariate analysis of prognostic factors was performed using the Cox proportional hazards model. Several multivariate analyses were conducted. The baseline model included age, radiotherapy use, and depth of myometrial invasion. Subsequently, the following factors were added separately: the FIGO grade, the binary grade, the proportion of solid growth, the pattern of myometrial invasion, and the presence of necrosis. All *p*-values are based on two-sided tests, with $p < 0.05$ considered statistically significant.

Results

Interobserver variability

FIGO grading system

The results of grading by the two pathologists according to the three-tiered FIGO grading system are shown in Table 2. The interobserver agreement for this grading system was fair (κ = 0.41; 70% agreement). When analysing the FIGO grading system as a two-tiered grading system (i.e., grade 3 vs. grades 1-2), interobserver agreement was much better (κ = 0.58; 85%

Table 2. Grading results from the FIGO grading system

	Pathologist 1: Number of patients (%)			
Results	**grade 1**	**grade 2**	**grade 3**	**Total**
Pathologist 2				
grade 1	430 (54)	18 (2)	4 (1)	452 (57)
grade 2	102 (13)	7 (1)	12 (1)	121 (15)
grade 3	77 (10)	27 (3)	123 (15)	227 (28)
Total	609 (76)	52 (7)	139 (17)	800 (100)
κ				0.41
Consensus	522 (65)	135 (17)	143 (18)	800 (100)

FIGO: International Federation of Gynecology and Obstetrics

Table 3. Grading results from the binary grading system

	Pathologist 1: Number of patients (%)		
Results	**low-grade**	**high-grade**	**Total**
Pathologist 2			
low-grade	462 (58)	15 (2)	477 (60)
high-grade	200 (25)	123 (15)	323 (40)
Total	662 (83)	138 (17)	800 (100)
κ			0.39
Consensus	570 (71)	230 (29)	800 (100)

Table 4. Results using individual grading criteria for the binary grading system

	Pathologist 1: Number of patients (%)					
	Solid growth		**Myometrial invasion**		**Necrosis**	
Results	**≤ 50% (-)**	**> 50% (+)**	**pushing (-)**	**infiltrating (+)**	**absent (-)**	**present (+)**
Pathologist 2						
(-)	499 (63)	17 (2)	195 (25)	29 (3)	488 (61)	16 (2)
(+)	146 (18)	138 (17)	204 (26)	363 (46)	194 (24)	102 (13)
κ		0.50		0.41		0.36
Consensus	623 (78)	177 (22)	368 (46)	432 (54)	554 (69)	246 (31)

agreement). After the combined grading session, 522 tumours (65%) were considered consensus FIGO grade 1 lesions, 135 tumours (17%) were considered FIGO grade 2 lesions, and 143 tumours (18%) were considered FIGO grade 3 lesions.

Binary grading system

The grading results according to the two-tiered grading system are shown in Table 3. The interobserver agreement for this grading system was almost similar to that of the FIGO grading system ($\kappa = 0.39$; 73% agreement). After the combined grading session, 570 tumours (71%) were considered binary low-grade lesions, and 230 tumours (29%) were considered binary high-grade lesions. The interobserver variability of the three separate criteria also was analysed. It can be seen in Table 4 that judgment of the proportion of solid growth had the best reproducibility ($\kappa = 0.50$; 80% agreement). Determining the pattern of myometrial invasion (Figure 1A-D) and the presence of tumour cell necrosis (Figure 2A-B) appeared to be more difficult ($\kappa = 0.41$ and 0.36, respectively; 71% and 74% agreement, respectively).

When analysing correlations between the three criteria, we found a strong correlation between the proportion of solid growth and the presence of necrosis. Sixty-seven percent of tumours with more than 50% solid growth had necrotic areas, compared with 20% of tumours with 50% or less solid growth ($p < 0.001$). The pattern of myometrial invasion was correlated significantly with the depth of invasion. Seventy-three percent of patients who had tumours with an infiltrating pattern showed deep myometrial invasion (50% or more), compared with 47% of patients who had tumours with pushing borders ($p < 0.001$). Both the pattern and the depth of myometrial invasion were correlated with patient age. Older patients (age 60 years or over) had tumours that more often showed deep myometrial invasion (67% vs. 46% for patients aged below 60 years; $p < 0.001$) and an infiltrating pattern of invasion (56% vs. 47%; $p = 0.03$). When comparing the FIGO grading system with the binary grading system, we found a significant correlation between both systems. Among the binary low-grade tumours, 95% were FIGO grade 1 or 2 lesions (82% and 13%, respectively), whereas only 24% and 27% of the binary high-grade tumours were FIGO grade 1 or 2 lesions, respectively ($p < 0.001$).

Outcome

Locoregional recurrences (vaginal, pelvic, or both) were diagnosed in 55 of 740 patients with stage I tumours (7%). The 5-year and 10-year locoregional recurrence rates were 7% and 9%, respectively. Distant metastases were found in 63 patients (9%), in most instances involving multiple sites. Distant recurrence rates at five years and ten years were 8% and 9%, respectively. During follow-up, 205 patients (28%) died. Sixty-six deaths (32%) were related to endometrial carcinoma; 130 patients (64%) died of intercurrent diseases; and, in nine patients (4%), the cause of death was unknown. The 5-year and 10-year cancer-specific survival rates were 93% and 90%, respectively. The prognostic value of postoperative radiotherapy, age

Table 5. Univariate analyses of prognostic factors for locoregional recurrence, distant recurrence, and cancer-specific survival

		LR*		DR*		CSS*	
Variable	***n* (%)**	**5-year %**	***p*-value**	**5-year %**	***p*-value**	**5-year %**	***p*-value**
Age							
< 60	179 (24)	3		5		97	
≥ 60	561 (76)	8	0.005	8	0.07	92	0.009
Radiotherapy							
yes	446 (60)	3		8		93	
no	294 (40)	13	< 0.001	7	0.82	93	0.95
Myometrial invasion							
< 50%	292 (39)	6		7		94	
≥ 50%	448 (61)	8	0.10	8	0.37	92	0.15
FIGO grade							
1	491 (66)	5		3		97	
2	126 (17)	8		8		94	
3	123 (17)	14	< 0.001	25	< 0.001	76	< 0.001
Binary grade							
low	537 (73)	5		3		96	
high	203 (27)	11	0.01	18	< 0.001	84	< 0.001
Solid growth							
≤ 50%	585 (79)	6		4		96	
> 50%	155 (21)	11	0.02	19	< 0.001	83	< 0.001
Infiltrating pattern							
pushing	346 (47)	7		6		94	
infiltrating	394 (53)	7	0.94	9	0.16	93	0.62
Necrosis							
absent	526 (71)	6		5		95	
present	214 (29)	8	0.37	14	< 0.001	87	< 0.001

LR: locoregional recurrence; DR: distant recurrence; CSS: cancer-specific survival; FIGO: International Federation of Gynecology and Obstetrics

* Total number of events: LR, 55; DR, 63; CSS, 66

(below 60 years vs. 60 years and over), and depth of myometrial invasion (less than 50% vs. 50% or more) was analysed for locoregional recurrence, distant recurrence, and cancer-specific survival. In univariate (Table 5) and multivariate (Table 6) analyses, no postoperative radiotherapy (Hazard ratio [HR]: 4.5) and age (HR: 3.6) were identified as prognostic parameters for locoregional recurrence. Age (HR: 2.0) was a significant prognostic factor for distant

Table 6. Multivariate analyses of prognostic factors for locoregional resurrence, distant recurrence, and cancer-specific survival

	LR		DR		CSS	
Variable	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Baseline model						
Age ≥ 60	3.6 (1.4-9.0)	0.007	2.0 (1.0-4.0)	0.045	2.7 (1.3-5.7)	0.009
No RT	4.5 (2.4-8.1)	< 0.001	0.9 (0.5-1.5)	0.65	0.9 (0.6-1.6)	0.87
Myometrial invasion ≥ 50%	1.8 (0.9-3.3)	0.05	1.6 (0.9-2.7)	0.10	1.8 (1.0-3.0)	0.04
Separate addition of the following factors						
FIGO grade						
2 vs. 1	2.0 (0.9-4.0)	0.06	2.2 (1.1-4.4)	0.03	1.4 (0.7-3.0)	0.37
3 vs. 1	3.6 (1.9-6.7)	< 0.001	7.7 (4.4-13.6)	< 0.001	7.4 (4.3-12.6)	< 0.001
3 vs. 1-2	1.7 (1.3-2.3)	< 0.001	2.5 (1.9-3.2)	< 0.001	2.6 (2.0-3.3)	< 0.001
Binary high-grade	2.1 (1.2-3.7)	0.006	4.1 (2.5-6.8)	< 0.001	3.8 (2.3-6.1)	< 0.001
Solid growth > 50%	2.4 (1.3-4.3)	0.004	3.9 (2.4-6.5)	< 0.001	3.8 (2.3-6.2)	< 0.001
Infiltrating pattern	0.8 (0.5-1.4)	0.51	1.4 (0.8-2.3)	0.23	1.0 (0.6-1.7)	0.99
Necrosis	1.3 (0.8-2.3)	0.33	2.9 (1.7-4.7)	< 0.001	2.6 (1.6-4.3)	< 0.001

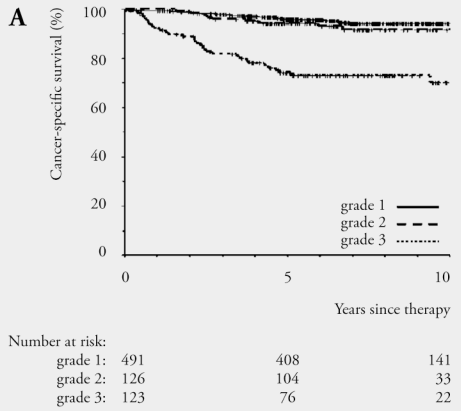
LR: locoregional recurrence; DR: distant recurrence; CSS: cancer-specific survival; HR: hazard ratio; 95%

CI: 95% confidence interval; RT: postoperative radiotherapy; FIGO: International Federation of Gynecology and Obstetrics

recurrence. Age (HR: 2.7) and the depth of myometrial invasion (HR: 1.8) were significant prognostic factors for cancer-specific survival.

FIGO grading system

The consensus FIGO grade was identified as a significant predictor of locoregional recurrence, distant recurrence, and overall survival. The 5-year locoregional recurrence rates were 5%, 8%, and 14% for grades 1, 2, and 3, respectively ($p < 0.001$). The 5-year distant recurrence rates were 3%, 8%, and 25% for grades 1, 2, and 3, respectively ($p < 0.001$); and the 5-year cancer-specific survival rates were 97%, 94%, and 76%, respectively ($p < 0.001$, Figure 3A). In multivariate analysis, FIGO grade proved to be of independent prognostic significance for locoregional recurrence (HR: 2.0 for grade 2, 3.6 for grade 3; $p = 0.06$ and $p < 0.001$, respectively, Table 6). FIGO grade also proved to be of independent prognostic significance for distant recurrence (HR: 2.2 for grade 2, 7.7 for grade 3; $p = 0.03$ and $p < 0.001$, respectively), and cancer-specific survival (HR: 7.4 for grade 3; $p < 0.001$). When converting the FIGO grading system into a two-tiered grading system (grade 3 vs. combined grades 1 and 2), FIGO grading still proved to be of independent prognostic significance for locoregional recurrence, distant recurrence, and cancer-specific survival (HR: 1.7, 2.5 and 2.6, respectively; all $p \leq 0.001$).



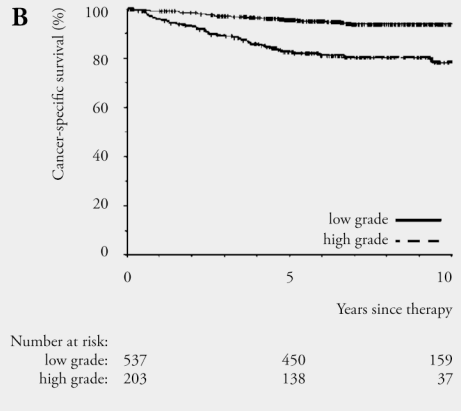
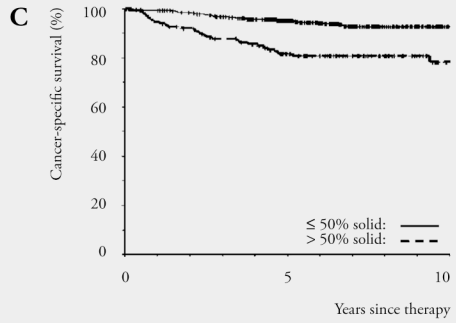


Figure 3. Cancer-specific survival. (A) According to FIGO grade ($p < 0.001$). (B) According to binary grade ($p < 0.001$).

Binary grading system

The consensus grade according to the binary grading system had prognostic significance for locoregional recurrence, distant recurrence, and cancer-specific survival. The 5-year locoregional recurrence rates were 5% and 11% for low-grade tumours and high-grade tumours, respectively ($p < 0.001$); the 5-year distant recurrence rates were 3% and 18%, respectively ($p < 0.001$); and the cancer-specific survival rates at five years were 96% and 84%, respectively ($p < 0.001$, Figure 3B). In multivariate analysis, the binary grade had independent prognostic significance for locoregional and distant recurrence and for cancer-specific survival (HR: 2.1, 4.1 and 3.8, respectively; $p \leq 0.006$, Table 6). Of the three separate criteria for the binary grading system, both more than 50% solid growth and the presence of necrosis proved to be significant independent adverse prognostic factors for distant recurrence and cancer-specific survival. The proportion of solid growth also was identified as a strong, independent prognostic factor for locoregional recurrence. The HRs for each endpoint were comparable to those of the binary grading system and were better than the HRs for the artificially created, two-tiered FIGO grading system. For tumours with 50% or less and for those with more than 50% solid growth, the 5-year locoregional recurrence rates were 6% and 11%, respectively ($p = 0.02$), and the cancer-specific survival rates at five years were 96% and 83%, respectively ($p < 0.001$, Figure 3C). Combining the proportion of solid growth and the presence of necrosis did not improve prognostic power (data not shown). The pattern of myometrial invasion did not have prognostic power.



(C) According to proportion of solid growth ($p < 0.001$).

Discussion

Determining histological tumour grade is an essential part of pathological diagnosis, because the histological grade has prognostic and therapeutic implications. In general, a grading system has to be practical, reproducible, and clinically relevant, with clear prognostic power. For endometrial carcinoma, the most widely used grading system is the three-tiered FIGO grading system. Several previous studies have questioned the reproducibility of this grading system. Reported percentages of interobserver agreement vary between 63% and 81%, with corresponding κ-values of 0.49-0.65.[6,9-11] This is comparable to the reproducibility that we observed for FIGO grading (70% agreement; $\kappa = 0.41$). In a previous study, we also showed that the reproducibility associated with identification of grade 2 tumours was poor.[5] At pathology review, a shift from grade 2 to grade 1 was seen in 78% of the original grade 2 tumours. Moreover, the outcome of patients with grade 1 and grade 2 tumours was practically identical (92% and 94% 5-year survival, respectively), which brings into question the clinical value of grade 2.

There are several features of the FIGO grading system that may be responsible for this lack of reproducibility. First, distinguishing between squamous and nonsquamous solid growth can be difficult, especially in patients with immature squamous metaplasia. Second, determining whether the percentage of nonsquamous solid growth is more or less than 5%, which distinguishes architecturally whether a tumour is a grade 2 or grade 1 lesion, is somewhat arbitrary. Finally, the determination of the degree of nuclear atypia is highly subjective, as illustrated by the 35% agreement ($\kappa = 0.22$) for nuclear grading reported by Lax *et al*.[6] Several alternative grading systems for endometrial carcinoma have been suggested to improve the reproducibility without loosing prognostic power. Lax *et al* [6] proposed a binary grading system based on the proportion of solid growth, the pattern of myometrial invasion, and the presence of tumour cell necrosis. Taylor *et al* [11] divided tumours into low-grade and high-grade lesions based solely on the proportion of solid growth.

We conducted the current study to compare the clinical value of the FIGO grading system, the binary grading system proposed by Lax *et al*,[6] and the three separate factors for that binary grading system (solid growth, pattern of myometrial invasion, and necrosis) by analysing their reproducibility and their prognostic value in 800 patients with endometrioid endometrial carcinoma. The interobserver agreement of the binary grading system was almost similar to that

of the three-tiered FIGO grading system (73% agreement; κ = 0.39), and was comparable to the 79% agreement reported by Lax *et al* [6] When we analysed the FIGO grading system as a two-tiered grading system (grade 3 vs. grades 1-2), interobserver agreement proved to be much better (85% agreement, κ = 0.58). With regard to the interobserver variability among the three separate criteria for the binary grading system, judgment of the proportion of solid growth showed 80% agreement (κ = 0.50). Determining the pattern of myometrial invasion was more difficult (71% agreement; κ = 0.41). This difficulty probably was caused by disagreement of the two pathologists on a considerable number of tumours with an essentially expansive growth pattern but with some masses or cords of tumour cells infiltrating the myometrium superficially. More detailed definitions of the pattern of myometrial invasion may improve the uniformity in differentiating between both patterns of invasion. It was found that the reproducibility of the presence of tumour cell necrosis was poor (74% agreement; κ = 0.36). Lax *et al* [6] stated that necrotic debris within masses of squamous epithelium did not qualify as tumour cell necrosis. Judging a necrotic mass as either debris or necrosis often was a matter of debate between the two pathologists. Furthermore, tumour cell necrosis was defined as areas of necrotic tumour immediately adjacent to viable tumour, and that criterion also proved to be somewhat subjective.

In the current study, it was found that the prognostic value of both the FIGO and the binary grading systems was good. In multivariate analyses, both FIGO grade 3 and the binary high-grade were identified as independent adverse prognostic factors for locoregional recurrence (HR: 3.6 and 2.1, respectively), distant recurrence (HR: 7.7 and 4.1), and cancer-specific survival (HR: 7.4 and 3.8). Although the HR for patients with FIGO grade 3 tumours versus patients with grade 1 tumours was greater than the HR for patients with binary high-grade tumours versus patients with low-grade tumours, the binary grading system was superior to the FIGO grading system when the FIGO grading system was converted into a two-tiered grading system. HRs for FIGO grade 3 versus grades 1-2 for locoregional and distant recurrences and for cancer-specific survival were 1.7, 2.5, and 2.6, respectively. Lax *et al* [6] stated that FIGO grade 1 and grade 2 tumours are not equivalent to low-grade tumours in the binary system; the low-grade tumour group was smaller than the FIGO grade 1 and grade 2 tumour groups combined (97 patients and 119 patients, respectively). The current results confirm this conclusion, because we found less low-grade tumours than FIGO grade 1 or grade 2 tumours (570 patients and 657 patients, respectively). Still, we found a strong correlation between both grading systems ($p < 0.001$), explaining why the prognostic value of both grading systems was so similar.

Taylor *et al* [11] compared the three-tiered FIGO grading system with another two-grade system. For 82 patients with stage I or II endometrioid adenocarcinoma, the percentage of nonsquamous solid areas (in increments of 10%) was scored. The 20% nonsquamous solid growth pattern was chosen to delineate high-grade and low-grade tumours. It was shown that the reproducibility of this grading system was very good (99% agreement; κ = 0.97), and the

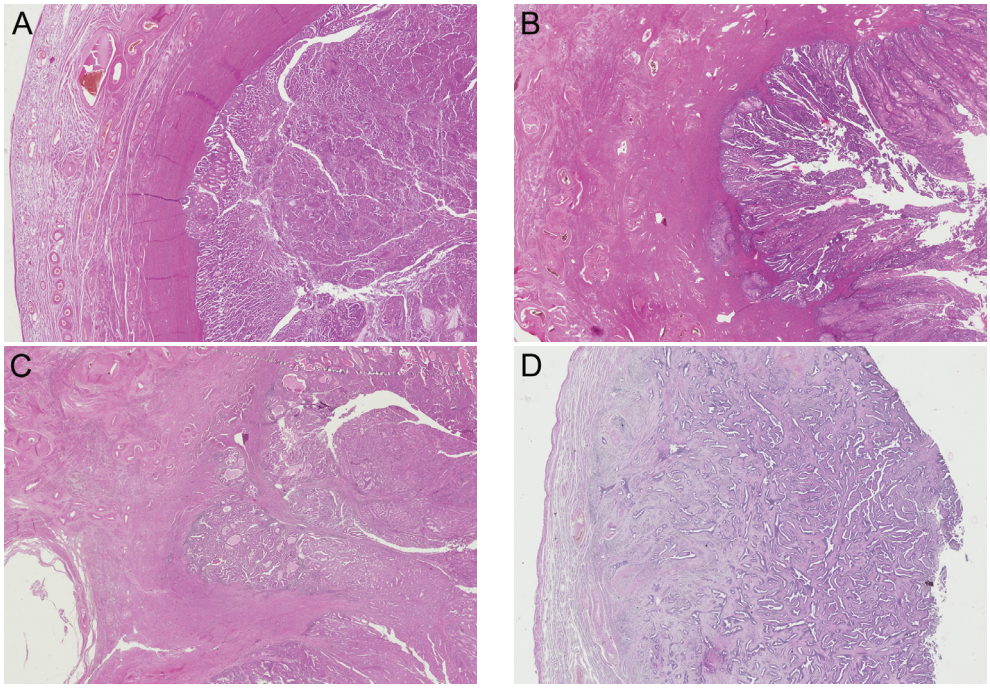


Figure 1. Patterns of myometrial invasion. (A) Pushing border. (B) Case of disagreement (consensus, pushing border). (C) Case of disagreement (consensus, infiltrating pattern). (D) Infiltrating pattern.

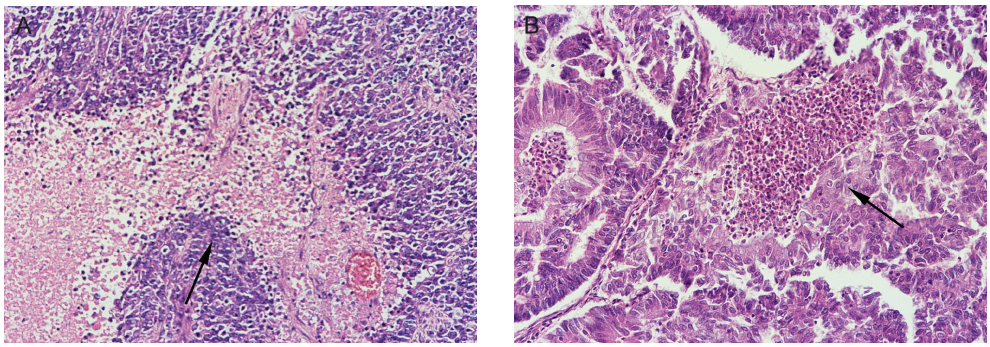


Figure 2. Necrosis. (A) Necrosis. (B) Case of disagreement (consensus, necrosis).

authors reported that their system had good prognostic significance, because disease recurred only in patients with high-grade tumours. We also analysed the reproducibility and prognostic value of the proportion of solid growth (defined as less than 50% vs. 50% or more areas of squamous and nonsquamous solid growth). In fact, this is a simplification of the FIGO grading system that leaves out the subdivision of more or less than 5% solid growth, and the differentiation between squamous and nonsquamous solid growth, and excludes the influence of nuclear atypia on the final grade. Although some have reported that nuclear grading is a good prognosticator and has good reproducibility,[12] others have questioned its prognostic value and its reproducibility.[6,9,10] Zaino *et al*,[9] in their study of 88 patients with endometrial carcinoma, found that the prognosis for patients who had architectural grade 2 tumours with either high or low nuclear grade was similar, bringing into question the need for the inclusion of nuclear atypia within the FIGO guidelines for grading.

Simply dividing patients into groups with high-grade and low-grade tumours based only on the proportion of solid growth (50% or less vs. more than 50%) proved to have an interobserver reproducibility that was better compared with both the binary grading system and the three-tiered FIGO grading system (80% agreement; $\kappa = 0.50$), and it was comparable to the 85% agreement ($\kappa = 0.58$) of the artificial, two-tiered FIGO-grading system. Furthermore, its prognostic value was superior to the two-tiered FIGO grading system and was comparable to the binary grading system.

In conclusion, although it was shown that the binary grading system proposed by Lax *et al* [6] had strong prognostic significance for locoregional and distant recurrence and cancer-specific survival, its reproducibility was poor. The traditional, three-tiered FIGO grading system also exhibited only fair reproducibility. However, the reproducibility of the combined FIGO grades 1 and 2 versus grade 3 was much better, but the prognostic value of this artificial, two-tiered FIGO grading system was less than the value of the binary grading system. Although a two-tiered grading system has advantages in terms of practical clinical utility, the reproducibility of the proposed binary system was too low to support its use instead of the traditional, three-tiered FIGO grading system. Converting the FIGO grading system into an artificial, two-tiered system improved its reproducibility and affected its prognostic power only moderately; therefore, it is a good alternative. However, a simple architectural grading system dividing tumours into low-grade and high-grade, based on the proportion of solid growth (less than 50% vs. 50% or more), was superior to the two-tiered FIGO grading system in prognostic power and was equally reproducible.

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Chapter 5

Combined E-cadherin, α-catenin and β-catenin expression is a favourable prognostic factor in endometrial carcinoma

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Abstract

Purpose: Cell adhesion molecules, such as E-cadherin, might be involved in the processes of tumour invasion and differentiation. The aim of this study was to investigate the expression of E-cadherin and α- en β-catenin in endometrial carcinoma, and to determine the prognostic value of these factors.

Materials and Methods: We have investigated the expression of E-cadherin, α- and β-catenin by immunohistochemistry in 225 endometrial carcinomas. The correlation between E-cadherin and the catenins, and their correlation with several histological and clinical parameters were analysed.

Results: Decreased E-cadherin, α- and β-catenin expression was observed in 44%, 47% and 33% of endometrial carcinomas, respectively, and was correlated with histological FIGO grade 3 ($p < 0.001$). Decreased E-cadherin expression was more often observed in non-endometrioid carcinomas (NEEC) than in endometrioid carcinomas (75% versus 43%, $p = 0.04$). Combined preserved E-cadherin, α- and β-catenin expression was an independent positive prognostic factor for survival in patients with a grade 1-2 carcinoma ($p = 0.02$).

Conclusions: Decreased E-cadherin expression was found to be associated with histological grade 3, and with NEEC. Combined preserved E-cadherin, α- and β-catenin expression was a significant prognostic factor.

Introduction

Epithelial cadherin (E-cadherin) is a member of the cadherin family of cell adhesion molecules. It is a transmembrane glycoprotein that joins adjacent epithelial cells together via a calcium-dependent binding mechanism. E-cadherin proteins of adjacent cells interact in the intercellular space. Binding of the cytoplasmic protein β-catenin to the intracellular part of E-cadherin and to α-catenin, which in turn is linked to the cytoskeleton, is necessary for a properly functioning E-cadherin protein.[1] Since E-cadherin is needed to keep neighbouring cells attached, it has been suggested that impaired function of this cadherin might lead to invasive potential of malignant epithelial tumours. Recently, it became clear that E-cadherin is not only a cell-cell adhesion molecule, but it is also somehow involved in the process of cell differentiation, although so far the underlying mechanism is unclear.[2,3] In several malignancies, such as breast and prostate cancer, a correlation has been found between the expression of E-cadherin and the histological grade.[3,4] So far, only a few studies have focused on the significance of E-cadherin expression in endometrial carcinoma. Decreased expression was found to be associated with increasing histological grade and with deep myometrial invasion.[5-7]
This study was done to investigate, by immunohistochemistry, the expression of E-cadherin and α- and β-catenin in a large series of 225 patients with endometrial carcinoma, with a follow-up of more than 10 years. We analysed the correlation between E-cadherin, α- catenin and β-catenin, their association with several histological and clinical characteristics, and the prognostic significance of these factors.

Material and methods

Cases

Between January 1984 and December 1993, 253 patients with endometrial cancer were treated with surgery and radiotherapy at the Leiden University Medical Center. Details of treatment and outcome have been published previously.[8] For most patients (91%) surgery consisted of a total abdominal hysterectomy and bilateral salpingo-oophorectomy. Peritoneal fluid was sent for cytological evaluation in 21% of cases. A pelvic staging lymphadenectomy was performed in 4% of cases; and pelvic and periaortic node sampling was performed in 5% of cases. Postoperative radiotherapy consisted of pelvic irradiation (median dose 40 Gy) followed by vaginal brachytherapy (median dose 15 Gy). In 225 cases (89%) formalin-fixed paraffin-embedded blocks from the tumour could be obtained for immunohistochemical analyses. All tumours were revised and graded by one pathologist (V.S.), according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines.[9] Two-hundred-and-thirteen tumours were endometrioid type adenocarcinoma, and 12 consisted of

non-endometrioid type carcinomas (4 malignant mixed mesodermal tumours and 8 papillary serous carcinomas). There were 168 stage I, 31 stage II and 26 stage III tumours. Most tumours were FIGO grade 1 (*n* = 154), 16 were grade 2 and 55 grade 3. For myometrial invasion not only the depth (less than 50%, *n* = 85 vs. 50% or more, *n* = 140) but also the pattern of invasion was recorded: diffusely infiltrating tumours, with nests of tumour cells infiltrating the myometrium haphazardly, versus tumours with an expansive growth pattern, with pushing borders and a continuous line between tumour and unaffected myometrium.[10] A pushing border was found in 83 cases, an infiltrating pattern in 131 cases, and in 11 cases there was no invasion or the pattern of invasion could not be assessed. All patients have been followed for a minimum of 6 years (median follow-up 11.6 years, range 6.3 - 16.2 years) or until death.

Immunohistochemistry

Paraffin sections (4 m) were mounted on saline-coated slides. Immunohistochemistry was performed with the MARK 5 Immunostainer (DPC, Breda, The Netherlands). Primary antibodies used in this study were anti-E-cadherin, clone 36 (1:2000, Transduction Laboratories), anti-β-catenin, clone 14 (1:1000, Transduction Laboratories) and anti-α-catenin, clone 5 (1:100, Transduction Laboratories). All the antibodies were monoclonal in type. Prior to incubation, all sections were subjected to antigen retrieval in a microwave with a boiling solution of 10mM citrate buffer, pH=6.0, for 10 minutes. In case of both anti-catenin antibodies 0,05% of a common detergent was added tot the citrate buffer as described previously.[11] Bound primary antibodies were visualised with subsequent incubations with biotinylated polyvalent IgG and HRP-labelled streptavidin (Ultravision, Labvision, Fremont, USA), followed by 0.05% diaminobenzidine and 0.0015% hydrogen peroxide. Slides were briefly counterstained in Mayer's haematoxylin. As negative controls, irrelevant IgG's at the same protein concentration as the primary antibody were used instead of the primary antibody. As positive controls, sections of cervix (E-cadherin), and skin (α- and β-catenin) were used.

Scoring

All specimens were evaluated semi-quantitatively and independently by two investigators (A.S. and R.A.), blinded to patient outcome and tumour characteristics. In case of disagreement, a third opinion was sought (V.S.) and consensus was achieved together. Slides were scored for the intensity of the membranous staining of E-cadherin, α- catenin and β-catenin (SI) and for the proportion of positive tumour cells (PTCs) according to a scoring system that has been used previously by Moreno-Bueno *et al.*[12] The SI could vary from 0 (absent), 1 (weak), 2 (moderate) to 3 (strong, as in normal endometrium, Figure 1). The PTCs varied from 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) to 4 (> 75%). The degree of membranous staining, the staining score, was calculated as the sum of SI and PTCs (range 0-7).

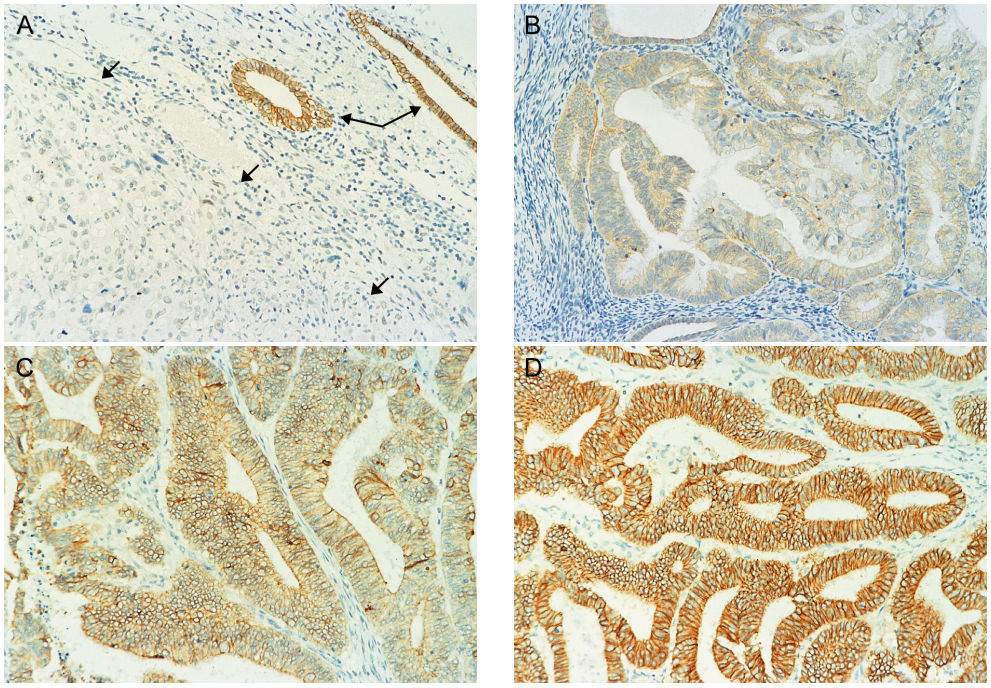

Figure 1. E-cadherin expression in endometrial carcinoma. (A) Absent in tumour cells (short arrows), strong in normal endometrial glands (long arrows). (B) Weak. (C) Moderate. (D) Strong.

Statistical analysis

Correlations between E-cadherin, α- and β-catenin, and other parameters were analysed using the chi-square test. Survival rates were calculated using the Kaplan-Meier method and differences between survival curves were assessed with the log-rank test. Multivariate analyses of prognostic factors were performed using the Cox proportional hazards model. We preferred a dichotomous scoring system for E-cadherin, α-catenin and β-catenin, since this would be more useful in daily clinical practice. Therefore we conducted several Cox regression analyses for E-cadherin, and similarly for α- and β-catenin, each time analysing the factor with a different cut-off point. Based on the change in log likelihood, the dichotomous scoring system with the strongest prognostic power was determined for each factor. The optimal cut-offs were found to be between a total score of 4 and 5, thus leading to the definition of preserved expression of E-cadherin, α-catenin and β-catenin when the total staining score was 5 or more, and decreased expression when the score was 4 or less. We have combined FIGO grades 1 and 2 in the outcome analyses of the current study, because in previous studies recurrence and survival rates for patients with grade 1 and grade 2 tumours were found to be comparable, and the reproducibility of grade 2 was shown to be poor.[8,13] All *p*-values are based on two-sided tests, with $p < 0.05$ considered statistically significant.

Table 1. Staining scores of E -cadherin and α-, and β-catenin, and combined scores*

	E-cadherin	**α-catenin**	**β-catenin**	**Combined score***
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Staining score				
7	14 (6)	6 (2.5)	17 (8)	
6	44 (19)	60 (26)	62 (27)	
5	67 (30)	54 (24)	71 (32)	
preserved expression	**125 (56)**	**120 (53)**	**150 (67)**	**69 (31)**
4	58 (26)	67 (30)	66 (29)	
3	34 (15)	33 (15)	9 (4)	
2	6 (3)	4 (2)	0	
0	2 (1)	1 (0.5)	0	
decreased expression	**100 (44)**	**105 (47)**	**75 (33)**	**156 (69)**
Total	**225 (100)**	**225 (100)**	**225 (100)**	**225 (100)**

* Combined score: preserved expression = preserved expression of E-cadherin, α-catenin, AND β-catenin

decreased expression = decreased expression of E-cadherin, α-catenin, OR β-catenin

Table 2. Correlations between E-cadherin, and α- and β-catenin and the histological grade

		Histological grade			
	Total	**1**	**2**	**3**	
Cell adhesion molecule	***n* (%)**	***n* (%)**	***n* (%)**	**N (%)**	***p*-value**
E-cadherin expression					
preserved	125 (56)	99 (44)	7 (3)	19 (9)	
decreased	100 (44)	55 (24)	9 (4)	36 (16)	< 0.001
α-catenin expression					
preserved	120 (53)	89 (40)	10 (4)	21 (9)	
decreased	105 (47)	65 (29)	6 (3)	34 (15)	< 0.001
β-catenin expression					
preserved	150 (67)	116 (52)	10 (4)	24 (11)	
decreased	75 (33)	38 (17)	6 (2)	31 (14)	< 0.001

Results

Staining results

Total staining scores of E-cadherin, α-catenin and β-catenin are shown in Table 1. For all factors there was a strong correlation between the staining intensity and the proportion of positive tumour cells ($p \leq 0.003$). Preserved E-cadherin, α- and β-catenin expression, defined as a staining score of 5 or more, was found in 125 (56%), 120 (53%) and 150 (67%) tumours, respectively. There was a strong correlation between the expression of E-cadherin, α-catenin and β-catenin. Among the tumours with decreased E-cadherin expression (staining score of 4 or less), significantly more tumours showed decreased α-catenin expression (61% of tumours with decreased E-cadherin versus 35% of tumours with preserved E-cadherin expression, $p < 0.001$) and decreased β-catenin expression (50% versus 20%, $p < 0.001$). There was also a strong correlation between α- and β-catenin expression ($p < 0.001$). Combining the E-cadherin, α-catenin and β-catenin scores resulted into 69 cases (31%) with preserved expression of all three factors. Among the 156 cases with decreased expression of one or more factors, there were 68 cases with decreased expression of one factor (44%), 52 cases with decreased expression of two factors (33%) and 36 cases with decreased expression of all three factors (23%).

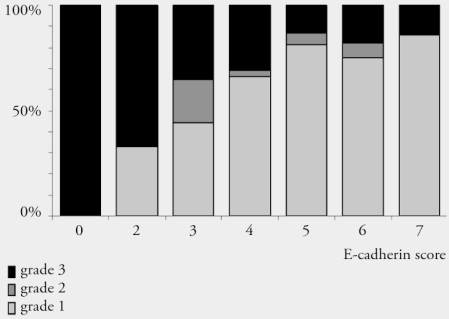

Figure 2. Distribution of histological grades according to the staining score of E-cadherin.

Correlations

A significant inverse correlation between the staining score of E-cadherin, α- and β-catenin and the histological grade was demonstrated (Table 2). The distribution of histological grades according to the staining score of E-cadherin (score 0-7) is shown in Figure 2. Expression of E-cadherin, α-catenin and β-catenin was not correlated to the pattern or the depth of myometrial invasion or stage. Tumours with an E-cadherin score of 0, 2, 3, 4, 5, 6 and 7, showed deep myometrial invasion in 100%, 33%, 68%, 62%, 61%, 64% and 57%, respectively, i.e. 62% of tumours with preserved E-cadherin and 63% of tumours with decreased E-cadherin expression had deep myometrial invasion ($p = 0.68$). Decreased E-cadherin expression was significantly more often observed in non-endometrioid type carcinomas (NEECs) than in endometrioid endometrial carcinomas (EECs): 75% (9/12) and 43% (91/213), respectively, ($p = 0.04$).

Table 3. Univariate (UV) and multivariate (MV) analyses of prognostic factors for endometrial cancer related death, for patients with grade 1-2 tumour

		UV	MV	
		***p*-value**	**HR* (95% CI)**	***p*-value**
FIGO stage	**II**		5.0 (1.9-13.3)	0.001
	III	< 0.001	5.7 (1.5-21.3)	0.009
Pattern of myometrial invasion	**infiltrating**	0.03	3.1 (1.0-9.4)	0.04
Depth of myometrial invasion	**≥ 50%**	0.12	1.5 (0.5-4.0)	0.48
E-cadherin, α-catenin and β-catenin	**decreased expression**	0.02	4.3 (1.2-14.8)	0.02

HR: hazard ratio; CI: confidence interval

* Hazard ratios for stage II and III compared with stage I; an infiltrating pattern of invasion compared with pushing borders; deep myometrial invasion (≥ 50%) compared with superficial invasion (< 50%); decreased expression of E-cadherin, α- and/or β-catenin compared with preserved expression of all three factors

Outcome

In univariate analyses, E-cadherin, α-catenin and β-catenin was all found to have prognostic significance, with decreased expression leading to decreased cancer-specific survival rates (CSS) (p = 0.008, 0.03 and 0.03, respectively). Five- and 10-year survival rates were 88% and 88% for patients with tumours showing preserved E-cadherin expression, versus 79% and 71% for those with decreased E-cadherin expression. Similar differences in survival rates were found for the catenin scores. Other significant prognostic factors for CSS were histological grade (p < 0.001), histological subtype (p < 0.001), stage (p < 0.001) and the pattern of myometrial invasion (p = 0.01). In multivariate analysis, however, E-cadherin, α- and β-catenin lost their statistical significance (p = 0.14, 0.17 and 0.32, respectively), and the significant independent prognostic factors for CSS were found to be histological grade and stage. When combining the scores of E-cadherin, α-catenin and β-catenin, we found that tumours with preserved expression of all three factors had significantly better CSS rates than tumours that with decreased expression of one or more factors (5-year CSS 91% and 81%, respectively, p = 0.02), although in multivariate analysis this statistical significance was lost (p = 0.06). No significant differences were found when comparing outcomes for tumours with decreased expression of one, two or three factors (details not shown). Among the patients with grade 1-2 tumours (n = 170), those with a tumour that had preserved expression of E-cadherin, α-catenin and β-catenin had a significantly better survival than patients with tumours with decreased expression of one or more of these factors (5-year CSS 97% and 89%, respectively, p = 0.02, Figure 3). This remained significant in multivariate analysis (p = 0.02, Table 3). The prognosis for patients with a grade 3 tumour (n = 55) was not influ-

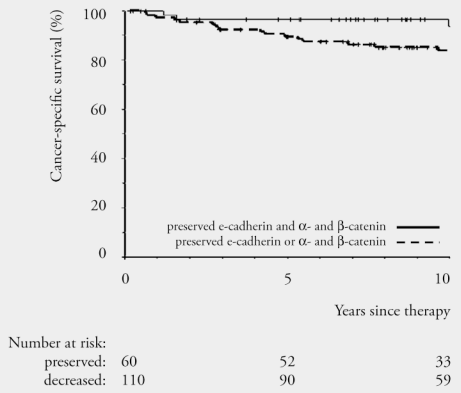

Figure 3. Cancer-specific survival in patients with a grade 1-2 tumour, according to combined E-cadherin, α- and β-catenin expression (p = 0.02).

enced by the combined expression of E-cadherin, α-catenin and β-catenin. Comparable results were obtained when analysing only stage I grade 1-2 tumours. Among these patients (n = 132), those with a tumour that showed preserved expression of E-cadherin, α-catenin and β-catenin had a significantly better survival than those with tumours that showed decreased expression of one or more of these factors (5-year CSS 100% and 93%, respectively, p = 0.02).

Discussion

In this series of 225 endometrial cancers, decreased E-cadherin expression was found in 100 tumours (44%). There was a strong correlation between expression of E-cadherin and expression of α- and β-catenin, which might have been expected since both catenins are necessary for a properly functioning E-cadherin.[1] Previously reported percentages of decreased or negative E-cadherin expression in endometrial carcinoma vary from 11% to 60%.[5-7,12] There are several possible explanations for this wide range of incidences. First, the relatively small size of most series (30 to 113 cases studied). Second, the use of different scoring systems, and different cut-off points. Generally, methods in which the intensity and quantity of E-cadherin expression are combined, are considered a more relevant evaluation of E-cadherin expression than intensity or proportion scores alone.[14-16] We have therefore scored both the intensity and the proportion of immunopositivity adding up to a total staining score of 0-7. We have chosen the cut-off values after analyses of the prognostic power of the resulting scoring systems, and thus tumours with a staining score of 4 or less were defined as having decreased expression, and tumours with a staining score of 5 or more as having preserved expression of E-cadherin, α-catenin or β-catenin. In contrast, Holcomb *et al* [7] considered tumours to be E-cadherin negative when less than 4% of the tumour cells were positive (in their series 11% were negative). Leblanc *et al* [6] reported 27% of endometrial carcinomas to have less than 10% positive cells. Sakuragi *et al* [5] divided endometrial cancers in three categories, with positive, heterogeneous or negative E-cadherin expression, and reported heterogeneous or negative

expression in 60% of the tumours. Moreno-Bueno *et al* [12] scored both the staining intensity and the percentage of positive tumour cells, however, they defined decreased expression as having a score of 5 or less, but did not explain why they chose this cut-off point. Decreased expression of E-cadherin was observed in 60% of tumours. A third reason for the wide range of reported E-cadherin expression rates is the difference between the studies in the proportion of endometrioid type (EEC) and non-endometrioid type carcinomas (NEEC). In our study, including 12 (5%) NEECs, significantly more NEECs showed decreased expression of E-cadherin as compared to EECs, 75% versus 43% (p = 0.04). In the study by Moreno-Bueno *et al* [12] a higher proportion (27%) of all cases were NEECs, which was most likely the major cause of the higher rate of decreased E-cadherin expression found in their study (60% versus 44% in ours). In other cancers, e.g. breast and gastric cancer, the association between histological subtype and expression of E-cadherin has been demonstrated as well, with decreased expression occurring more often in lobular than in ductal carcinomas of the breast,[17,18] and in diffuse type gastric cancer.[19] Studies on endometrial carcinoma are less conclusive. One study, comparing E-cadherin expression in 10 papillary serous carcinomas and 14 papillary endometrioid carcinomas, found no difference between these histological subtypes.[20] Another study, comparing 17 EECs and 17 serous carcinomas, found E-cadherin expression to be more prominent in serous carcinomas than in EECs,[21] whereas a third larger study analysing 113 endometrial carcinomas found, similar to our results, that decreased expression of E-cadherin significantly more often occurred in NEECs than in EECs (87% versus 50%).[12] This difference of E-cadherin expression between different histological subtypes is in concordance with previous studies, which showed that different molecular abnormalities were involved in carcinogenesis of EECs and NEECs.[22]

Since cell adhesion molecules such as E-cadherin are needed to retain adhesion between neighbouring cells, it has been suggested that an impaired function of E-cadherin could lead to invasive potential in malignant epithelial tumours. This was confirmed in an experimental study that showed in a variety of human cancer cell lines that non-invasive cell lines expressed E-cadherin protein, whereas invasive carcinoma cell lines had lost E-cadherin expression.[3] In vivo, however, reduced E-cadherin expression has been demonstrated in lobular carcinoma in situ of the breast[23] and in atypical endometrial hyperplasia.[12] This indicates that decreased expression of E-cadherin is not purely a predictor of invasiveness, but can also occur in non-invasive lesions. We did not find a correlation between decreased expression of E-cadherin and deeper myometrial invasion or a more infiltrating pattern of invasion. Some studies on endometrial carcinoma did[5] and some did not[7] show a correlation between decreased expression of E-cadherin and deeper myometrial invasion. It seems that a complex biological process such as stromal invasion is not regulated by a single molecular alteration, but caused by a sequence of changes, including alterations in several cell adhesion molecules.

Cadherins not only function as cell-cell adhesion molecules, but are also somehow involved in the process of differentiation, although so far the underlying mechanism is unclear.[2,3] In

endometrial carcinoma a correlation between the expression of E-cadherin and the histological grade has been shown.[5-7] We confirmed this strong correlation between decreased E-cadherin, α- or β-catenin expression and histological grade ($p < 0.001$). Preserved expression of E-cadherin was significantly more often observed in grade 1 tumours, predominantly consisting of glands, than in grade 3 tumours, with areas of solid fields. Thus decreased expression of E-cadherin is associated with a diffuse growth pattern, lacking tubular or gland formation, just as in lobular breast cancer[17,18] and diffuse type gastric cancer.[19] This supports the theory that E-cadherin may somehow be involved in the process of gland formation.

E-cadherin expression has been shown to be an independent prognostic factor in breast cancer.[16] So far, in endometrial cancer no independent prognostic power of E-cadherin has been reported. We demonstrated that patients with a tumour that showed combined preserved E-cadherin, α-catenin and β-catenin expression had a better prognosis than patients with decreased expression of one of these factors. No significant difference was found when comparing outcome for tumours with decreased expression of respectively one, two or three factors. This underlines the fact that these three factors are closely related, and that a defect in one of these factors may already cause a disturbance in their function. Since E-cadherin, α-catenin and β-catenin expression was correlated to histological grade, which is known to be a strong independent prognosticator,[8,13] we further analysed their prognostic significance in grade 1-2 tumours as opposed to grade 3 tumours. It was found that within the group of patients with a grade 1-2 tumour ($n = 170$), combined preserved E-cadherin, α-catenin and β-catenin expression was an independent prognostic factor for survival (5-year CSS 97% versus 89%, hazard ratio: 4.3, $p = 0.02$).

In the treatment of patients with stage I endometrial carcinoma, the indication for postoperative radiotherapy is tailored to the prognostic factors histological grade 3, deep myometrial invasion and advanced age.[13] Within the subset of patients with a stage I, grade 1-2 tumour, we found that none of the patients with preserved expression of E-cadherin, α-catenin and β-catenin recurred within the observed period (minimum follow-up 6 years). This might imply that analysis of the expression pattern of these factors could be used to identify patients with such a favourable prognosis that adjuvant treatment could be omitted. Larger, prospective studies of E-cadherin and catenin expression would be needed to confirm this.

In conclusion, this study shows in a large group of endometrial carcinomas, with a long follow-up, that decreased E-cadherin expression is associated with histological grade 3, and is more often seen in NEECs than in EECs. Furthermore, combined E-cadherin, α- and β-catenin expression was shown to be an independent prognostic factor for survival. Within the group of patients with a grade 1-2 tumour, preserved expression of all three factors identified a subset of patients with favourable outcome.

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Chapter 6

Nuclear β-catenin is a molecular feature of type I endometrial carcinoma

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Abstract

Purpose: Two types of endometrial carcinoma can be distinguished: type I tumours, which are oestrogen-related and are typically low-grade endometrioid carcinomas; and type II tumours, which are unrelated to oestrogen stimulation and are often non-endometrioid carcinomas. The molecular abnormalities involved in carcinogenesis appear to be different for these tumour types. The aim of this study was to test the hypothesis that an abnormality in the Wnt/β-catenin signalling pathway is a molecular feature of type I endometrial carcinoma.

Materials and methods: This study investigated nuclear β-catenin by immunohistochemistry in 233 endometrial carcinomas and analysed its correlations with several immunohistochemical, histological, and clinical parameters, such as proliferation rate (Ki-67), expression of oestrogen and progesterone receptors, and survival.

Results: Nuclear β-catenin expression was observed in 39 cases (16%). All tumours expressing nuclear β-catenin were endometrioid adenocarcinomas, were significantly better differentiated, and were more often hormone receptor-positive than tumours without nuclear β-catenin. No correlation with proliferation rate was found.

Conclusions: It was found that several features of type I endometrial carcinoma occur significantly more often in tumours expressing nuclear β-catenin, suggesting that an abnormality in the Wnt/β-catenin signalling pathway, resulting in nuclear β-catenin immunopositivity, is a molecular feature of a subset of type I endometrial carcinomas.

Introduction

β-catenin was originally identified as a component of the cadherin-mediated intercellular adhesion system.[1] Subsequent studies have demonstrated that it also functions as a downstream transcriptional factor in the Wnt signalling pathway, functioning as an oncogene.[2] In normal epithelial cells, in the absence of a Wnt signal, a cytoplasmic complex comprising β-catenin, adenomatous polyposis coli (APC), axin, and GSK-3β mediates the phosphorylation of β-catenin and, as a consequence, the targeted degradation of this protein via the ubiquitin-proteasome pathway.[3,4] Mutations of genes affecting this complex (e.g. β-catenin in endometrial carcinoma[5-7] or, in the case of colon cancer, mutation of APC[8]) severely impair the down-regulation of β-catenin, which leads to an excess of cytoplasmic protein. This results in concomitant translocation of β-catenin into the nucleus, where it can form transcriptionally active complexes with T-cell factor/lymphoid enhancer factor (Tcf/Lef), resulting in the activation of several genes, including c-myc and cyclin D1[9,10] (Figure 1). In colon carcinoma cells, this has been shown to result in uncontrolled cellular proliferation and polyp formation.[8,11,12]

Only a few studies have focused on the significance of nuclear β-catenin in endometrial cancer.[5,6,13] In 1983, Bokhman was the first to describe two different types of endometrial carcinoma.[14] Type I tumours are oestrogen-related, often preceded by a pre-cancerous condition (hyperplasia), and are typically low-grade endometrioid carcinomas of the endometrium (EECs). They usually develop in pre- and peri-menopausal women and in general have a good prognosis. On the other hand, type II tumours are unrelated to oestrogen stimulation, mostly developing in atrophic endometrium, presumably preceded by endometrial intraepithelial carcinoma (EIC), and are often papillary serous and clear cell carcinomas, i.e. non-endometrioid endometrial carcinomas (NEECs). They affect older women and usually have a poor clinical outcome.[15] The molecular abnormalities involved in the development of endometrial carcinomas appear to be different for both types of tumour. In type II carcinomas, p53 mutations have been reported in both the invasive and the intraepithelial components in up to 90% of cases.[16,17] No molecular abnormality has been found to be responsible for the majority of tumours of the endometrioid phenotype. The type I tumour category seems to encompass a more heterogeneous group of tumours, with different molecular alterations, such as PTEN mutations and microsatellite instability, being reported to occur during tumorigenesis.[18] In this study, we have analysed correlations of nuclear β-catenin with several immunohistochemical, histological, and clinical parameters, such as the proliferation rate, the expression of the oestrogen (ER) and progesterone (PR) receptors, and survival, in a large series of 233 endometrial tumour samples. The aim of the study was to test the hypothesis that abnormalities in the Wnt/β-catenin signalling pathway occur only in type I endometrial carcinoma. The following questions were addressed: Are tumours expressing nuclear β-catenin endometrioid carcinomas? Are they mostly well-differentiated tumours, with less myometrial invasion? Do they present at a lower stage? Are these tumours more often hormone receptor-positive, affecting predominantly pre-menopausal women? And, finally, is the prognosis for patients with a tumour expressing nuclear β-catenin better?

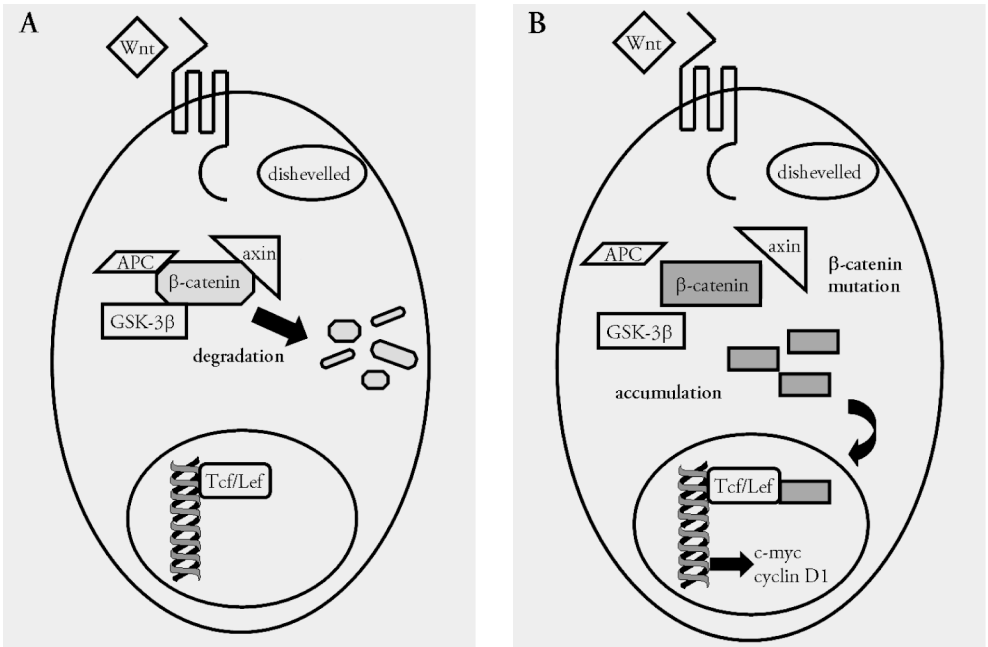

Figure 1. Wnt/β-catenin pathway. (A) In the absence of a Wnt signal, β-catenin is degraded through interaction with a complex comprising APC, GSK-3β and axin. (B) Mutations of β-catenin suppress the degradation of β-catenin, leading to the accumulation of β-catenin in the cytoplasm and nucleus and activation of Tcf/Lef target genes, such as c-myc and cyclin D1.

Materials and methods

Tissue samples

Between January 1984 and December 1993, 253 patients with endometrial cancer were treated with surgery and radiotherapy at the Leiden University Medical Center. Patient and tumour characteristics have been published previously.[19] In 233 cases (92%), formalin-fixed, paraffin-embedded blocks from the tumour could be obtained for immunohistochemical analyses.

Immunohistochemistry

Paraffin sections (4μm) were mounted on saline-coated slides. Immunohistochemistry was performed with the MARK 5 Immunostainer (DPC, Breda, The Netherlands). The primary antibodies used in this study were anti-β-catenin, clone 14 (1:1000; Transduction Laboratories), anti-Ki-67, clone MIB-1 (1:200; DAKO, Glostrup, Denmark), anti-oestrogen

receptor, clone 1D5, (1:100; DAKO) and anti-progesterone receptor (1:100; DAKO). All the antibodies were monoclonal in type except the progesterone receptor antibody, which was a rabbit polyclonal. Prior to incubation, all sections were subjected to antigen retrieval in a microwave with a boiling solution of 10 mM citrate buffer, pH 6.0, for 10 minutes. Bound primary antibodies were visualized with subsequent incubations with biotinylated polyvalent IgG and HRP-labelled streptavidin (Ultravision, Labvision, Fremont, USA), followed by 0.05% diaminobenzidine and 0.0015% hydrogen peroxide. Slides were briefly counterstained in Mayer's haematoxylin. As negative controls, irrelevant IgGs at the same protein concentration as the primary antibody were used instead of the primary antibody. As positive controls, sections of skin (β-catenin), tonsil (Ki-67), and breast carcinoma (ER and PR) were used.

Scoring

Specimens were scored by one observer (A.S.), who was blinded to the patients' outcome and other histological and non-histological covariates. β-catenin slides were scored for the presence or absence of a nuclear staining pattern. In accordance with a previous study,[7] nuclear staining was considered positive when at least 1% of the cells showed nuclear immunopositivity. Furthermore, the degree of membranous β-catenin staining was determined. Both the intensity of the membranous staining (staining intensity, SI) and the proportion of positive tumour cells (positive tumour cells, PTCs) were scored; the SI could vary from 0 (none), 1 (weak), 2 (moderate) to 3 (strong) and the PTCs varied from 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) to 4 (> 75%). The degree of membranous staining (staining score) was calculated as the sum of SI and PTC (range 0, 2-7). On the ER and PR slides, the tumour was judged to be positive when at least one area showed clear positive nuclear staining. The proliferation rate was evaluated quantitatively, by counting the number of nuclei positive for Ki-67 per 200 cells, in five different high-power fields (200x).

Statistical analysis

Survival rates were calculated using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test.[19] Correlations between nuclear β-catenin and other parameters were analysed using the chi-square test. Ki-67 was analysed semi-quantitatively, dividing the scores into quartiles. All *p*-values are based on two-sided tests, with $p < 0.05$ considered statistically significant.

Results

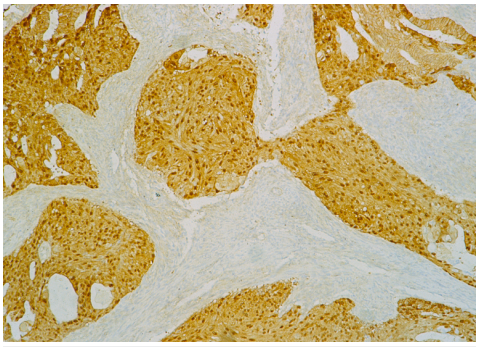

Figure 2. Nuclear β-catenin staining in a well-differentiated endometrioid-type adenocarcinoma with abundant squamous metaplasia.

Nuclear β-catenin expression was observed in 39 of the 233 cases (16%). In many of these cases, the nuclear staining was most prominent in areas of squamous metaplasia (Figure 2). All 233 tumours showed some degree of membranous staining, reflecting the function of β-catenin in the cadherin-mediated intercellular adhesion system.

We analysed whether a correlation existed between nuclear β-catenin expression and the degree of membranous staining, as determined by the combination of the staining intensity and the proportion of positive tumour cells. Decreased membranous staining, defined as a staining score of 4 or less, was found in 33% of tumours with nuclear β-catenin and in 48% of tumours without nuclear β-catenin staining. This difference was not statistically significant (p = 0.09). The presence of nuclear β-catenin staining did not correlate with the rate of proliferation, as determined by the percentage of positive Ki-67 cells (X^2 = 5.666, p = 0.34). Several differences were observed between the tumours with and those without nuclear β-catenin staining (Table 1).

All 39 tumours with nuclear β-catenin were endometrioid adenocarcinomas (EECs). In the group without nuclear β-catenin, 6% (n = 12) consisted of non-endometrioid type carcinomas (NEECs) [4 malignant mixed Müllerian tumours (MMMTs) and 8 papillary serous carcinomas]. This difference in histological subtypes between the two groups was not statistically significant. Considering the very small number of NEECs (n = 12), these were excluded from further analyses, which were focused on the comparison of the EECs with and without nuclear β-catenin. There was a significant difference in histological grade between EECs with and without nuclear β-catenin. In the group of tumours expressing nuclear β-catenin, 90% were grade 1 and 8% grade 3, in contrast to 68% grade 1 and 23% grade 3 in the group without nuclear β-catenin (p = 0.02). Patients with tumours showing nuclear β-catenin-expression were significantly more often pre-menopausal (15% vs. 4%, p = 0.01) and had somewhat higher survival rates, although this difference was not statistically significant (5- and 10-year cancer-specific survival rates of 92% and 87%, vs. 85% and 82%, respectively, p = 0.78).

The number of tumours with a positive hormone receptor was significantly higher in the EECs with nuclear β-catenin: 82% were ER-positive and 77% PR-positive, compared with 56% (p = 0.003) and 46% (p = 0.001) in the group without nuclear β-catenin. No significant correlation was found between nuclear β-catenin staining and tumour stage, or between nuclear β-catenin and the depth of myometrial invasion.

Table 1. Correlations between the pattern of β-catenin staining and several histological and non-histological parameters

	β-catenin		
	nuclear staining *n* (%)	**no nuclear staining *n* (%)**	***p*-value**
Tumour grade			
1	35 (90)	123 (68)	
2	1 (2)	17 (9)	
3	3 (8)	42 (23)	0.02
FIGO stage			
I	28 (72)	140 (77)	
II	8 (20)	22 (12)	
III	3 (8)	20 (11)	0.35
Myometrial invasion			
< 50%	12 (31)	72 (40)	
≥ 50%	27 (69)	110 (60)	0.31
Menopausal state			
pre-menopausal	6 (15)	8 (4)	
post-menopausal	33 (85)	174 (96)	0.01
Estrogen Receptor			
positive	32 (82)	97 (56)	
negative	7 (18)	76 (44)	0.003
Progesterone Receptor			
positive	30 (77)	81 (46)	
negative	9 (23)	94 (54)	0.001
Cancer-specific survival			
5-year (%)	92	85	
10-year (%)	87	82	0.78

The hormone receptors were statistically significant prognostic factors for survival. Patients with an ER-positive EEC (129/212 tumours, 61%) had a 5-year cancer-specific survival rate of 89%, as opposed to 80% for those with ER-negative tumours ($p = 0.003$). Women with a PR-positive positive tumour (111/214 tumours, 52%) had a 5-year cancer-specific survival rate of 91%, versus 80% for those with PR-negative tumours ($p = 0.02$). There was a strong correlation between both hormone receptors: 43% of all cases were positive for both receptors, 30% were negative for both receptors, and only 27% were positive for just one of the hormone receptors ($p < 0.001$). Patients who had a tumour with a high proliferation rate had a lower cancer-specific survival rate than those with less proliferating tumours, but the differ-

ence in survival rate was not statistically significant. When dividing tumours into groups with 0-25%, 26-50%, 51-75% and 76-100% cells positive for Ki-67, the 5-year cancer-specific survival rates were 87%, 87%, 75% and 75%, respectively (p = 0.61).

Discussion

In this series of 233 endometrial carcinomas, nuclear β-catenin expression was found in 39 of 233 tumours (16%), which is in accordance with the percentages reported in other studies, varying from 13 to 44%.[5-7,18,20-23] In the positive cases, nuclear β-catenin staining was most prominent in squamous metaplastic areas, an observation that has previously been reported by Saegusa and Okayasu.[23] The biological meaning of this phenomenon remains to be clarified, especially since there were also well-differentiated tumours with abundant squamous metaplastic areas that did not show nuclear β-catenin staining. In normal epithelial cells, abnormalities in the Wnt/β-catenin signalling pathway, leading to a nuclear β-catenin staining pattern, result in the activation of several genes, including c-myc and cyclin D1.[9,10] In colon carcinoma cells, this has been shown to cause uncontrolled cellular proliferation.[8] In normal endometrium, nuclear β-catenin has been observed during the mid- and late proliferative phase of the menstrual cycle, which supports the idea that the nuclear localization of β-catenin indicates a condition in which cell proliferation signals are activated in the endometrium.[20] We therefore analysed whether tumours with nuclear β-catenin showed a higher proliferation rate than tumours without nuclear β-catenin. However, we did not find such a correlation. This is probably due to the fact that in all malignant tumours, by definition, there is an imbalance between the rate of proliferation and the amount of cell loss. Apparently, in this study, the type of genetic abnormality causing this imbalance (an abnormality in the Wnt/β-catenin signalling pathway or some other abnormality) did not result in a significantly different proliferation rate. β-catenin mutations and the subsequent nuclear accumulation of β-catenin protein have only been found in the endometrioid type of endometrial carcinomas.[18,22] We therefore hypothesized that abnormalities in the Wnt/β-catenin signalling pathway occur only in type I endometrial carcinoma. The aim of this study was to test this hypothesis in a large series of endometrial carcinomas, by analysing different features of type I carcinoma in tumours with and tumours without nuclear β-catenin. We found that all tumours with nuclear β-catenin were endometrioid adenocarcinomas (EECs). The same observation was made by Palacios *et al*,[18] who analysed the presence of nuclear β-catenin in 40 endometrial carcinomas. Thirteen expressed nuclear β-catenin and, similar to our observation, all were EECs, in contrast to tumours without nuclear β-catenin, of which nine (33%) were non-endometrioid type carcinomas (NEECs). The difference in histological subtype that we observed in our study, i.e. 0% (0/39) NEECs in tumours with nuclear β-catenin, versus 6%

(12/194) NEECs in tumours without nuclear β-catenin, was not statistically significant. This is probably due to the small number of NEECs (*n* = 12) in our study. Considering the very small number of NEECs, these were excluded from further analyses, which were focused on comparison of the EECs with and without nuclear β-catenin. We found that EECs with nuclear β-catenin were significantly better differentiated than tumours without nuclear β-catenin: 90% of the tumours with nuclear β-catenin were well differentiated. Experimental studies have shown that the Wnt/β-catenin signalling pathway is involved in several processes concerning morphological differentiation. It has an important role during embryogenesis and stem cell differentiation,[8,24] and tcf-1 (a downstream factor in the Wnt/β-catenin signalling pathway) has been found to be essential for T-cell differentiation.[25] This might explain why tumours with nuclear β-catenin are predominantly well differentiated. We found patients who had EECs with nuclear β-catenin to be significantly more often pre-menopausal than those without nuclear β-catenin. Furthermore, tumours with nuclear β-catenin were significantly more often PR- and ER-positive than tumours without nuclear β-catenin. Nei *et al* [20] have looked at β-catenin in normal endometrium, endometrial hyperplasia, and endometrial cancer samples. They found no correlation between the intensity of ER and PR staining and the nuclear staining of β-catenin in hyperplasia and cancer. However, in normal endometrium, they found a tendency for the samples in the proliferative phase, with strong staining for ER, to show nuclear staining for β-catenin. Furthermore, oestrogens have been reported to stimulate resting cells (G_0 phase) to enter the cell cycle, a process that is preceded by increased expression of c-myc and cyclin D1,[26] two of the genes that are also activated in the Wnt/β-catenin signalling pathway. This suggests that the Wnt/β-catenin signalling pathway can be stimulated by serum oestrogen, but it might also just be that both pathways converge towards a common step that includes up-regulation of cyclin D1 and c-myc to promote proliferation.[21] Although tumours with nuclear β-catenin were more often well differentiated and hormone receptor-positive, we did not find a significant difference in prognosis. The cancer-specific survival rates were better for patients with tumours expressing nuclear β-catenin than for those with tumours without nuclear β-catenin (92% vs. 85% at 5 years, respectively), but this difference was not statistically significant. This may be related to the relatively small number of tumours expressing nuclear β-catenin (39/221).

In conclusion, we found several features of type I endometrial carcinoma to occur significantly more often in EECs with nuclear β-catenin than in EECs without nuclear expression: well-differentiated tumours, expression of hormone receptors and pre-menopausal status. These results strongly suggest that abnormalities in the Wnt/β-catenin signalling pathway, resulting in a nuclear β-catenin staining pattern, are molecular features of a subset of type I endometrial carcinomas. Although the number of NEECs (type II) in this series is relatively small, the absence of nuclear β-catenin staining in these cases at least suggests that this molecular event is less important in the development of these carcinomas. We are currently investigating whether nuclear β-catenin develops together with or apart from other molecular

abnormalities in type I carcinomas. More research into this area might enable us to divide the large, heterogeneous group of type I endometrial carcinomas into several subgroups, each with its specific molecular abnormalities and clinical aspects.

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Chapter 7

Summary and general discussion

Summary and general discussion

Endometrial cancer is the most common malignancy of the female genital tract in Western countries, affecting around 1500 new patients per year in the Netherlands. After breast, colorectal, and lung cancer, endometrial cancer is the fourth most common invasive tumour in Dutch women. It accounts for about 5% of all tumours. The prognosis of endometrial cancer patients depends on several clinical and pathological factors, such as age, International Federation of Gynaecology and Obstetrics (FIGO) stage, depth of myometrial invasion, histological subtype, lymph vascular space invasion and histological grade. In general, patients with endometrial carcinoma have a good prognosis, since most patients (75-80%) present with early-stage disease (FIGO stage I). Five-year survival rates for FIGO stage I endometrial cancer are 80-90%. In the Netherlands, about 300 women per year die of the disease, mainly of distant metastases. Standard treatment consists of a total abdominal hysterectomy with bilateral salpingo-oophorectomy. The indication for postoperative pelvic radiotherapy is tailored to prognostic factors. The purpose of this thesis was to evaluate the significance of these prognostic factors in endometrial carcinoma and to investigate the incidence and significance of molecular abnormalities and their potential role as prognostic factors for clinical practice.

In this thesis the patient data and histology specimens of two patient cohorts have been analysed. First, the data and specimens of a retrospective study from the Leiden University Medical Center (LUMC) were analysed. Secondly, data and histology specimens from the multicentre Post Operative Radiation Therapy in Endometrial Carcinoma (PORTEC) trial were analysed. In **chapter 2** long-term outcome of the patients from the LUMC study is reported. This study included 253 patients with endometrial carcinoma stages I to III. The histological slides were reviewed and the prognostic value of stage, age, histological grade, histological subtype, depth of myometrial invasion (less than 50% vs. 50% or more) and pattern of invasion (infiltrating pattern vs. pushing borders) was analysed. Median follow-up was 11.7 years. Overall and cancer-specific (CSS) actuarial survival rates at 5 years were 77% and 85%, and 10-year survival rates were 64% and 82%, respectively. The 5-year rates of locoregional

recurrences and distant metastases were 7% and 15%. These results are in accordance with rates reported in previous studies.[1-4] In multivariate analysis, stage (hazard ratios [HRs]: 3.4 for stage II, 3.7 for stage III), histological grade (HR: 3.6 for grade 3), age (HR: 2.4 for age 60 years or over), and pattern of myometrial invasion (HR: 2.2 for infiltrating pattern) had independent prognostic significance for CSS. In 1988 Mittal and Barwick[5] were the first to describe the prognostic significance of the pattern of myometrial invasion. They reported that patients with a diffusely infiltrating pattern of growth were at increased risk of death from endometrial carcinoma. Five-year mortality of the five patients with a diffusely infiltrating pattern was 40%, compared with 13.3% mortality at five years for a control group of 15 patients with carcinomas without this diffusely infiltrating pattern. However, this difference was not statistically significant,[6] most likely due to the small numbers in this study. The results of three subsequent studies on the prognostic value of the pattern of myometrial invasion are not uniform.[7-9] Two studies did find the pattern of myometrial invasion to have prognostic power,[7,9] one study did not find a difference in recurrence or survival rates according to the pattern of myometrial invasion.[8] In other malignancies, such as cervical cancer[10] and several head and neck malignancies,[11,12] the pattern of invasion of the surrounding stromal tissue has been found to be a significant prognostic factor for survival. The number of studies that have focussed on the prognostic significance of the pattern of invasion in endometrial cancer is small, as well as the number of patients in these studies (n = 93-112). Furthermore, the definition of an infiltrating pattern of invasion differs between the studies. In our study, the pattern of invasion was defined as infiltrative if glands or tumour cells haphazardly infiltrated the myometrium, by which there was no longer a more or less smooth border between tumour and myometrium, even when this happened in only a small part of the tumour. This pattern was found to be a strong independent prognosticator for a decreased CSS. The depth of invasion, on the other hand, had only borderline prognostic significance in univariate analysis (p = 0.07), and lost its statistical significance in multivariate analysis. In theory, deep invasion into the outer part (outer 50% to 33%) of the myometrial width increases the risk of vessel invasion, since the major afferent lymph and blood vessels of the myometrium are located in the subserosal areas. However, when a tumour invades the myometrium for more than 50% with an expansive growth pattern, it pushes the myometrium away rather than infiltrating it, without reaching the vessels in the outer part of the myometrium, and thus without an increased risk of regional or distant spread. That might explain the observed superior prognostic power of the pattern of myometrial invasion as compared to the depth of invasion. Furthermore, determination of the depth of myometrial invasion can be difficult, and depends on the way the macroscopic specimen has been processed, which may be subject to sampling errors. Determination of the pattern of myometrial invasion is less dependent on the way the macroscopic specimen has been processed, and might therefore be more reproducible. Since it has strong prognostic power, the pattern of myometrial invasion is a good alternative for the depth of myometrial invasion as prognostic factor for clinical practice. At pathology review, a

shift from grade 2 to grade 1 was seen in 112 of the 144 grade 2 tumours (78%). There are several features of the FIGO grading system that may be responsible for this considerable shift of the tumour grade at revision. The FIGO grading system is based on the extent of nonsquamous solid growth and on nuclear atypia.[13] Grade 1 tumours have 5% or less areas of solid growth, grade 2 6% to 50%, and grade 3 more than 50% solid growth. In case of marked nuclear atypia, a grade 1 or 2 is raised by one grade. Distinguishing between squamous and nonsquamous solid growth can be difficult. Furthermore, determining whether the percentage of nonsquamous solid growth is more or less than 5% is somewhat arbitrary. And the determination of the degree of nuclear atypia is highly subjective, as illustrated by the 35% agreement ($\kappa = 0.22$) for nuclear grading reported by Lax *et al.*[14] Furthermore, at time of review, slides were consistently scored according to the FIGO 1988 grading criteria, whereas these criteria had not yet been implemented in all hospitals at the time of the patients' treatment (between 1984 and 1993). No difference in CSS between grade 1 and grade 2 was found (94% vs. 90% 5-year CSS for original grade and 92% vs. 94% for grade after review), whereas grade 3 was found to be a significant adverse prognostic factor ($p < 0.001$). A similar shift from grade 2 to grade 1 was observed at pathology review of the PORTEC trial. The PORTEC trial included 715 patients with a stage IC, grade 1 or 2, or stage IB, grade 2 or 3 endometrial carcinoma, of which 714 could be evaluated. The slides of 569 patients (80%) could be obtained for pathology review. Originally, 21%, 68% and 11% of tumours were assigned grade 1, 2 and 3, respectively, while after pathology review, this changed to 69%, 16% and 15%. Eligibility for the trial was based on the grade assigned by the regional pathologist. After surgery, patients were randomised to receive pelvic radiotherapy (RT) or no further treatment. In 2000 the first results of this trial were published. The analysis described in **chapter 3**, with a median follow-up for patients alive of 97 months, was conducted to evaluate long-term outcome and to analyse prognostic factors after central pathology review. The 10-year locoregional relapse rates were 5% and 14% in the RT group and in the control group, respectively ($p < 0.001$). There was no significant survival difference between the treatment arms, with 10-year overall survival rates of 66% in the RT group and 73% in the control group ($p = 0.09$). In multivariate analyses, postoperative radiotherapy was found to be an independent prognostic factor for locoregional control (HR: 3.9; $p < 0.001$). Due to the shift in tumour grade that was observed at pathology review, 134 tumours were diagnosed as grade 1 tumours with superficial myometrial invasion (stage IB), and would not have met the inclusion criteria for the study. After exclusion of these 134 cases from the analyses, the outcome of the trial remained essentially the same, with 10-year recurrence rates of 5% for the RT group and 17% for the control group ($p < 0.001$), and 10-year overall survival rates of 65% and 70%, respectively ($p = 0.23$). This analysis, with long-term follow-up and central pathology review, confirmed the previous PORTEC trial results, that postoperative pelvic radiotherapy for stage I endometrial carcinoma significantly decreases the risk of locoregional recurrence, but does not provide a survival advantage.

In view of the poor reproducibility and limited value of the intermediate grade, as described in chapter 2 and chapter 3, a two-tiered grading system seems preferable. In 2000 Lax *et al* [14] proposed a binary grading system that discriminates between high-grade and low-grade tumours based on the proportion of solid growth (50% or less vs. more than 50%), the pattern of myometrial invasion (infiltrating vs. pushing border), and the presence of tumour cell necrosis. A review of 800 patients with endometrioid adenocarcinoma was conducted (**chapter 4**), in order to compare the interobserver variability, and the clinical utility of the two- and three-tiered grading systems. The prognostic significance and the interobserver variability, of the three-tiered FIGO grading system and the binary grading system, as published by Lax *et al*,[14] were analysed and compared. All slides were reviewed and graded independently by two pathologists. The interobserver agreement for both systems was moderate, with 70% and 73% agreement rates for the FIGO (κ = 0.41) and binary (κ = 0.39) grading systems, respectively. When converting the FIGO grading system into an artificial, two-tiered grading system (grade 3 vs. grades 1-2), the agreement was much better (agreement rate, 85%; κ = 0.58). Of the 3 criteria for the binary grading system, amount of solid growth (less than 50% vs. 50% or more) had the greatest reproducibility (agreement rate, 80%; κ = 0.50). Both the two-tiered FIGO and the binary grading systems were significant predictors of locoregional recurrence, distant recurrence, and cancer-specific survival (hazard ratios [HRs]: 1.7, 2.5, and 2.6, for FIGO and 2.1, 4.1, and 3.8, for the binary grading system). The amount of solid growth was a strong prognostic factor for these three endpoints as well (HRs: 2.4, 3.9, and 3.8, respectively). This simple architectural two-tiered grading system based on the proportion of solid growth (less than 50% vs. 50% or more) had superior prognostic power and greater reproducibility than both the FIGO and the binary grading systems.

Determining the histological tumour grade is an essential part of pathological diagnosis, as the histological grade has prognostic and therapeutic implications. In general, a grading system has to be practical, reproducible and clinically relevant, having clear prognostic power. For a diversity of gynaecological and non-gynaecological malignancies, such as colorectal adenomas,[15] the value of the intermediate grade or the intermediate degree of dysplasia is a matter of debate, and a trend is observed to change traditionally used three-tiered grading systems into two-tiered grading systems. In 1988, in the United States of America, the classification of squamous intraepithelial cervical neoplasia (CIN) was changed from CIN I, CIN II, and CIN III to low-grade squamous intraepithelial lesion (SIL) and high-grade SIL, because of an observed lack of reproducibility.[16] For malignant ovarian germ cell tumours a higher degree of interobserver agreement for a two-tiered grading scale as compared to a three-tiered grading scale was observed (κ = 0.66 and κ = 0.54, respectively), leading to the recommendation of the use of a two-tiered grading system for this malignancy.[17] Furthermore, an analysis of the impact of pathological review in more than 500 gynaecological cancers revealed a change in diagnosis in 33% of cases (including a change in the histological grade in the majority of cases), which would have altered patient management in 12%.[18] Comparable observations

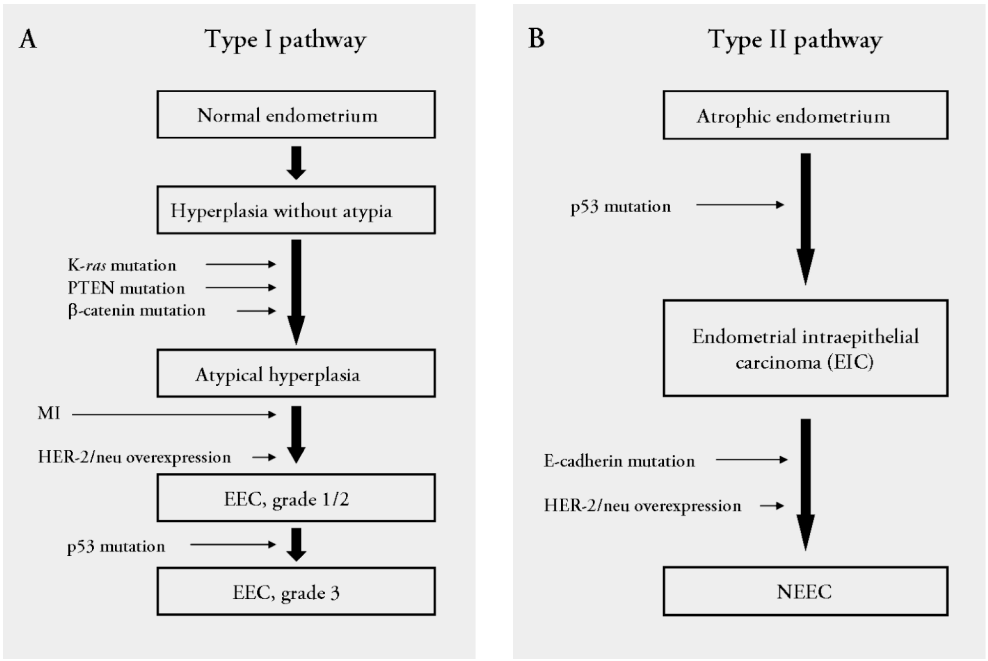

Figure 1. Dualistic model of endometrial carcinogenesis. (A) Type I pathway. (B) Type II pathway. (EEC: endometrioid endometrial carcinoma, NEEC: non- endometrioid endometrial carcinoma, MI: microsatellite instability).

were made in our study. After pathology review, 34 irradiated patients (13.4%) were diagnosed as having a stage I, grade 1 tumours with less than 50% myometrial invasion, implying that there was no need for postoperative radiotherapy in these patients. Similarly in the PORTEC trial, 134 tumours (24%) were diagnosed at pathology review as grade 1 with superficial myometrial invasion, for which radiotherapy would not have been indicated. Approximately one half of these patients where randomised to receive postoperative radiotherapy. There are disadvantages attached to changing the grading system that is used worldwide. It would be difficult to compare outcome of new therapies or new treatment strategies with historical control groups when criteria for prognostic variables have been changed. Still, the use of this simple binary grading system that divided tumours into low-grade lesions and high-grade lesions based on the proportion of solid growth (less than 50% vs. 50% or more) might eventually lead to better patient care, considering its superior reproducibility without loss of prognostic power compared with the FIGO grading system.

Two types of endometrial carcinoma can be distinguished. Type I tumours are oestrogen-related and are typically low-grade endometrioid carcinomas, with positive oestrogen- (ER) and progesterone receptors (PR). They usually develop in an oestrogen rich environment (pre- and

peri-menopausal state), and in general have a good prognosis. Type II tumours are unrelated to oestrogen stimulation and are often non-endometrioid carcinomas. The molecular abnormalities involved in carcinogenesis appear to be different for these tumour types. In **chapter 5** and **chapter 6** the potential role of cell adhesion molecule E-cadherin, and α- and β-catenin as prognostic factors for clinical practice has been investigated. Furthermore, their significance in the carcinogenesis of either of the two types of endometrial carcinoma was evaluated. The expression of E-cadherin, α- and β-catenin was investigated by immunohistochemistry in 225 endometrial carcinomas (**chapter 5**). E-cadherin is a member of the cadherin family of cell adhesion molecules. α- and β-catenin are necessary for a properly functioning E-cadherin protein. Since E-cadherin is needed to keep neighbouring cells attached, it has been suggested that impaired function of this cadherin might lead to invasive potential of malignant epithelial tumours. Furthermore, E-cadherin is also somehow involved in the process of cell differentiation, although so far the underlying mechanism is unclear. The intensity of the membranous staining (score 0-3) and the proportion of positive tumour cells (score 0-4) were scored, adding up to a total score of 0-7. Normal endometrial glands show strong membranous staining. Decreased expression was defined as a score of 4 or less. A score of 5 or more was defined as preserved expression. Decreased E-cadherin, α- and β-catenin expression was observed in 44%, 47% and 33% of endometrial carcinomas, respectively. It was found that decreased expression of E-cadherin, α- or β-catenin was correlated with histological grade 3 ($p < 0.001$), implying that it is associated with a diffuse growth pattern, lacking tubular or gland formation, just as in lobular breast cancer and diffuse type gastric cancer. This supports the theory that E-cadherin may somehow be involved in the process of gland formation. Decreased E-cadherin expression was more often observed in non-endometrioid carcinomas than in endometrioid carcinomas (75% versus 43%, $p = 0.04$). This might imply that decreased expression of E-cadherin, possibly caused by an E-cadherin mutation,[19] is a molecular abnormality that is more often involved in the carcinogenesis of type II than in the carcinogenesis of type I endometrial carcinoma. Expression of E-cadherin, α-catenin and β-catenin was not correlated to the depth, or to the pattern of myometrial invasion. It seems that a complex biological process such as stromal invasion is not regulated by a single molecular alteration, but is caused by a sequence of changes, including alterations in several cell adhesion molecules. Assessment of E-cadherin, α- and β-catenin had prognostic value in a subset of patients. Among patients with grade 1 or grade 2 tumours, those with preserved expression of E-cadherin, α- and β-catenin had a significantly better 5-year CSS of 97% compared with 89% 5-year CSS for those with decreased expression of one or more of these factors ($p = 0.02$).

β-catenin is not only a component of the cadherin-mediated intercellular adhesion system, it also functions as an oncogene in the Wnt signalling pathway. In normal epithelial cells a cytoplasmic complex comprising β-catenin mediates the phosphorylation of β-catenin and, as a consequence, the targeted degradation of this protein. A mutation in the β-catenin gene

severely impairs the down-regulation of its protein, which leads to an excess of cytoplasmic β-catenin. This results in concomitant translocation of β-catenin into the nucleus, where it can form transcriptionally active complexes with T-cell factor/lymphoid enhancer factor (Tcf/Lef), resulting in the activation of several genes, including c-myc and cyclin D1, which can cause uncontrolled cellular proliferation. Since β-catenin mutations and the subsequent nuclear accumulation of β-catenin protein have only been found in the endometrioid type endometrial carcinomas, it was hypothesized in **chapter 6** that abnormalities in the Wnt/β-catenin signalling pathway occur only in type I endometrial carcinoma. Of all 233 endometrial carcinomas investigated, nuclear β-catenin expression was observed in 39 cases (16%). All tumours expressing nuclear β-catenin were endometrioid adenocarcinomas (EECs). EECs expressing nuclear β-catenin were more often well differentiated than EECs without nuclear β-catenin, and were more often ER-positive and PR-positive. Furthermore, patients with tumours showing nuclear β-catenin-expression were significantly more often pre-menopausal and had higher survival rates. These results suggest that an abnormality in the Wnt/β-catenin signalling pathway, leading to a nuclear β-catenin staining pattern, is a molecular feature of a subset of type I endometrial carcinomas.
Other molecular abnormalities found in type I endometrial carcinoma are PTEN mutations (found in 35-50% of type I carcinomas), microsatellite instability (20-30%), K-*ras* mutations (15-30%), p53 mutations (10-20%) and HER-2/neu overexpression (10-15%). So far, no molecular abnormality has been found to be responsible for the majority of tumours of the endometrioid phenotype. K-*ras* mutations and β-catenin mutations have been found in simple hyperplasic lesions. Microsatellite instability, however, has only been observed in atypical hyperplasic lesions adjacent to endometrial carcinoma and may therefore be a late event in the transition from atypical hyperplasia to carcinoma. Since p53 mutations are mostly found in grade 3 EEC, and never in endometrial hyperplasia, p53 mutations in EEC seem to be late events, related to dedifferentiation of these tumours. In NEEC, on the other hand, p53 mutations are reported in up to 90% of the cases, both in the invasive and in the pre-malignant intraepithelial components, suggesting this to be an early event in carcinogenesis of type II endometrial carcinoma (Figure 1).

Better understanding of the carcinogenesis of the two different types of endometrial carcinoma might lead to improved means of treatment of endometrial cancer, and may provide the foundation for molecularly directed therapies. Results of the first microarray studies support the concept of the two types of endometrial carcinoma.[20,21] In these studies, the global gene expression patterns of EEC and NEEC were compared. The differences in gene expression between these histological subtypes might provide a basis for further investigations into the carcinogenesis of the two types of endometrial carcinoma. Such comprehensive genomic analyses are probably the only way to fully understand tumour biology and will give the best information about the carcinogenesis of endometrial cancer. However, fresh tumour material

is required for these analyses, and in daily practice, most macroscopic specimens of endometrial cancer are put in formalin immediately after the operation. Recently, in the Netherlands a prospective microarray study was started to analyse the differences in gene expression patterns between endometrial carcinomas of patients previously treated for breast cancer with tamoxifen, and endometrial carcinomas of patients previously treated for breast cancer without tamoxifen. Despite numerous studies on molecular abnormalities in endometrial carcinoma, this has not yet resulted in implementation of molecular features as prognostic factors in clinical practice. Their prognostic power, if present at all, has not been found to be superior to other, traditional factors. More research into this area, especially studies analysing patterns of gene expression, rather than focussing on a limited number of genes, will hopefully result in identification of significant molecular prognostic factors that will benefit patient care.

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Nederlands Samenvatting

Samenvatting

In Nederland krijgen per jaar ongeveer 1500 vrouwen baarmoederkanker. Doorgaans ontstaat dit in de binnenste slijmvlieslaag van de baarmoeder, het endometrium. Dit wordt een endometriumcarcinoom genoemd. De prognose van patiënten met een endometriumcarcinoom is in het algemeen gunstig, en hangt samen met een aantal factoren.

- De leeftijd van de patiënt, waarbij oudere patiënten een slechtere prognose hebben dan jongere.
- Het stadium van de ziekte op het moment van behandeling, d.w.z. de mate waarin de ziekte is doorgegroeid in het omringende weefsel en in hoeverre deze is uitgezaaid naar de lymfeklieren of andere organen. Bij het merendeel van de patiënten (75-80%) is de kanker beperkt tot de baarmoeder (stadium I).
- De mate van ingroei in de omringende spierlaag (myometrium invasie diepte). Diepe ingroei in deze omringende spierlaag verhoogt de kans op invasie van de bloed- en lymfevaten die gelegen zijn in het buitenste deel van het myometrium, waardoor de tumorcellen versleept kunnen worden naar de lymfeklieren in het bekken of elders in het lichaam (bijvoorbeeld de longen).
- Het type kankercel (histologisch subtype).
- De differentiatiegraad van het endometriumcarcinoom, dit is de mate van uitrijping van de kankercellen, ofwel in hoeverre zij nog lijken op gewone endometriumcellen. Dit zegt iets over delingssnelheid en de mate van agressiviteit van de tumor. Het endometriumcarcinoom wordt gegradeerd volgens het International Federation of Gynecology and Obstetrics (FIGO) graderingsysteem, en wordt onderverdeeld in drie graden: goed gedifferentieerde (graad 1), matig gedifferentieerde (graad 2) en slecht gedifferentieerde tumoren (graad 3).

Omdat het endometriumcarcinoom meestal snel klachten geeft in de vorm van vaginaal bloedverlies, wordt het vaak vroeg ontdekt en zijn de overlevingskansen over het algemeen goed. Vijf jaar na het stellen van de diagnose is 80-90% van de patiënten met een stadium I endometriumcarcinoom nog in leven. Uiteindelijk overlijden in Nederland per jaar ongeveer 300 vrouwen aan endometriumcarcinoom.

De standaardbehandeling van het endometriumcarcinoom bestaat uit chirurgische verwijdering van de baarmoeder, de eileiders en de eierstokken. Afhankelijk van de leeftijd van de patiënt, de differentiatiegraad van de tumor en de myometrium invasie diepte, worden patiënten na de operatie (postoperatief) bestraald op het operatiegebied en de lymfklierstations in het bekken. Bij elke vorm van kanker is er sprake van een ongecontroleerde aanmaak van cellen. Dit wordt veroorzaakt door een verstoring (mutatie) in één of meer van de genen (het erfelijk materiaal van de cel), die verantwoordelijk zijn voor de controle op de aanmaak of afbraak van cellen. Door middel van moleculair biologisch onderzoek kunnen deze genafwijkingen of de gevolgen hiervan (veranderde expressie van eiwitten) worden aangetoond.
Het doel van dit proefschrift is het belang van verschillende prognostische factoren voor het endometriumcarcinoom te onderzoeken. Daarnaast is onderzoek gedaan naar het vóórkomen van moleculaire afwijkingen bij het endometriumcarcinoom en naar hun mogelijke prognostische betekenis voor de dagelijkse klinische praktijk.

In dit proefschrift worden de patiëntgegevens en het pathologisch materiaal van twee cohorten geanalyseerd. Allereerst wordt een cohort geanalyseerd van 253 patiënten die tussen 1984 en 1993 in het Leids Universitair Medisch Centrum (LUMC) zijn bestraald vanwege een endometriumcarcinoom. Ten tweede wordt de pathologische beoordeling beschreven van de Postoperatieve Radiotherapie bij Endometriumcarcinoom (PORTEC) studie, die tussen 1990 en 1997 in de meeste centra in Nederland is uitgevoerd om de waarde van postoperatieve radiotherapie bij patiënten met een stadium I endometriumcarcinoom te bepalen. Na operatie werd er geloot of de patiënt bestraald zou worden of geen verdere therapie zou ontvangen. In deze studie zijn 715 patiënten geïncludeerd met een stadium I, graad 1 of 2 endometriumcarcinoom dat diep in het myometrium infiltreerde (50% of meer), of een stadium I, graad 3 tumor met oppervlakkige myometrium invasie (minder dan 50%). Er konden 714 patiënten geëvalueerd worden. Het proefschrift is als volgt opgebouwd. **Hoofdstuk 1** bevat een algemene inleiding. In **hoofdstuk 2** en **hoofdstuk 3** worden de lange termijn resultaten van de patiënten uit respectievelijk de LUMC studie en de PORTEC studie beschreven. In **hoofdstuk 4** wordt de waarde van verschillende graderingsystemen voor het endometriumcarcinoom vergeleken. En in **hoofdstuk 5** staat de prognostische betekenis van E-cadherine, α-catenine en β-catenine centraal, waarna **hoofdstuk 6** beschrijft wat de rol van β-catenine is bij het ontstaan van het endometriumcarcinoom.

Hoofdstuk 2 beschrijft de lange termijn overleving van de 253 patiënten uit de LUMC studie. Retrospectief is geanalyseerd wat het prognostisch belang was van het tumorstadium, de leeftijd van patiënte, de differentiatiegraad, het histologisch subtype, de diepte van de myometrium invasie (meer of minder dan de helft) en het patroon van de invasie (een infiltrerende tumor versus een tumor die het myometrium meer wegdrukt dan infiltreert, een zogenaamde "pushing border"). De mediane follow-up bedroeg 11.7 jaar.

Na vijf en tien jaar waren nog respectievelijk 77% en 64% van de patiënten in leven. De kanker-specifieke overleving (cancer-specific survival, CSS), d.w.z. het percentage patiënten dat nog in leven is of is overleden aan andere oorzaken dan het endometriumcarcinoom, was na vijf en tien jaar respectievelijk 85% en 82%. Na vijf jaar was bij 7% van de patiënten een loco-regionaal recidief ontstaan (hernieuwde tumorgroei op de voormalige plaats van de baarmoeder of in de omringende lymfeklieren), en bij 15% waren uitzaaiingen elders in het lichaam ontstaan. Sterke, onafhankelijk prognostische factoren voor de CSS bleken te zijn:

- het tumorstadium (een 3.5 maal hoger risico om te overlijden aan de ziekte bij een verder gevorderd stadium van de ziekte t.o.v. stadium I),
- de differentiatiegraad (3.6 maal hoger overlijdensrisico bij een slecht gedifferentieerde tumor t.o.v. een goed gedifferentieerde tumor) en
- het patroon van myometrium invasie (2.2 maal hoger overlijdensrisico bij een infiltrerend invasie patroon t.o.v. een patroon met "pushing borders").

De diepte van de myometrium invasie bleek in onze studie geen onafhankelijk prognostische factor te zijn. Diepe ingroei in het myometrium verhoogt de kans op invasie van de vaten die gelegen zijn in het buitenste deel daarvan. Echter, in het geval van een tumor met een "pushing border" die het myometrium voor meer dan de helft lijkt in te groeien, duwt de tumor de omringende spierlaag meer weg dan dat deze het myometrium daadwerkelijk ingroeit, waardoor de vaten in het buitenste deel van het myometrium niet bereikt worden. Daarnaast blijkt het in de praktijk lastig te zijn om de precieze diepte van de myometrium invasie te bepalen, en hangt deze beoordeling af van de manier waarop de baarmoeder na de operatie verwerkt is. Aangezien het patroon van myometrium invasie beter reproduceerbaar lijkt en een sterkere prognostische factor is, is dit een goed alternatief voor de myometrium invasie diepte als prognostische parameter in de dagelijkse praktijk.

Voor deze LUMC studie zijn alle pathologie coupes van de tumoren opnieuw beoordeeld en gegradeerd. Hierbij bleken 112 van de 144 (78%) tumoren die oorspronkelijk als matig gedifferentieerd waren gegradeerd, nu als goed gedifferentieerd beoordeeld te worden. Deze grote verschuiving had geen effect op de CSS voor de groep goed gedifferentieerde en de groep matig gedifferentieerde tumoren (94% versus 90% 5-jaars overleving volgens de originele graad, en 92% versus 94% volgens de gereviseerde graad), terwijl een slecht gedifferentieerde tumor een duidelijk slechtere prognose bleek te hebben (5-jaars overleving 63%). Een soortgelijke verschuiving van graad 2 naar graad 1 werd gevonden tijdens de pathologische revisie van de PORTEC studie. Van 569 (80%) patiënten uit deze studie konden de coupes verkregen worden voor pathologische revisie. Oorspronkelijk waren 21%, 68% en 11% van alle tumoren respectievelijk graad 1, 2 en 3. Na revisie waren dit respectievelijk 69%, 16% en 15%. In 2000 zijn de eerste resultaten van de PORTEC studie gepubliceerd. In **hoofdstuk 3** wordt de analyse beschreven met een mediane follow-up van 97 maanden, die verricht is teneinde de lange termijn resultaten te bekijken en de prognostische factoren opnieuw te evalueren, maar nu met de gereviseerde differentiatiegraad. Oorspronkelijk waren de tumoren

door regionale pathologen gegradeerd.
Na tien jaar werden 5% en 14% loco-regionale recidieven gevonden in respectievelijk de radiotherapie (RT) groep en de controle groep ($p < 0.001$). Postoperatieve radiotherapie bleek een sterke, onafhankelijke prognostische factor voor het optreden van een loco-regionaal recidief (3.9 maal hoger risico op een loco-regionaal recidief in de controle groep vergeleken met de RT groep). Er was geen significant verschil in overleving tussen de beide behandelgroepen, met 10-jaars overlevingspercentages van respectievelijk 66% (RT) en 73% (controle, $p = 0.09$). Ten gevolge van de verschuiving van graad 2 naar graad 1 die tijdens de pathologische revisie gevonden werd, werden 134 tumoren gediagnosticeerd als graad 1 met oppervlakkige myometrium invasie. Dit betekent dat deze eigenlijk niet voor inclusie in de studie in aanmerking zouden zijn gekomen. Wanneer deze 134 patiënten uit de analyses geëxcludeerd werden, veranderden de uitkomsten nauwelijks, met 10-jaars loco-regionale recidiefpercentages van 5% in de RT groep en 17% in de controle groep ($p < 0.001$) en 10-jaars overlevingspercentages van 65% en 70% ($p = 0.23$).
Deze analyse met lange follow-up en met gereviseerde pathologie, bevestigt de eerdere uitkomsten van de PORTEC studie dat postoperatieve radiotherapie voor stadium I endometriumcarcinomen de kans op een loco-regionaal recidief significant vermindert, maar zonder overlevingswinst. Vanwege deze verbetering van de loco-regionale controle, blijft postoperatieve radiotherapie geïndiceerd voor patiënten met een stadium I endometriumcarcinoom met een verhoogd risico op een loco-regionaal recidief. Momenteel is er voor patiënten met een stadium I endometriumcarcinoom een indicatie voor postoperatieve radiotherapie, indien tenminste twee van drie negatief prognostische factoren aanwezig zijn (graad 3, meer dan 50% myometrium invasie, en leeftijd ouder dan 60).

Gezien de slechte reproduceerbaarheid en beperkte klinische waarde van graad 2, zoals beschreven in hoofdstuk 2 en 3, lijkt een tweedelig graderingsysteem zinvoller dan het huidige driedelige graderingsysteem, aangezien dit beter correleert met de prognose van de patiënt en waarschijnlijk beter reproduceerbaar is. Daarom werd een graderingstudie opgezet met tumorweefsel van 800 patiënten uit de beide studies (**hoofdstuk 4**). Verschillende graderingsystemen zijn met elkaar vergeleken: enerzijds het traditioneel gebruikte driedelige FIGO graderingsysteem (goed, matig, slecht gedifferentieerd, resp. graad 1, 2 en 3), en anderzijds een nieuw tweedelig (binair) graderingsysteem (laag- en hooggradig) dat gebaseerd is op de hoeveelheid solide tumorgroei (in tegenstelling tot groei in buizen, zoals dat ook gezien wordt in normaal endometrium), het patroon van de myometrium invasie (infiltrerend versus "pushing borders") en de aanwezigheid van necrose (afgestorven tumorcellen). Alle coupes zijn, onafhankelijk van elkaar, door twee pathologen gegradeerd. Als maat voor de reproduceerbaarheid werd gekeken naar het percentage waarin overeenstemming was tussen beide pathologen. De overeenstemming tussen de beide pathologen voor de twee systemen was matig, gezien de 70% overeenstemming voor het FIGO graderingsysteem en 73% voor het

binaire systeem. Wanneer het FIGO systeem omgezet werd in een kunstmatig tweedelig systeem (graad 1 en 2 versus graad 3), was de overeenstemming veel beter (85% overeenstemming). Wanneer gekeken werd naar de drie afzonderlijke criteria van het binaire systeem, bleek de beoordeling van de hoeveelheid solide groei (meer of minder dan 50%) het best reproduceerbaar (80% overeenstemming). Zowel het kunstmatige FIGO graderingsysteem (graad 1 en 2 vs. graad 3) als het binaire graderingsysteem waren sterke prognostische factoren voor de kanker-specifieke overleving. Patiënten met een FIGO graad 3 tumor hadden een 2.6 maal hoger risico om te overlijden aan de tumor dan patiënten met een graad 1 tumor. Hooggradige tumoren volgens het binaire systeem gaven een 3.8 maal hoger overlijdensrisico t.o.v. laaggradige tumoren. Ook de hoeveelheid solide groei bleek een sterke prognostische factor. Tumoren met meer dan 50% solide groei gaven een 3.8 maal hoger overlijdensrisico t.o.v. tumoren met minder dan 50% solide groei. Dit eenvoudige tweedelige graderingsysteem, waarin tumoren worden verdeeld in laaggradige en hooggradige lesies op basis van de hoeveelheid solide groei (meer of minder dan 50%), had een betere prognostische waarde en reproduceerbaarheid dan zowel het FIGO als het binaire graderingsysteem. Er zijn nadelen verbonden aan het veranderen van het graderingsysteem dat wereldwijd gebruikt wordt. Zo zal het moeilijker zijn om het resultaat van nieuwe behandelmethoden te vergelijken met historische controle groepen als de criteria voor prognostische factoren veranderd zijn. Toch zou het gebruik van dit simpele tweedelige graderingsysteem, gebaseerd op de hoeveelheid solide groei, uiteindelijk kunnen leiden tot betere patiëntenzorg, gezien de hogere reproduceerbaarheid en gelijke prognostische waarde van dit graderingsysteem, vergeleken met het FIGO graderingsysteem.

Er worden twee typen endometriumcarcinomen onderscheiden.

- Type I tumoren zijn gerelateerd aan het vrouwelijk geslachtshormoon oestrogeen. Het zijn meestal goed gedifferentieerde endometrioid type carcinomen, met aanwezigheid van hormoonreceptoren (positieve expressie). Ze ontstaan vaak vanuit een goedaardige celproliferatie van het baarmoederslijmvlies (hyperplasie), bij vrouwen die nog niet of kort in de overgang zijn (pre- of peri-menopausaal) en hebben over het algemeen een goede prognose.
- Type II tumoren zijn vaak niet-endometrioid type carcinomen die frequenter bij oudere vrouwen voorkomen en over het algemeen een slechtere prognose hebben.

De moleculaire afwijkingen die betrokken zijn bij het ontstaan van het endometriumcarcinoom lijken voor beide types verschillend te zijn. In **hoofdstuk 5 en 6** is de rol van E-cadherine, α-catenine en β-catenine bij het ontstaan van beide typen endometriumcarcinomen onderzocht, evenals hun mogelijke prognostische betekenis. E-cadherine is een zogenaamd cel-adhesie molecuul. Het heeft α- en β-catenine nodig om goed te kunnen functioneren. Aangezien E-cadherine zorgt voor de samenhang tussen cellen, is in de literatuur gesuggereerd dat een verstoorde functie van E-cadherine zou kunnen leiden tot de doorgroei

van kwaadaardige tumoren naar het omringende weefsel. Verder blijkt E-cadherine ook betrokken te zijn bij het proces van celdifferentiatie, alhoewel het onderliggende mechanisme daarvan tot op heden nog onduidelijk is. **In hoofdstuk 5** wordt verslag gedaan van een onderzoek waarbij 225 endometriumcarcinomen middels een immunohistiochemische techniek zijn onderzocht op de expressie van E-cadherine en α- en β-catenine. Verminderde expressie voor E-cadherine, α-catenine en β-catenine werd gevonden in 44%, 47% en 33% van de tumoren. Er werd geen verband gevonden tussen de expressie van E-cadherine en de diepte of het patroon van myometrium invasie. Wel werd een sterke correlatie gevonden tussen een verminderde expressie van E-cadherine, α-catenine en β-catenine, en differentiatiegraad 3 van de tumor ($p < 0.001$). Verminderde expressie van E-cadherine werd vaker gevonden bij niet-endometrioide type dan bij endometrioide type carcinomen ($p = 0.04$), hetgeen er wellicht op duidt dat verminderde expressie van dit eiwit (mogelijk het gevolg van een E-cadherine mutatie) een kenmerk is van type II endometriumcarcinomen. De expressie van E-cadherine en α- en β-catenine was van prognostisch belang voor een deel van de patiënten met een endometriumcarcinoom. Van alle patiënten met een goed of matig gedifferentieerde tumor, hadden diegenen met een normale expressie van zowel E-cadherine als α- en β-catenine een significant betere 5-jaars kanker-specifieke overleving van 97% vergeleken met de 89% 5-jaars overleving van patiënten met een tumor die verminderde expressie toonde voor een of meerdere van deze factoren.

β-catenine is niet alleen een onderdeel van het celadhesie complex waar ook E-cadherine onderdeel van uitmaakt, maar het functioneert ook als onderdeel van de zogenaamde Wnt-signalling pathway. Deze pathway bestaat uit een serie processen binnen een cel, uiteindelijk leidend tot de aanmaak van genen die betrokken zijn bij de proliferatie van de cel. Een verstoring binnen deze pathway kan leiden tot een verhoogde aanmaak van deze genen, en dus tot een ongecontroleerde proliferatie van cellen (een tumor). Een van die verstoringen is een mutatie van het β-catenine gen. Hierdoor kan het β-catenine eiwit minder goed afgebroken worden, waardoor een overschot ontstaat, dat naar de kern van de cel verplaatst wordt.

Het β-catenine eiwit kan zichtbaar gemaakt worden d.m.v. immunohistochemie. Membraneuze aankleuring van β-catenine is een uiting van de functie van dit eiwit binnen het celadhesie systeem. Nucleaire (kern) aankleuring van β-catenine is een gevolg van een verstoring binnen de Wnt-signalling pathway zoals hierboven beschreven. Tot op heden is nucleaire aankleuring van β-catenine alleen aangetroffen bij endometrioid type endometriumcarcinomen. Daarom ontstond de hypothese in hoofdstuk 6 dat abnormaliteiten in de Wnt-signalling pathway alleen voorkomen bij type I endometriumcarcinomen. In deze serie werden 233 endometriumcarcinomen immunohistochemisch onderzocht op de aanwezigheid van β-catenine in de kern. Dit werd gezien bij 39 van de 233 tumoren (16%).

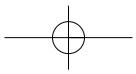
Alleen endometrioid type carcinomen vertoonden nucleaire aankleuring. Tumoren met nucleaire β-catenine expressie waren in vergelijking met tumoren zonder deze vorm van aankleuring significant vaker goed gedifferentieerd ($p = 0.02$), hadden vaker positieve expressie van hor-

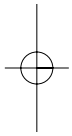
moonreceptoren ($p \leq 0.003$), patiënten waren significant vaker pre-menopausaal ($p = 0.01$) en zij hadden een betere kanker-specifieke overleving. Dit alles suggereert derhalve sterk dat een afwijking in de Wnt-signalling pathway inderdaad een moleculair kenmerk is van type I endometriumcarcinomen.

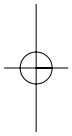
Meer kennis omtrent het ontstaan (de carcinogenese) van de twee typen endometriumcarcinomen zou kunnen leiden tot nieuwe, verbeterde behandelmethoden voor het endometriumcarcinoom. De beste manier om de carcinogenese zo volledig mogelijk te doorgronden, is via veelomvattende genanalyses. Voor zulke analyses is echter vers materiaal nodig, en in praktijk worden de meeste baarmoederpreparaten direct na de operatie in formaline gedaan. Tot op heden zijn moleculaire kenmerken van het endometriumcarcinoom nog niet in gebruik als prognostische factor. Dit komt voornamelijk omdat hun prognostische waarde, voor zover deze al gevonden werd, niet beter was dan die van andere, traditionele prognostische factoren. Meer onderzoek op dit gebied, met name studies die patronen van genexpressie onderzoeken, in plaats van zich te richten op een beperkt aantal genen, zal hopelijk resulteren in de identificatie van significante moleculaire prognostische factoren waar de patiënt baat bij heeft.

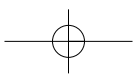
Curriculum Vitae

Astrid Scholten werd op 15 juni 1972 geboren te Haarlem. In 1990 behaalde zij het diploma gymnasium β aan de Alexander Hegius Scholengemeenschap te Deventer, waarna zij begon met de studie geneeskunde aan de Universiteit Leiden. Tijdens haar studie deed zij onderzoek op de afdeling Radiotherapie van het LUMC (onder leiding van prof. dr. J.W.H. Leer), en liep zij in 1994 gedurende 4 maanden stage op de afdeling Radiotherapie van het St. Thomas' Hospital te Londen. Vlak voor haar artsexamen in 1997, verbleef zij gedurende een half jaar in Australië, alwaar zij stages liep op de afdeling Radiotherapie van het Westmead Hospital te Sydney, en bij de Royal Flying Docters Service te Broken Hill. In 1998 heeft zij één jaar gewerkt als arts-assistent Interne Geneeskunde in het Groene Hart Ziekenhuis te Gouda (opleider: dr. K.J. Heering). In 1999 begon zij met de opleiding tot radiotherapeut-oncoloog in het Leids Universitair Medisch Centrum (opleider: prof. dr. E.M. Noordijk). De opleiding werd vanaf juni 2000 gedurende een jaar onderbroken, om de basis te leggen voor het onderzoek dat beschreven staat in dit proefschrift, hetgeen in nauwe samenwerking met de afdeling pathologie werd uitgevoerd (hoofd: prof. dr. G.J. Fleuren, begeleider: dr. V.T.H.B.M. Smit).
In de periode 2000-2003 kreeg zij drie dochters.









Nawoord

Zonder de steun en hulp van velen, was dit boekje nooit tot stand gekomen. Een paar mensen wil ik met name bedanken. Carien Creutzberg voor de vele waardevolle uren die zij gestoken heeft in het meedenken over, en lezen en bijsturen van alle stukken. Lambert van den Broek voor zijn praktische hulp, en het geduld waarmee hij me wegwijs gemaakt heeft in de wereld van de immuunhistochemie. Klaas van der Ham voor het vervaardigen van alle prachtige kleurenplaatjes. Voor het scoren van vele honderden gekleurde coupes ben ik Riena Aliredjo zeer erkentelijk. En alle mede-auteurs dank ik voor hun opbouwende commentaar op de stukken. Pap en mam, dank. Niet alleen voor die vele ukken-oppasdagen, maar vooral voor jullie onvoorwaardelijke steun, betrokkenheid en liefde. Lieve Jan-Kees, jouw nuchtere, relativerende, en vaak humoristische kijk op de zaken was de beste remedie tegen elk onderzoeksdipje. Dank voor alles. En tenslotte Sarah, Jasmijn en Suze. Lieve dametjes, hoe blij en voldaan ik me ook voel als ik kijk naar het resultaat van mijn onderzoek in de vorm van dit boekje, jullie moeder te zijn, is en blijft het mooiste wat er is!