Endometrial Carcinoma: Description of a Novel Grading System and Identification of Additional Predictors of Patient Outcome

By

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ABSTRACT

The aims of our studies were to examine the prognostic value of several pathological parameters in early stage papillary serous carcinoma of endometrium (PSCE), to evaluate the value of tumor proliferative activity in predicting patient outcome in early stage endometrial carcinomas, and to describe a novel grading system for endometrial carcinoma.

To address our first aim, we studied 65 cases of PSCE from patients with International Federation of Gynecology and Obstetric (FIGO) stage I or II disease who were treated at British Columbia Cancer Agency (BCCA) between 1985-1995. In each case, the tumor grade, lymphovascular invasion, and amount of the serous carcinoma component were assessed. p53 immunostaining was performed on 45 tumors. Only FIGO stage was of prognostic significance, and none of the other pathological features examined showed a significant correlation with patient outcome.

To address our second aim, the mitotic index, MIB-1 staining index, and p53 immunostaining in 39 tumors from patients with low grade, stage Ia or Ib endometrioid carcinoma, and 23 tumors from patients with stage I PSCE were assessed. p53 overexpression and proliferative indices (mitotic and MIB-1) were strongly correlated with each other in low grade endometrioid carcinoma. These markers of proliferative activity are also independent prognostic indicators in these tumors. On the other hand, PSCE are rapidly proliferative tumors even at an early stage, and quantification of proliferative activity in these tumors does not allow prediction of patient outcome.
In addressing our third aim, 200 cases of endometrial carcinoma, treated by hysterectomy, were retrieved from the archives of the Dept of Pathology, Vancouver General Hospital, for the period 1983-1998. Each tumor was graded by using three grading systems; FIGO grading, binary grading, and a new grading system, based on the Nottingham grading system used for breast carcinoma. With the new grading system, the tumors are classified based on a combination of the tumor architecture, nuclear grade, and mitotic index. This new grading system has strong prognostic value, and was found to be an independent predictor of patient outcome when the patient survival was adjusted for FIGO stage, patient age, and tumor cell type.
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CHAPTER 1

INTRODUCTION
Epidemiology and Risk Factors:

Endometrial carcinoma is the most common invasive cancer of the female genital tract and the fourth most frequently diagnosed cancer in women in North America\textsuperscript{143,145}. There is a wide difference in its incidence in various countries, with the highest risk areas being the United States, Canada, and Europe, whereas rates in developing countries and Japan are four to five times lower\textsuperscript{133}. The American Cancer Society estimated that there would be 36,100 new cases in the United States in the year 2000, directly leading to 6,500 deaths\textsuperscript{13}. In the USA and Canada endometrial cancer accounts for about 8-10\% of all cancer in women. Similarly high rates were found in Western European populations. With respect to racial differences, the incidence is about twice as high in whites compared to blacks. In low risk areas, endometrial cancer represents only 2-4\% of all cancers. Worldwide, approximately 150,000 new cases of endometrial cancer are diagnosed every year, making this neoplasm the fifth most common cancer in women worldwide\textsuperscript{133}. It is the most curable of the 10 most common cancers in women and the most frequently curable of the gynecologic cancers\textsuperscript{145}.

Endometrial carcinoma occurs during the reproductive and menopausal years. The median age of patients at the time of diagnosis is 63 years, however, 20-25\% of cases are diagnosed before the onset of menopause\textsuperscript{138,58}.

Approximately 50\% of endometrial carcinomas occur in women who have particular risk factors for the tumor. Excessive estrogen is associated with most of the risk factors that have been identified with endometrial carcinoma. Obesity, nulliparity, and late
menopause are classically associated with endometrial carcinoma; all are associated with a hyperestrogenic state\textsuperscript{133, 143, 145}.

Diabetes mellitus and hypertension historically were stated to be associated with endometrial carcinoma. Most investigators feel these diseases are probably not associated with endometrial cancer per se, but are more prevalent in elderly obese women\textsuperscript{43}.

Unopposed exogenous estrogen administered for the treatment of menopausal symptoms, and endogenous estrogen secreted from granulosa or theca cell ovarian tumors are well documented risk factors for the development of endometrial cancer. Unopposed exogenous estrogen was popular in the late 1960s and 1970s in the treatment of menopausal symptoms. It was associated with a relative risk of 6.0\textsuperscript{145}. The practice of prescribing unopposed estrogen has been discontinued and combined estrogen-progesterone preparations have been adopted\textsuperscript{145}.

Progesterone is the dominant component of today's oral contraceptive agents, which confer protection against endometrial carcinoma. There is a strong evidence to suggest that oral contraceptives (OC) reduce the risk of endometrial carcinoma. A protection by about 50\% for women who had ever used OC versus those who never use them has been reported in the literature. Such protection increases with duration of OC use\textsuperscript{133, 139}.

Tamoxifen is a synthetic antiestrogen that is used for the treatment of breast cancer. In addition, it has been shown to have estrogenic effects on endometrium and to increase the
risk of endometrial carcinoma. The association between tamoxifen and endometrial carcinoma was first recognized in 1985, when endometrial carcinoma developed in three women with breast cancer after only 7 to 14 months of tamoxifen therapy. Many studies found that the cumulative dose and duration of tamoxifen therapy affect the relative risk of endometrial carcinoma. The relative risk of endometrial carcinoma was reported as 2.2 in women on tamoxifen therapy. Although two studies have found an increase in the frequency of high grade and aggressive endometrial carcinoma with tamoxifen therapy, most of endometrial carcinomas arising in tamoxifen treated women are well differentiated endometrioid carcinomas.

Endometrial cancer is the most common extracolonic cancer in women with the hereditary nonpolyposis colonic cancer syndrome, also known as Lynch syndrome. This syndrome manifests primarily with colon cancer but is also associated with endometrial, breast and ovarian cancer.
Classification:

The recent World Health Organization (WHO) and International Society of Gynecological Pathologists (ISGYP) classification of endometrial carcinoma is based primarily on the cell type of the tumor (TABLE 1)\textsuperscript{36,143}.

\begin{table}
\centering
\caption{Classification of endometrial carcinoma}
\begin{tabular}{ll}
\hline
Endometrioid adenocarcinoma & \\
Typical & \\
Variants & \\
Villoglandular & \\
With squamous differentiation & \\
Secretory & \\
Ciliated & \\
Oxyphilic & \\
Sertoliform & \\
Serous papillary carcinoma & \\
Clear cell carcinoma & \\
Mucinous carcinoma & \\
Squamous cell carcinoma & \\
Undifferentiated carcinoma & \\
Small cell & \\
Large cell & \\
Mixed carcinoma & \\
\hline
\end{tabular}
\end{table}
Endometrioid Adenocarcinoma:

Endometrioid adenocarcinoma is the most common form of endometrial carcinoma, accounting for approximately 80% of cases of endometrial carcinoma. This subtype is referred to as endometrioid because it resembles proliferative phase endometrium morphologically. These tumors are composed of tubular glands lined by stratified or pseudostratified columnar cells with rounded nuclei and variably prominent nucleoli. Nuclear atypia is variable but most often only mild to moderate. Mucin is usually absent or confined to luminal tips of the cells. A solid component may variably be present in these tumors. The architectural grade is determined by the percentage of solid component. By definition these tumors do not contain more than 10% of serous, mucinous, clear cell, or squamous carcinoma component. Tumors with foci of more than 10% non-endometrioid adenocarcinoma are referred as mixed carcinoma according to the WHO classification.

Several morphological variants of endometrioid adenocarcinoma have been recognised, two of which are more frequently seen. Villoglandular carcinomas are composed of long, slender, delicate papillae. The tumor cells are usually columnar in shape with low nuclear grade and associated with typical endometrioid adenocarcinoma and hyperplasia. This variant accounted for 13% and 31% of cases of endometrioid adenocarcinomas in the two largest studies in the literature. The WHO recommends that endometrioid adenocarcinomas associated with squamous elements to be designated as endometrioid adenocarcinoma with squamous differentiation, and these tumors account for approximately 25% of endometrioid carcinomas. It is generally believed that estrogen
stimulation induces squamous metaplasia of the endometrium, although squamous metaplasia can also be induced by progestin therapy for endometrial hyperplasia.  

Other less commonly seen variants of endometrioid carcinomas include secretory, ciliated, oxyphilic, and sertoliform carcinomas. Secretory carcinomas consist of crowded, well formed glands lined by columnar cells with subnuclear or supranuclear cytoplasmic glycogen vacuoles. Ciliated carcinomas are composed of cells possessing eosinophilic cytoplasm and cilia. Oxyphilic endometrioid carcinoma is otherwise typical endometrioid carcinoma that is composed predominantly or entirely of cells with abundant oxyphilic cytoplasm. Sertoliform endometrioid carcinomas are a rare variant with focal to predominant pattern resembling that of ovarian Sertoli cell tumors.  

The median age of presentation of endometrioid adenocarcinoma is 59 years. Most women are postmenopausal, with only 1-8% of these tumors are seen in women under 40 year of age. The typical presentation is postmenopausal bleeding.  

Serous Papillary Carcinoma:  
This uncommon aggressive subtype of endometrial carcinoma was first described by Hendrickson in 1982 as a tumor that had a distinctive microscopic features and an unusually high rate of relapse. It accounts for 1-10% of cases of endometrial carcinoma and for a disproportionately high percentage of patients who die of endometrial cancer. This tumor resembles its ovarian counterpart in morphology and in its propensity for transperitoneal spread. Although papillary architecture is a common finding in serous carcinoma of endometrium, most other types of endometrial carcinoma can display
papillary architecture but are usually not highly aggressive tumors. What distinguishes this subtype is the uniformly high grade nuclear atypia. Although a papillary pattern typically predominates, glandular and solid patterns are also seen. Originally the papillae of serous carcinoma were described as thick, short papillae, but subsequent studies have shown that thin papillae may be present in more than half of cases. Cells of serous carcinoma are frequently polygonal with eosinophilic and clear cytoplasm, but hobnail cells are also observed. Marked nuclear atypia is always present. Psammoma bodies are seen in one third of tumors. Myometrial invasion is a common finding, seen in 70% of tumors, and lymph node metastasis is present in 37% of cases.

Patients with serous carcinoma are typically postmenopausal; the median age at diagnosis is 10 years older than that of endometrioid adenocarcinoma. The typical clinical presentation of serous carcinoma of endometrium is postmenopausal bleeding. These tumors are unassociated with estrogenic excess or endometrial hyperplasia and develop from atrophic endometrium, accordingly the well recognised risk factors identified with endometrioid carcinoma and related to estrogen excess are not seen in patients with serous carcinoma.

**Clear Cell Carcinoma:**

This is another uncommon aggressive subtype of endometrial carcinoma; it accounts for 1-6% of endometrial carcinomas. It is seen in elderly women with median age at diagnosis of approximately 65 years. Clear cell carcinoma may exhibit solid, papillary, tubular, and cystic patterns. The tumor cells can be polygonal, cuboidal, or hobnail-
shaped cells, with abundant clear cytoplasm indicative of the presence of glycogen.

Hobnail cells usually possess a granular eosinophilic cytoplasm. Marked nuclear atypia is usual.3, 36, 89, 143

**Mucinous Adenocarcinoma:**

This is a rare subtype of endometrial carcinoma that has an appearance similar to mucinous carcinoma of endocervix. It represents the dominant cellular population in 1-9% of endometrial carcinoma; pure mucinous carcinoma is rare. To qualify as a mucinous carcinoma, more than one half the cell population of the tumor must contain periodic acid-Schiff (PAS) positive, diastase-resistant intracytoplasmic mucin. Mucinous carcinomas are almost invariably stage I. The median age at presentation is 60 years.36, 143

**Mixed carcinoma:**

Mixed type endometrial carcinoma shows combination of two or more of the pure types, described above. By convention, a mixed carcinoma has at least two components, each comprising at least 10% of the tumor.36, 143

**Squamous Carcinoma:**

Primary squamous carcinoma of endometrium is extremely rare. Three criteria must be met before a diagnosis of endometrial squamous carcinoma is made: 1) no coexisting glandular carcinoma in endometrium, 2) no connection between the tumor in the
endometrium and the squamous epithelium of the cervix, and 3) no primary squamous carcinoma of the cervix.

**Undifferentiated carcinoma:**

This is a rare subtype of endometrial carcinoma and the term is applied to endometrial carcinoma that is too poorly differentiated to be categorised as one of the other specific types described above. It accounts for 1.6% of cases of endometrial carcinoma. It has two variants based on cell size, large cell undifferentiated carcinoma, and small cell variant.
Molecular Genetics and Pathogenesis of Endometrial Carcinoma:

Although the molecular events involved in the development and progression of endometrial carcinoma are far from being fully understood, substantial progress has been made over the past decade in characterization of this tumor at the molecular level.

Based initially on epidemiological studies, endometrial carcinoma was divided into two clinically distinguishable broad categories. Subsequently, it was recognised that these clinical entities correlated with the two major histological types of endometrial carcinoma: endometrioid, and the less common but more aggressive serous. Bokhman\(^\text{18}\) proposed based on clinicopathological observations in 366 endometrial carcinomas that there are two main types of endometrial carcinomas: type 1 tumors related to hormonal imbalances of estrogen and type 2 tumors that seem largely unrelated to estrogen. Several other studies had provided epidemiological and clinicopathological insights into the two pathways of endometrial carcinogenesis. In a study by Sherman\(^\text{153}\ et\ al.\), 328 endometrioid, 26 serous carcinomas, and 320 population-based controls without endometrial cancer were analysed. This study had provided epidemiologic support for the dualistic model for endometrial carcinogenesis. In their analysis, the average age of patients with serous carcinomas was 6 years greater than that of endometrioid carcinoma patients. Obesity and exogenous estrogen hormone use were associated with increased risk for endometrioid carcinoma, but these were not related to risk for serous carcinoma. This study had also demonstrated statistical differences in serum sex hormones and sex hormone binding globulin levels between patients with these two types of tumors. Both estrogens and androgens were elevated in women with
endometrioid carcinoma compared to serum levels in women with serous carcinoma, or controls. In contrast, levels of sex-hormone-binding globulin, a circulating protein that reduces the bioavailability of estrogen, were higher among serous carcinoma patients than controls or endometrioid carcinoma patients. These results suggest a fundamental difference in the hormonal milieu in which these two types of tumors develop.  

This hormonal difference in pathogenesis between these two tumor types is further supported by studies on precursor lesions for endometrioid and serous carcinoma. Endometrial hyperplasia, a lesion characterised by architectural alteration of endometrial glandular epithelium in endometrium, is frequently associated with hyperestrinism (i.e., exogenous estrogen use, obesity, polycystic ovarian disease). Atypical endometrial hyperplasia, when cytological atypia of hyperplastic endometrial glands exist, undoubtedly represents the precursor of many, but possibly not all, endometrioid carcinomas (type 1). This is supported by: 1) similar appearance of atypical hyperplasia and grade 1 endometrioid carcinoma, 2) atypical hyperplasia and endometrioid carcinoma are often present concurrently and in topographic proximity in hysterectomy specimens, and 3) natural history studies have demonstrated that a significant percentage of atypical hyperplasias progress to endometrioid carcinoma if untreated. Kurman et al. reported the follow-up of 170 patients with endometrial hyperplasia who were followed for at least 1 year without hysterectomy. Hyperplasia without atypia progressed to carcinoma only rarely, whereas 8% of atypical simple and 29% of atypical complex hyperplasias progressed. These results suggest that most hyperplasias without atypia
probably represent early, highly reversible lesions in the pathogenesis of endometrioid carcinoma (type 1).

In contrast, the uninvolved endometrium in uteri containing serous carcinoma (type 2) is usually atrophic and not hyperplastic. Ambros et al.\textsuperscript{11} reported that 76% of serous carcinomas were associated with atrophy and 5% with hyperplasia, whereas 29% of endometrioid carcinomas were associated with atrophy and 46% with hyperplasia. A recently described precursor lesions for serous carcinoma had been identified. This lesion was termed endometrial intraepithelial carcinoma (EIC) and it appears to represent malignant transformation of atrophic surface endometrium. In the study by Ambros et al.\textsuperscript{11}, EIC was described in 89% of uteri containing serous carcinoma.

The clinical behaviour and patient outcome also points towards two distinct tumors, Hendrickson et al.\textsuperscript{71} reported that serous carcinomas accounted for 50% of recurrences in a series of 256 clinical stage I endometrial carcinoma, even though this tumor represent and only 10% of endometrial carcinomas reviewed.

Recent molecular studies have provided additional support for this dualistic model of endometrial carcinogenesis by demonstrating differences in the molecular alterations that underlie the two types of tumors\textsuperscript{19,89,95,154}. \textit{K-ras}, \textit{p53}, \textit{PTEN}, and DNA mismatch repair genes are the most frequently altered genes in endometrial carcinoma, with a detailed description of each genetic alteration and the frequency of its occurrence in each tumor type to follow.
In summary, the proposed dualistic model of endometrial carcinogenesis incorporates two pathways that differ with respect to epidemiologic risk factors, histopathologic features, and molecular events. Based on this proposed model, type 1 tumors are indolent neoplasms that are associated with estrogen excess (i.e. obesity, exogenous estrogen therapy), proceeded by endometrial hyperplasia, of low grade endometrioid morphology, occur in relatively younger women, and exhibit a stable clinical course with good prognosis. Type 2 tumors are unrelated to estrogen excess, associated with atrophic endometrium with or without EIC, not associated with endometrial hyperplasia, typically seen in older women, of high grade serous morphology, and exhibit an aggressive course and poor prognosis.
Molecular Genetics in endometrial carcinoma:

* p53 tumor suppressor gene

p53 is a tumor suppressor gene that resides on the short arm of chromosome 17 (mapped on chromosome 17p13.1). It encodes a short lived protein (half-life 5-45 minutes) that plays a major role in suppression of cell proliferation; it can induce apoptosis or prevent cells from dividing if there is DNA damage (G₁ arrest). It mediates its action by binding to specific regions of DNA and up regulates the transcription of several target genes. p53 induced cell cycle arrest occurs late in the G₁ phase and is caused by the p53 dependent transcription of the cyclin-dependent kinase inhibitor p21. p53 also helps in the process of DNA repair by inducing the transcription of *Growth Arrest and DNA Damage (GADD45)*, a protein involved in DNA repair. If the DNA damage is repaired successfully, p53 activates the *mdm2* gene, whose product binds to and down regulates p53, thus the cell cycle arrest is released. If the DNA damage is not repaired, p53 can induce cell apoptosis by transcriptional up regulation of apoptosis-inducing genes (*bax*, and IGF-BP3). Mutation of the p53 gene diminishes the cell ability to repair damage to DNA before entry into S-phase. This can lead to a greater chance that damaged DNA will be passed on in successive generations of cells (Figure 1)³⁹,⁶⁹,⁹⁹,¹⁷³.
Ionizing radiation
Carcinogenes
Mutagens

Normal cell
(p53 normal)

DNA damage

p53 activated and binds to DNA

Transcriptional up-regulation of target genes

P21 (CDK inhibitor)

GADD45 (DNA repair)

G1 arrest

Successful repair

Normal cell

Cell with p53 mutation

DNA damage

P53 dependent gene not facilitated

No cell cycle arrest

No DNA repair

Mutant cells

Expansion and additional mutation

bax Apoptosis gene

Repair fails

Apoptosis

Malignant tumor

Fig. 1 The role of p53 in maintaining the integrity of the genome. CDK: cycline dependant kinase.
Mutant p53 protein is resistant to degradation and accumulates in the nucleus, and has a longer half-life (4-8 hours) than native protein. Using standard immunohistochemical techniques, wild type p53 protein is essentially not detected because of its short half-life. However, it should be remembered that wild type p53 protein may accumulate within cells after a number of different external stresses and be detectable when highly sensitive techniques are used. Several reports have shown a close correlation between immunohistochemical detection of overexpression of p53 and the presence of mutations in the gene. The concordance between gene mutations and overexpression is not absolute, however, and the presence of overexpression does not always indicate gene mutation and vice versa. In a large series of human carcinomas, Soong et al. found discordant information in 24% of endometrial carcinoma when immunohistochemical detection of p53 and p53 mutational analysis were compared.

Mutations in p53 gene are found in approximately 10%-20% of all endometrioid carcinoma, with most occurring in grade 3, and occasionally in grade 2 tumors. By immunohistochemistry, p53 overexpression has been detected in 17%-30% of endometrioid carcinomas. Overall, p53 mutations and/or overexpression occur in approximately 43%-50% of grade 3 tumors, and have not been identified in grade 1 tumors or endometrial hyperplasia. p53 mutation is less frequent in early stage, being shown by 9% of stage I/II tumors but by 41% at stage III/IV. These findings suggest a role for p53 mutation in the progression, but not the initiation of endometrioid carcinoma (type 1).
On the other hand p53 mutations have been identified in approximately 90% of serous carcinomas. Furthermore, approximately 75% of EIC have p53 mutations. These findings suggest that in serous carcinoma p53 mutations occur relatively early and are central to the development of this tumor type. This suggests a role for p53 mutation in the initiation of serous carcinoma, i.e. this tumor may evolve via a p53-driven pathway.

By immunohistochemistry, p53 overexpression has been detected in 71%-86% of serous carcinomas, a frequency close to the rate of p53 mutation as detected by direct sequence analysis.

**Mutations in PTEN:**

Phosphatase and Tensin homolog (PTEN) is a tumor suppressor gene located on chromosome 10q23.3 and encodes a lipid phosphatase. The primary target is the lipid molecule phosphatidylinositol 3,4,5-triphosphate (PIP3) that is involved in a signal transduction pathway that regulates cell growth and apoptosis. Germline PTEN mutations have been identified in Cowden syndrome, an inherited hamartomatous syndrome that carries increased risk of thyroid, breast, and possibly endometrial cancer. Somatic PTEN mutations have been identified in multiple tumor types including glioblastoma multiforme, prostate, breast, thyroid, and endometrial cancer. PTEN is mutated in 30-50% of sporadic endometrioid endometrial carcinomas, representing the most frequently mutated gene in this tumor, and the highest frequency of PTEN mutations in any primary tumor. The frequency of gene mutation is similar in all three grades of endometrioid carcinoma. It is also found to be mutated in approximately 20% of cases of endometrial hyperplasia. Levine et al. found PTEN
mutations in 27% of complex atypical hyperplasia associated with carcinoma and in 22% of those without concurrent carcinoma. Another study by Maxwell et al. \textsuperscript{112} found equivalent frequencies of \textit{PTEN} mutations in hyperplasia with or without atypia, 19% and 21% respectively. These findings suggest that inactivation of this gene is an important early event in the pathogenesis of endometrioid carcinoma, and possibly plays a role in initiation of at least some cases of endometrioid carcinoma.

Recent immunohistochemical studies of endometrial carcinoma and hyperplasia by using monoclonal antibody against \textit{PTEN} protein further supports the mutational studies. Mutter \textit{et al.}\textsuperscript{123} have reported 61% of endometrioid carcinomas have lost \textit{PTEN} expression by immunohistochemical staining, and 97% showed decreased expression or complete loss of \textit{PTEN} protein. Confluent groups of endometrial glands lacking \textit{PTEN} expression were found in the majority of cases of complex atypical hyperplasia.

Furthermore occasional isolated glands lacking detectable \textit{PTEN} expression were found in hyperplastic lesions without atypia. A subsequent study by Mutter \textit{et al.}\textsuperscript{122} found loss of \textit{PTEN} expression and \textit{PTEN} genetic alterations (mutations, deletions) in clusters of otherwise benign appearing endometrial glands. These studies confirm that \textit{PTEN} plays an early and central role in development of endometrial carcinoma of endometrioid type. The latter study suggests that \textit{PTEN} inactivation and/or lack of expression may occur before the development of a recognizable histopathologic lesion. \textit{PTEN} may provide a novel marker for detecting endometrial carcinoma precursor lesions such as atypical hyperplasia and even benign endometrial glands predisposed to progress to malignancy. This may provide targets for therapeutic intervention before the development of
histologically recognizable malignant lesions. Of note, PTEN mutations are almost never found in papillary serous carcinoma.

Mutations of PTEN have been identified in up to 86% of endometrioid carcinomas with microsatellite instability, suggesting a relationship between these molecular alterations, although the mutations in PTEN do not involve microsatellite sequences \(^{154}\).

**Mismatch Repair System and Microsatellite Instability:**

Microsatellites are short, highly polymorphic, repetitive sequence of DNA that are widely distributed throughout the genome and are usually in non-coding regions. These are usually less than 1kb in length and are characterised by a repeat size of 2 to 6 base pairs \(^{39,154}\). Microsatellite sequences of an individual are fixed for life and are the same in every tissue. The lengths of these repeats are maintained in normal cells by DNA mismatch repair (MMR) system. This is a fundamental cellular system responsible for detection and correction of mismatched base pairs that often develop during DNA replication. There are at least 5 human genes that encode proteins essential for MMR system (MMR genes). Among the known MMR genes, only two have been found to be inactivated in endometrial carcinoma; hMLH1 (3p21-23), and hMSH2 (2p21-22) \(^{67,82,135}\).

Microsatellite instability (MSI) refers to alterations in the length of the microsatellite repetitive sequences in tumor DNA compared with DNA from normal tissue obtained from the same person \(^1\). MSI represents a marker for defects in the mismatch repair mechanism. It takes multiple mutations in a single cell to develop cancer, which is
unlikely to occur in normal cells with intact repair mechanisms and low spontaneous mutations rates. However, MMR defects could result in increased spontaneous mutation rates, potentially permitting a cell to acquire sufficient mutations for the development of cancer.\textsuperscript{106}

MSI was first described in a subset of sporadic colorectal cancer and in tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. Endometrial carcinoma is the most common non-colorectal cancer in women with HNPCC. MSI was also found in approximately 20% of sporadic endometrioid endometrial carcinoma, but almost never found in pure serous carcinoma of endometrium\textsuperscript{21, 28, 135, 167}. MSI has been found in all three grades and in all stages of endometrioid carcinoma, a finding that suggests the possibility that MSI is a relatively early event in the pathogenesis of endometrioid carcinoma. MSI has been also found in complex atypical hyperplasia that is associated with endometrioid carcinoma, but not in lesser degrees of hyperplasia or hyperplasia without associated carcinoma. Although MSI is an early event in the pathogenesis of endometrioid carcinoma, theses findings suggest that MSI may occur late in the transition from atypical hyperplasia to carcinoma.

Malfunction in the MMR system is responsible for MSI in most cases. Only a small proportion showed mutations in \textit{hMLH1}, whereas the vast majority of \textit{hMLH1} gene inactivation is due to promoter hypermethylation\textsuperscript{135}. In some tumors, however, the cause of MSI remains unknown. The importance of \textit{hMLH1}-promoter hypermethylation is that it leads to lack of expression of the gene product, which can be demonstrated by
immunohistochemistry. Immunohistochemical studies have shown that majority of MSI-positive endometrial carcinoma lack expression of hMLH1 and lack of expression is extremely rare in MSI-negative tumors. Immunohistochemistry appears to offer a relatively convenient, specific, rapid, and thus cost-effective method of MMR analysis.

In a study by Esteller et al., 7 of 14 complex atypical hyperplasia cases showed hMLH1 promoter hypermethylation, 6 of which were associated with carcinoma. Only 4 of the 7 hyperplasias also demonstrated MSI. These findings suggest there may be an observable temporal interval between hMLH1-promoter hypermethylation and the development of MSI.

Mutation in K-ras:
K-ras is a proto-oncogene that encodes a guanine nucleotide-binding protein of 21 kDa that plays a role in the regulation of cell growth and differentiation by transducing signals from activated growth factor trans-membrane receptors. In the mutant form, K-ras is constitutively active even in the absence of activated growth factor receptor. Mutations of K-ras have been identified in approximately 20% of endometrioid carcinomas but not found in serous carcinoma. The mutations have been found in all grades of endometrioid carcinoma and have been reported in complex atypical hyperplasia, suggesting that K-ras mutations are an early event in endometrioid carcinoma pathogenesis.
Summary of Molecular Data:

In summary, molecular data support the proposed dualistic model of endometrial carcinogenesis. Type 1 endometrial carcinomas (endometrioid morphology) are associated with mutations in K-ras, PTEN, MMR genes (mainly hMLH1), and MSI, whereas type 2 carcinomas (mostly of serous morphology) are associated with p53 mutation.

Proposed Pathways for Endometrial Carcinogenesis:

Based on the accumulating data that support a dualistic model for endometrial carcinogenesis, Sherman et al.\textsuperscript{154} proposed two distinct pathways for the development of endometrial carcinoma; classic and alternative pathways.

Classic Pathway of endometrial carcinogenesis (for Type 1 tumors):

Figure 2 shows the details of the proposed classic pathway. Most of endometrioid carcinomas (type 1 tumors) develop in a setting of estrogen excess. This hormonal imbalance may result from absolute excesses of endogenous or exogenous estrogen or relative deficiencies of progesterone. Prolonged relative estrogen excess may act directly, promoting endometrial carcinogenesis by stimulating rapid proliferation of epithelial cells. However, recent studies have shown that estrogen may produce diverse effects and at least some of its action on epithelial cells may be mediated through endometrial stroma. Moreover, new data suggests that the role of estrogen in carcinogenesis is not limited to stimulating epithelial proliferation but also producing DNA damage. Therefore, relative estrogen excess could promote carcinogenesis via two mechanisms: direct and
indirect stimulation of epithelial proliferation and producing DNA damage. Development of endometrial hyperplasia, including atypical hyperplasia, is not sufficient for progression to carcinoma. Furthermore, the progression of these lesions to carcinoma, when it does occur, is neither inevitable nor rapid. Additional molecular alterations are presumably needed. As well, the acquisition of cytological atypia in hyperplasia is poorly understood and probably develops over a period of years. Molecular studies have shown that PTEN and K-ras mutations are early events in endometrial carcinogenesis. These molecular alterations have been identified in atypical hyperplasia but not in hyperplasia without atypia. It is possible that at least some cases of hyperplasia acquired PTEN and/or K-ras mutations before progression into atypical hyperplasia and carcinoma. Defect in MMR may occur in the transition between atypical hyperplasia and carcinoma, since MSI (marker of MMR defect) is noted only in atypical hyperplasia seen in association with carcinoma.

Histopathologic examination suggests that grade 3 endometrioid carcinomas develop from grade 1 tumors that have undergone clonal evolution and dedifferentiation. This process of tumor progression may be associated with loss of hormone receptor expression and p53 mutation.

Finally, it is possible that there is not always a step-wise progression as depicted in Figure 2. In some instances there may be the ability to bypass early or intermediate stages en route to development of adenocarcinoma.
Fig. 2 Classic pathway of endometrial carcinogenesis for type 1 tumors. MMR: mismatch repair, MSI: microsatellite instability.
Alternative Pathway of endometrial carcinogenesis (for type 2 tumors):

Figure 3 shows the details of the proposed alternative pathway. Serous carcinoma (type 2 tumor) typically develops in elderly women with atrophic endometrium. The only definite risk factor for serous carcinoma is aging. Although there is accumulating evidence for the existence of this second pathway for the development of serous carcinoma, distinct from molecular pathogenesis of endometrioid carcinoma, this pathway is poorly understood compared to the classic one. p53 mutation is the only genetic alteration consistently identified with this tumor and its precursor lesions (EIC).
Alternative Pathway (for type 2 tumors)

Aging

Menopause

Atrophic endometrium

?p53 mutation

Endometrial Intraepithelial carcinoma

Genetic instability

Rapid uncontrolled growth

Invasive serous carcinoma

Fig. 3 Proposed alternative pathway of endometrial carcinogenesis (type 2 tumors).
Proliferation and Apoptosis in Normal and Neoplastic Endometrium:

Endometrium is a dynamic and actively proliferating tissue that undergoes cyclic morphologic changes during the normal reproductive cycle. These changes are particularly evident in the upper two thirds of endometrial mucosa, the functionalis layer. Morphologic alterations are minimal in the lower one third of endometrium, the basalis layer. These cyclic changes consist of three morphological phases in the endometrium: proliferative, secretory, and menstrual phases. These morphological alterations are under tight control by cyclically released ovarian hormones, estradiol and progesterone. The release of these ovarian hormones is regulated through the hypothalamopituitary-ovarian axis. The ovarian steroid hormones mediate their effect on endometrial epithelium, stroma, and possibly endothelial cells through estrogen and progesterone receptors. These are nuclear receptors that bind with high affinity to estradiol and progesterone, respectively. The plasma levels of these ovarian hormones show cyclic changes corresponding to ovarian follicular maturation in one hand and the cyclic morphological phases of endometrium on the other hand.

Estradiol has a crucial role in the proliferation of endometrium. Estradiol effects are mediated indirectly by a polypeptide growth factor, epidermal growth factor (EGF). EGF promotes the transition of cells from G0 to G1 phase of cell cycle. EGF receptors have been demonstrated in both endometrial glands and stroma, with higher concentration in the glands than stroma, and concentrations parallel the cyclic fluctuation of ovarian steroid hormones.
It appears that the regulation of EGF receptor concentration is under control of ovarian estradiol and progesterone. It has been shown that EGF alone failed to induce endometrial cells proliferation, but in combination with estradiol it increased the mean gland (but not stromal) cell counts more than 50% in vitro\textsuperscript{143,174}.

The concentrations of estrogen and progesterone receptors in normal endometrium show cyclic fluctuation corresponding to the cyclic plasma levels of estradiol and progesterone. Estrogen receptors are highly expressed in glandular endometrial cells during the proliferative phase and decrease markedly in the late secretory phase, reflecting the suppressive effect of progesterone. Estrogen and progesterone receptors rise gradually to reach a peak by the time of ovulation, reflecting their induction by estradiol. They subsequently decrease in the late secretory phase, mainly in the glands, while they remain present in the stroma\textsuperscript{29}. Estradiol promotes the synthesis of both estrogen and progesterone receptors whereas progesterone inhibits the synthesis of estrogen receptors\textsuperscript{143}.

After ovulation, the proliferative endometrium undergoes rapid secretory differentiation. This is primarily due to the effect of progesterone. As shown in women with inactive ovaries, progesterone alone is able to induce full secretory transformation of the human estradiol-primed endometrium\textsuperscript{161}. The mechanism of progesterone effect is poorly understood. Insulin-like growth factor II (IGF-II) may play a role in endometrial differentiation. Transforming growth factor β (TGF-β) mRNA expression increases
progressively during the cycle, suggesting that it may contribute to inhibition of cellular proliferation.\(^{29}\)

In the last phase of the normal endometrial cycle, the menstrual phase, the endometrial mucosa rapidly degenerates and is expelled. Accumulation of highly potent proteolytic enzymes confined to membrane-bound lysosomes is observed during the first half of the postovulatory period. Coinciding with the fall of estrogen and progesterone on Day 25 of the cycle, the integrity of the lysosomal membrane is no longer maintained. Lysosomal autodigestion destroys the glandular and stromal cells and the vascular endothelium. Ischemia due to vasoconstriction of endometrial vessels is observed at the beginning of the menstrual period, and rupture of the capillaries initiates bleeding. The significant increase of prostaglandins (PGF\(_2\)) in the late secretory endometrium may also release acid hydrolases from lysosomes and enhance expulsion of degenerated endometrium by PGF\(_2\)-mediated myometrial contractions.\(^{143}\)

Parallel to these enzymatic and vascular mechanisms, apoptotic phenomena have recently been described in the menstrual endometrium.\(^{29,131,132}\) Apoptosis in human endometrium was first described by Hopwood and Levison\(^{75}\) in 1976. Their study suggested that apoptotic bodies are a useful additional marker in the dating of endometrial curettings, as they increase significantly during late secretory, premenstrual, and menstrual phases. Quantitative studies of apoptosis during the different phases of the menstrual cycle have confirmed their observations.\(^{78,163}\)
Dynamic remodelling of the endometrium results from a delicate balance of proliferation and apoptosis with specific subpopulation of stromal and epithelial cells, a process that is likely hormone dependent and plays a role in normal tissue homeostasis \(^{143}\). The apoptotic effects of steroid hormones are likely mediated through a complex network of inhibitors and initiators \(^{163}\). In normal endometrium bcl-2, an apoptosis inhibitor, displays a cycling pattern of expression, opposite to that of apoptosis, with peak expression during late proliferative phase, decreasing during late secretory and menstrual phases. The cyclic expression pattern of bcl-2 in endometrial glandular cells is related to changes in estrogen and progesterone receptors expression throughout the cycle \(^{65, 78, 132, 140}\). In contrast to glandular cells, myometrial smooth muscle cells showed consistent bcl-2 immunoreactivity throughout the menstrual cycle \(^{132}\). Progesterone has been shown to decrease endometrial secretion of bcl-2, positively promoting apoptosis by increasing levels of the apoptosis inducer BAK \(^{166}\).

In endometrial hyperplasia, bcl-2 expression was observed with a higher level comparable to late proliferative phase of normal endometrium. This is particularly true for hyperplasias without atypia. The levels of bcl-2 expression drop in atypical complex hyperplasia and carcinoma \(^{32, 70, 140, 179}\).

Down regulation of bcl-2 expression was closely related to progesterone therapy in endometrial hyperplasia and carcinoma \(^{14, 147}\). Amezecua et al.\(^{14}\) found that bcl-2 expression was lower following successful progestin treatment of endometrial hyperplasias, whereas it remained expressed in hyperplasias which persist despite
progestational therapy. This suggests that bcl-2 may represent a therapeutic target during progestational therapy in the treatment of hyperplasia. Saegusa et al.\textsuperscript{147} found significant reduction in mitotic index but not apoptotic index in endometrial carcinoma treated with prolonged progesterone therapy.

In a study by Ioffe et al.\textsuperscript{78} the proliferative activity of endometrium, as measured by mitotic index and MIB-1, showed a striking decrease from benign proliferative endometrium to simple hyperplasia. This was explained by the fact that benign proliferative endometrium has a very high proliferative rate to compensate for the monthly shedding. In endometrial hyperplasia, however, there is no regular shedding of the tissue and the net result is glandular crowding. The proliferative activity did not show significant difference between complex versus simple hyperplasia, or hyperplasia with atypia versus hyperplasia without atypia. In endometrial carcinoma the proliferative activity as measured by MIB-1 index was found to be significantly higher than proliferative endometrium and endometrial hyperplasia without atypia \textsuperscript{78,80,119,140}. There were no significant differences between atypical hyperplasia and adenocarcinoma \textsuperscript{80}. A number of studies have shown that measuring the proliferative activity of endometrial carcinoma can be useful in predicting patient outcome \textsuperscript{60,61,126,149}. Pizer et al.\textsuperscript{137} have reported higher MIB-1 index in grade 2-3 endometrial carcinoma than in grade 1 tumors, but this difference was not statistically analysed.
Apoptotic index as measured on hematoxylin and eosin (H&E) slides showed progressive increase from benign endometrial glands to dysplastic and malignant ones. The observed increase was significantly different when complex hyperplasia and endometrial carcinoma were compared. Apoptotic index was higher in hyperplastic and malignant endometrium than mitotic index, but the opposite is true for benign proliferative endometrium.\textsuperscript{78, 160}

Correlation of apoptotic index with the MIB-1 index showed higher MIB-1 index as compared to apoptotic index in endometrial hyperplasia and carcinoma, showing that the balance is in fact in favour of proliferation and net increase of cells. \textit{bcl-2} expression did not correlate with the apoptotic index in the study of Ioffe \textit{et al.} \textsuperscript{78}. This is in agreement with a study of follicular lymphoma showing that apoptotic index is independent of \textit{bcl-2} expression.\textsuperscript{162} Apoptosis is a very complex process and \textit{bcl-2} protein is only one member of a family of proteins with different roles in the regulation of cell death.\textsuperscript{78}

Risberg \textit{et al.} \textsuperscript{140} have reported a significant association between MIB-1 index and \textit{bcl-2} expression in proliferative endometrium, but this association is lost in neoplastic endometrium. They suggested that an imbalance between proliferation and apoptosis may be an important factor in the development of different endometrial lesions, benign and malignant. This is in agreement with a report by Kuwashima \textit{et al.} \textsuperscript{92} who found an inverse correlation between \textit{bcl-2} expression and MIB-1 index in endometrial carcinoma.
Staging of Endometrial Carcinoma:

Staging of cancer, in general, is based on the size of the primary lesion, extent of local spread to adjacent tissue, extent of spread to regional lymph nodes, and the presence of distant metastasis at the time of diagnosis \(^{39}\).

Staging of endometrial carcinoma is useful to determine prognosis, plan treatment, and provide a standardized method of reporting data from different centres \(^{143}\). The most recent and widely accepted staging for endometrial carcinoma is the one adopted by the FIGO \(^{42}\) (International Federation of Gynecology and Obstetrics) cancer committee in 1988 (Table 2).

Table 2. Surgical staging classification. FIGO nomenclature Rio de Janeiro, 1988

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>Tumor limited to the endometrium</td>
</tr>
<tr>
<td>Ib</td>
<td>Invasion to less than half of the myometrium</td>
</tr>
<tr>
<td>Ic</td>
<td>Invasion equal to or more than half of the myometrium</td>
</tr>
<tr>
<td>IIa</td>
<td>Endocervical glandular involvement only</td>
</tr>
<tr>
<td>IIb</td>
<td>Cervical stromal invasion</td>
</tr>
<tr>
<td>IIIa</td>
<td>Tumor invades the serosa of the corpus uteri and/or adnexae and/or positive cytological findings</td>
</tr>
<tr>
<td>IIIb</td>
<td>Vaginal metastases</td>
</tr>
<tr>
<td>IIIc</td>
<td>Metastases to pelvic and/or para-aortic lymph nodes</td>
</tr>
<tr>
<td>IVa</td>
<td>Tumor invasion of bladder and/or bowel mucosa</td>
</tr>
<tr>
<td>IVb</td>
<td>Distant metastases, including intra-abdominal metastasis and/or inguinal lymph nodes</td>
</tr>
</tbody>
</table>
The first FIGO staging for endometrial carcinoma was adopted in 1950 and was used for approximately 10 years. This early staging was a simple system based on two criteria. The first was whether the cancer was confined to uterine corpus or had spread beyond corpus. The second was whether the patient was medically operable or not. Stage I included tumor confined to the corpus, and stage II indicated spread beyond the uterus. Then these stages were subdivided into whether the patient was operable or not. The spread of the tumor was defined by clinical examination and included the findings at the time of diagnostic dilatation and curettage with endocervical curettage.

In 1961, the FIGO Committee on Cancer added a special stage for cervical involvement and one for extrauterine spread that was confined to the pelvis. Stage IV was added for bladder and/or rectal mucosal involvement or spread beyond the pelvis.

The FIGO Committee on Cancer in 1971 changed the staging by subdividing Stage I into Ia and Ib based on the depth of the uterine cavity. The measurement of the uterine cavity was determined by sounding the uterus, from the external cervical os to the top of interior portion of uterus. Stage Ia was defined as a measurement of less than 8 cm, and stage Ib as greater than 8 cm. Also, the grading of the tumor was included in the staging and was based primarily on the degree of glandular versus non-glandular components. Stages II, III, and IV were essentially unchanged from 1962. This staging is basically a clinical staging system since it was based on clinical data obtained from examination, sounding of uterine cavity, and findings on fractional dilatation and curettage.
In the following years, there came to be general recognition of risk factors that are now thought to be essential in the management of patients with endometrial carcinoma and were not included in the 1971 FIGO clinical staging. These factors include depth of myometrial invasion, cervical extension, pelvic node metastases, adnexal metastases, penetration of uterine serosa, and positive peritoneal cytological findings. Recognizing the advances in information with respect to natural history of endometrial carcinoma and the influence of this on survival, the FIGO cancer committee in 1988 decided to develop a staging system incorporating this new information. This resulted in the currently used staging system, widely used as of January 1989 (Table 2). This is a surgicopathologic staging system. It is evident that the pathologic findings could be altered by pre-operative radiation therapy, that might modify the appropriate delineation of the surgicopathological findings. This new staging assumes that most patients will be treated by a primary surgical approach. If prior radiation has been used or no surgical evaluation was feasible, the clinical staging system of 1971 is applied\textsuperscript{115}.

As a result of the changes in staging, the fractional curettage, used in earlier staging, is no longer necessary. It is recommended that the depth of myometrial invasion is identified in relation to the full thickness of the myometrium, on the hysterectomy specimen\textsuperscript{40,115,143}. Tumor in vascular spaces beyond the deepest point of invasion should not be used for this measurement. Involvement of adenomyosis with carcinoma should not be counted as myometrial invasion\textsuperscript{143}.
The majority of patients diagnosed with endometrial carcinoma have FIGO stage I disease. In a population-based study of women with endometrial carcinoma, 81% of tumors were stage I, 11% were stage II, 6% were stage III, and 2% were stage IV.
Grading of Endometrial Carcinoma:

Cancer grading, in general, assesses the degree of tumor differentiation i.e. how closely the tumor resemble its tissue of origin. Accordingly tumors are classified into grades that are marked by increasing anaplasia. The most widely used grading system for endometrial carcinoma is the FIGO system. The importance of grading endometrial carcinoma in predicting patient outcome has been recognized for many years. For this reason, the Cancer Committee of FIGO added histological grading to the staging classification for stage I tumors in 1973. Stage I tumors were subdivided into three grades: highly differentiated adenomatous carcinoma as grade 1, differentiated adenomatous carcinoma as grade 2, and predominantly solid or entirely undifferentiated carcinoma as grade 3. In 1982, grading was applied to tumors of all stages, and in 1988, the grading was more precisely defined. For endometrioid endometrial carcinoma the 1988 FIGO grading system is based primarily on the tumor architectural growth and secondarily on nuclear criteria. The architectural component of this grading is specifically based on the amount of nonsquamous solid growth (Table 3).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Growth Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5% or less of solid growth</td>
</tr>
<tr>
<td>2</td>
<td>6-50% solid growth</td>
</tr>
<tr>
<td>3</td>
<td>More than 50% solid growth</td>
</tr>
</tbody>
</table>

*exclusive of areas of squamous differentiation
Marked differences in architectural grade can be seen within a tumor. When a tumor displays this type of heterogeneity, the architectural grade should be based on the overall appearance\textsuperscript{143}.

The nuclear grade is based on nuclear size and shape, chromatin distribution, and the size of nucleoli. Grade 1 nuclei are small or mildly enlarged (approximately two to three the times the diameter of a lymphocyte), oval in shape, with little variation in size and shape, have evenly dispersed chromatin, and infrequent indistinct nucleoli. Grade 3 nuclei are markedly enlarged (more than six times the diameter of a lymphocyte), markedly pleomorphic, with coarse, clumped and sometimes smudged chromatin, and frequent prominent nucleoli. Grade 2 nuclei have features intermediate between those of grade 1 and 3. The nuclear grade is based on the grade of the majority of the nuclei\textsuperscript{143,186}.

The 1988 FIGO grading system recommends that tumors be graded using both architectural and nuclear criteria. The tumor is graded primarily by architecture with the overall grade modified by the nuclear grade when there is discordance. The grade of tumors that are architecturally grade 1 or 2 are increased by one grade in the presence of a notable degree of nuclear atypia, inappropriate for the architectural grade\textsuperscript{42,186}. For example, a tumor that is grade 2 by architecture but in which there is marked nuclear atypia (grade 3 nuclei) should be upgraded to grade 3. Serous and clear cell histological subtypes are graded based on their nuclear grade only. Most of these tumors have grade 3 nuclei and only few have grade 2 nuclear atypia\textsuperscript{42}. 

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Despite its wide use, there are several pitfalls of the FIGO grading system that make it difficult to apply. Determining whether solid growth is squamous or nonsquamous can at times be difficult. In addition, determining whether solid nonsquamous growth comprises less than or greater than 5% of the tumor is often problematic and arbitrary. Defining what represents notable nuclear atypia is particularly subjective. Since grading of endometrial carcinoma not only has prognostic significance, but plays a role in subdividing patients into treatment groups, particularly for patients with stage I tumors, an explicit grading system with unambiguous definitions of criteria for categories is mandatory.

Several studies have looked at the reproducibility of 1988 FIGO grading. Neilsen et al. found that the interobserver reproducibility was acceptable for the architectural grade (kappa value 0.70) but unacceptably low for the assessment of notable nuclear atypia (kappa value 0.55). Both intraobserver and interobserver reproducibility for the combined FIGO grading was acceptable (kappa values 0.65 and 0.66), but it is obvious that this is a primarily a reflection of the architectural component of the grade. Another study comparing FIGO grading to pure nuclear grading of endometrioid carcinoma demonstrated fair interobserver reproducibility for FIGO grading but only poor interobserver reproducibility for nuclear grading. Zaino et al. examined the utility of FIGO grading by using a database of 715 patients with low clinical stage endometrioid endometrial carcinoma. Patients with architectural grade 1 or 2 carcinomas but with predominantly grade 3 nuclei were moved up one grade. The risk of recurrence and death for those upgraded patients was similar to that of the other patients in the grade into
which they were reassigned. This study provided support for the FIGO modification of architectural grade based on inappropriate nuclear atypia and reinforced the need for a uniform definition of nuclear grade\textsuperscript{143,186}.

It thus seems reasonable to study methods to improve reproducibility and prognostic power of endometrial carcinoma grading. Several studies proposed either a modification of the current FIGO grading or a new grading system. A quantitative and morphometric method has been shown to provide reproducible information by examining the mean of the shortest nuclear axis of tumor cells\textsuperscript{16,127}. A semiquantitative nuclear grading has also been suggested based on the proportion of nuclei with large nuclear shortest axes\textsuperscript{68}. Morphometric analyses are labour intensive and the equipment and technical expertise are not widely available.

The clinical utility of the intermediate grade (grade 2) of the three part grading system has been questioned\textsuperscript{169}. A simplified grading system was proposed by Lax \textit{et al.}\textsuperscript{94} who suggested a two tiered grading system. This binary system divides endometrioid carcinoma into two grades, high and low, based on the presence of greater than 50\% solid growth, tumor cell necrosis, and diffusely infiltrative rather than pushing pattern of myometrial invasion; high grade tumors display at least two of these three features whereas low grade tumor have at most one.
Behaviour and Prognosis of endometrial carcinoma:

Behaviour of endometrial carcinomas varies according to the tumor cell type or, more precisely, according to the histopathogenetic type. Endometrioid carcinoma, the prototype of type 1 endometrial carcinoma, spreads by lymphatic and vascular dissemination, direct extension to contiguous organs, and transperitoneal and transtubal seeding. Lymphatic metastasis is more common than hematogenous spread, but involvement of the lungs without metastasis to mediastinal lymph nodes indicates that hematogenous spread may occasionally occur early in the course of the disease. The tumor tends to spread to the pelvic lymph nodes before involving paraaortic lymph nodes and transperitoneal spread tends to occur late in the course of the disease 143.

The majority of endometrioid carcinomas are limited to the uterine corpus (FIGO stage I) at the time of presentation (81% of tumors in one population based study) 4,145. These tumors tend to be low FIGO grade (grade 1 or 2) at time of diagnosis (ranging from 74% to 83%) 4,94. These stage I, low grade tumors have an excellent prognosis. In a Gynecologic Oncology Group (GOG) study the 5-year survival for endometrioid carcinoma of FIGO stage Ia was 94% 183. Using the current FIGO staging system, another report found the 5-year disease free survival was 90% for stage I, 83% for stage II, and 43% for stage III patients 110.

Serous carcinoma of endometrium, the prototype of type 2 tumors, has a propensity for myometrial and lymphatic invasion. The hysterectomy specimen often discloses tumor in lymphatic involvement within the myometrium, cervix, broad ligament, fallopian tube,
and ovarian hilus. It is similar to its ovarian counterpart in its propensity for transperitoneal metastasis. Involvement of peritoneal surfaces in the pelvis and abdomen occurs early in the course of the disease. In addition to intraperitoneal spread, serous carcinoma can metastasize to the liver, brain, and skin. Compared to endometrioid carcinoma, serous carcinoma is associated more frequently with lymphatic invasion, deep myometrial invasion, and lymph node metastasis. Most studies have reported that serous carcinoma is clinically understaged in approximately 40% of cases. Cirisano et al. have reported upstaging to surgical stage III-IV occurred in 47% of serous carcinomas, and 39% of clear cell carcinomas, compared to only 12% of endometrioid carcinomas.

Serous carcinoma is more frequently, as compared to endometrioid carcinoma, found at higher stage (stage III and IV) at the time of presentation. Extraperitoneal metastasis was reported in 55% of serous carcinomas and 45% of clear cell carcinomas which were confined to the inner half of myometrium compared, to 17% of grade 3 endometrioid carcinomas.

The 5-year actuarial survival rate for all stages of serous carcinoma was reported as 36%. The range reported for 5-year survival rates for patients with clinical stage I and II serous and clear cell carcinomas was 44-72%. Cirisano et al. found the 5-year survival rate for patients with stage Ia tumors was 57%, and 53% for patients with stage Ib or Ic tumors.
Prognostic Factors for Endometrial Carcinoma:

Several prognostic factors for patients with endometrial carcinoma have been detected; the importance of each factor in predicting the patient’s outcome is quite variable. Some of these factors are highly significant and independent of others prognosticators in predicting the patient’s outcome. On the other hand, some other factors have lower significance in predicting the patient’s outcome and their significance is lost in multivariable analyses when the patient’s survival is adjusted for other prognosticators.

Prognostic factors can be broadly divided into three main groups: clinical factors, surgicopathological factors, and biological and molecular markers (Table 4).

**Clinical Factors:**

Age, race, and socioeconomic status are prognostic factors in endometrial carcinoma. Age was found to be the most important prognostic factor for patient survival independent of FIGO stage, grade, patient race, and socioeconomic status \(^{143}\). Younger patients have better outcomes than older patients. A recent study has shown significantly decreased survival for patient older than 50 years of age, independent of surgical stage or grade of the tumor \(^{54}\). Another recent study has shown that age was a significant predictor for tumor recurrence in univariate analysis but not independent of other predictors in multivariate analysis \(^{121}\). In the United States, white women have a significant survival advantage when compared to African-American women even after adjusting the survival for other prognosticators.
Surgicopathological Factors:

Several important prognostic factors fall in this category. The most important is the FIGO stage. The prognostic utility of the revised 1988 FIGO surgical-pathological staging of endometrial carcinoma has been confirmed in several studies of large number of patients using both univariate and multivariate analyses.

The prognostic significance of the individual components of the revised FIGO staging has been also documented. These include myometrial invasion, uterine cervix involvement, peritoneal cytology, and lymph node metastasis. Among those, myometrial invasion has received a great deal of attention as an independent prognostic factor. Both the depth of myometrial invasion and pattern of invasion have been examined. Depth of myometrial invasion independent of tumor grade, is an important predictor of prognosis. In fact, it probably is the single most important predictor of behaviour of stage I endometrioid carcinoma. Diffusely infiltrative endometrial carcinoma with little or no stromal response has been termed adenoma malignum. Although two earlier studies suggested that this pattern might have adverse prognostic significance, a larger more recent study found it did not. Lax et al. have recently described two patterns of myometrial invasion, diffusely infiltrative, and expansive growth patterns, and shown that the former was associated with adverse outcome.

Histologic grade is among the most important predictors of patient outcome in endometrial carcinoma, and this is particularly true for early stage tumors (stage I and II). Surprisingly, this has been true irrespective of the method for assignment of grade,
including grading based primarily on architectural pattern, nuclear grading, and nucleolar size. 

The histologic type of endometrial carcinoma is a prognostic factor for survival. When compared on stage for stage basis, serous and clear cell carcinomas have more aggressive course and adverse outcome than endometrioid or mucinous carcinoma. The presence of a tiny component of serous or clear cell carcinoma in endometrioid carcinoma may have an adverse prognostic significance, even if this component is less than 10% which is the cut-off of mixed tumor according to WHO classification.

Other pathological factors of prognostic significance include: lymphovascular invasion, perivascular lymphocytic infiltrate, and coexistence of endometrial hyperplasia. Significant correlation between lymphovascular invasion and patient survival, independent of tumor grade and depth of myometrial invasion has been reported. Perivascular lymphocytic infiltrate is frequently associated with vascular invasion and hence is a useful marker of lymphovascular invasion. The presence of perivascular lymphocytic infiltrate has also been shown to be an independent adverse prognostic marker in some studies but was not as strong as lymphovascular invasion in one study. Coexistence of endometrial hyperplasia was found to be a marker of favourable outcome but it is probably it is related to the common association of this precursor lesion with low grade endometrioid carcinoma.
Biological and Molecular Markers:

Several biological and molecular markers have been identified in recent years and at least some of them have shown a strongly significant association with patient survival and/or tumor recurrence. Other markers are better described as adjuvant prognosticators in endometrial carcinoma, since they show only a weak association with patient outcome and their prognostic significance is lost when the analysis of survival is adjusted for other important prognostic factors.

Markers of adverse outcome include tumor aneuploidy, loss of estrogen (ER) and progesterone receptor (PR) expression, loss of bcl-2 expression, p53 and HER-2/neu overexpression, and high mitotic and MIB-1 indices.
Table 4. Prognostic Factors for Endometrial Carcinoma

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Associated with adverse outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Older patient (&gt;60 year old)</td>
</tr>
<tr>
<td>Race</td>
<td>African-American Low status</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
</tr>
<tr>
<td><strong>Surgicopathological Factors</strong></td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td>Higher stages (stage III and IV)</td>
</tr>
<tr>
<td>Stage components:</td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>Deep invasion</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td>presence</td>
</tr>
<tr>
<td>Peritoneal cytology</td>
<td>positivity</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>positivity</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>Higher grade (grade 3)</td>
</tr>
<tr>
<td>Histologic type</td>
<td>Serous and clear cell carcinoma</td>
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<tr>
<td>Lymphovascular invasion</td>
<td>presence</td>
</tr>
<tr>
<td>Perivascular lymphocytic infiltrate</td>
<td>presence</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td>absence</td>
</tr>
<tr>
<td><strong>Biological and Molecular Markers</strong></td>
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<td>DNA ploidy</td>
<td>Aneuploid</td>
</tr>
<tr>
<td>ER &amp; PR receptors</td>
<td>absence</td>
</tr>
<tr>
<td>bcl-2 expression</td>
<td>loss</td>
</tr>
<tr>
<td>Apoptotic bodies</td>
<td>increase</td>
</tr>
<tr>
<td>p53 immunoreactivity</td>
<td>overexpression</td>
</tr>
<tr>
<td>HER-2/neu immunoreactivity</td>
<td>overexpression</td>
</tr>
<tr>
<td>Markers of proliferation</td>
<td>Elevated</td>
</tr>
<tr>
<td>Mitotic index</td>
<td></td>
</tr>
<tr>
<td>MIB-1 (Ki-67) index</td>
<td></td>
</tr>
<tr>
<td>AgNOR counting</td>
<td></td>
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<tr>
<td>S-phase fraction</td>
<td></td>
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<tr>
<td>Proliferating cell nuclear antigen (PCNA)</td>
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</tbody>
</table>
Treatment Guidelines for Endometrial Carcinoma:

Surgery is the primary treatment for 92% to 97% of women with endometrial carcinoma. Once the diagnosis of endometrial carcinoma is confirmed by dilatation and curettage, the patient should be preoperatively evaluated. The pathology report for the dilatation and curettage specimen should include the tumor grade, subtype, and whether or not there is cervical involvement, if fractional dilation and curettage was performed. This information is necessary to define proper treatment for the patients.

At time of surgery, peritoneal cytologic sampling, abdominal exploration, palpation and biopsy of any suspicious lymph nodes or lesions, and abdominal hysterectomy and bilateral salpingo-oophorectomy are performed. Pelvic and para-aortic lymphadenectomy is appropriate when any of the followings are present: serous, clear cell, or undifferentiated carcinoma, grade 3 endometrioid carcinoma, cervical involvement in fractional dilatation and curettage, evidence of extrauterine spread, greater than 50% myometrial invasion, or palpably enlarged lymph nodes. Several studies have shown that the depth of myometrial invasion can be assessed by gross inspection and possibly intraoperative frozen sections. The overall accuracy of the intraoperative frozen sections examination for identifying high-risk pathological factors is 94%, as compared with a sensitivity of gross inspection alone of 71%.

On the basis of clinicopathological prognostic factors and surgical staging of endometrial carcinoma, patients are classified into 3 risk groups (treatment groups) reflecting their
risk of recurrence and/or death, and postoperative adjuvant therapy should be considered in this context (Table 5) \(^{145}\).

**Table 5.** Classification of the risk of recurrence in women with endometrial carcinoma

<table>
<thead>
<tr>
<th>Low risk</th>
<th>Intermediate risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I, grade 1 or 2 non-serous and non-clear cell adenocarcinoma with invasion of the inner half or less of myometrium (Stage Ia or Ib only).</td>
<td>Stage Ia or Ib, grade 3 non-serous and non-clear cell carcinoma or Stage Ic, of any grade non-serous non-clear cell carcinoma.</td>
<td>Stage II, III, or IV disease or serous or clear cell carcinoma.</td>
</tr>
</tbody>
</table>

No further adjuvant therapy is recommended for low risk group. Adjuvant radiation therapy is indicated for patients in the other two groups. After the primary surgical treatment, the extent of disease can be determined and the field for radiation therapy can be more appropriately tailored to treat the pelvis, the pelvis and para-aortic region, or the whole abdomen \(^{145}\).

In view of the highly aggressive behaviour of serous carcinoma of endometrium, adjuvant therapy should be considered for all these patients except those that qualify as having minimal uterine serous carcinoma. EIC or minimal uterine serous carcinoma (stage Ia with less than 1 cm of carcinoma in endometrium and no vascular invasion) can
be adequately treated with surgery alone. Patients with stage Ia serous carcinoma or serous carcinoma confined to an endometrial polyp, who are carefully surgically staged, have a good outcome when treated by surgery alone, and the need for further therapy for this group of patients is questionable. Whole abdominal radiotherapy is the adjuvant therapy offered to patients with early stage (stage I and II) serous carcinoma at BCCA. A recent study has shown this to be associated with improved patient outcomes compared to radiotherapy less than whole abdominal radiotherapy.

The use of hormonal therapy in endometrial carcinoma is controversial. Serous tumors are unresponsive to this therapy, and almost always lack expression of hormone receptors. The use of adjuvant progestational therapy has been studied in five randomized trials; no benefit has been demonstrated. A review of estrogen replacement in patients with stage I or II endometrial carcinoma found no difference in progression-free or overall survival associated with estrogen use.

Preoperative radiation therapy followed by hysterectomy has become less popular because it may overtreat women who have less extensive disease, and it makes the pathological evaluation of the uterus more difficult. Primary radiation therapy is reserved for women for whom the risks of surgery are high, including very elderly women and those with multiple medical problems, who together make up 4 to 9 percent of women with endometrial carcinoma. In this high-risk group, up to 36 percent of patients die of intercurrent disease. A case–control study with matching according to clinical stage and tumor grade found no significant difference in survival between women treated...
surgically and those who received primary radiation therapy. Improved intraoperative and postoperative care has made it possible to treat all but a few women surgically.
CHAPTER 2

Early Stage Uterine Papillary serous Carcinoma of Endometrium:

Pathological Predictors of Patient Outcome
OBJECTIVE AND HYPOTHESIS

Papillary Serous Carcinoma of Endometrium (PSCE) is an aggressive subtype of endometrial carcinoma even at an early stage. Patients with early stage tumors may benefit from adjuvant therapy that could reduce the risk of tumor recurrence and/or prolong patient survival. Identification of pathological factors that can predict patient outcome would help in better tailoring adjuvant therapy. This is achieved by reducing unnecessarily aggressive therapy, with potential adverse side effects, for patients having less aggressive tumors and, on the other hand, using appropriately aggressive therapy in patients who are at high risk of recurrence.

Pathological factors such as depth of myometrial invasion, lymphovascular invasion, and FIGO grade and stage were found to be of prognostic value in endometrioid carcinoma. The value of these factors in predicting the tumor behaviour has not been firmly established in PSCE. Furthermore, p53 overexpression is seen occasionally in low grade endometrioid carcinoma, and it has been widely reported to be strongly correlated with the aggressive tumor behaviour. Overexpression of p53 appears to arise very early in the natural history of PSCE and to occur with equally high frequency in early and late stages. It is unclear whether overexpression of p53 is associated with an adverse prognosis in PSCE.

One more factor evaluated in this study is the prognostic value of percentage of papillary serous carcinoma component in endometrial carcinoma. It is not uncommon to find a small component of papillary serous carcinoma within a tumor containing other less
aggressive variants of endometrial carcinoma. This component could represent either more than 10% of the tumor bulk to qualify the tumor as mixed type according to the WHO classification or less than 10%. It is unclear whether endometrial carcinoma that harbours less than 10% of papillary serous carcinoma component has a similar aggressive behaviour as does tumors with larger fraction of papillary serous carcinoma.
MATERIALS AND METHODS

The British Columbia Cancer Agency (BCCA) is a regional cancer center with a catchment population of approximately 4 million people. Patients who have early stage endometrial carcinoma usually undergo investigations, staging, and total abdominal hysterectomy including bilateral salpingo-oophorectomy in their home community by general gynecologists. Pelvic and para-aortic lymphadenectomy are not done routinely. Pathology is reviewed centrally at our institution, and treatment recommendations are made accordingly. Between 1985–1995, there were 87 cases of PSCE limited to the uterus with or without positive washings (Stage I, II, or IIIa on the basis of positive washings). Of these, 2 had PSCE in the endometrial curetting specimen only and no residual tumor was found on hysterectomy, 2 had concurrent ovarian carcinomas, 3 had relapsed before referral to our center, and 2 had radiologically detected metastases, and these patients were excluded from the study. The remaining 78 patients formed the basis of this study. Patient records of these 78 patients were reviewed. Median follow up was 52 months (3–139 months) and median age at diagnosis was 68 (42–93) years. Stage distribution was Ia =10, Ib =31, Ic =16, IIa =11, IIb =6, IIIa washings only 4. Initial pathologic diagnosis of cancer was usually made by dilatation and curettage (60 cases, 6 cases by endometrial biopsy, and 12 unknown), but the diagnosis of PSCE was recognized before hysterectomy in only 16 cases. Peritoneal washings were performed in 45 patients and were positive in 4 patients. Eleven patients had lymph node biopsy or dissection; all were negative. By policy, patients who had completely debulked Stage I, II, or IIIa washings only PSCE were offered adjuvant radiotherapy.
Hematoxylin and eosin stained slides were retrieved from community hospitals if possible; slides from 65 patients were available for review. Blinded to patient outcome, these were reviewed for percentage papillary serous component of the tumor, grade, depth of myometrial invasion, lymphatic or vascular invasion, and cervical involvement. Paraffin-embedded blocks and/or unstained slides were available from 49 patients for p53 immunostaining. The avidin-biotin (ABC) method was used for immunostaining and applied to formalin-fixed and paraffin-embedded tissue. Serial sections of the paraffin blocks were cut at 3μm thickness, deparaffinized with xylene, and rehydrated through a series of graded alcohols. The immunostaining procedure was performed using an automated stainer (Ventana, Tucson, AZ). The primary antibody used was the mouse monoclonal anti-p53 antibody clone DO-7 (Dako, 1:400). The anti-p53 antibody DO-7 recognizes both wild type and mutant p53. In contrast to wild-type p53, which is relatively unstable and expressed in small amounts, mutant p53 is overexpressed, and therefore remains longer in the nucleus. Sections were counterstained with light hematoxylin. Nuclear staining of < 5% of nuclei was considered negative, while ≥ 5–50% was considered weak positive and > 50% strong positive.

The cause of death was known for all patients. Two patients who died of possible treatment-related complications were considered to have died free of disease. Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL). Survival was calculated using the Kaplan–Meier method, and survival differences were determined using log-rank test. Multivariate analysis was performed using Cox regression.
RESULTS

Actuarial 5-year disease-specific survival of all 78 patients was 66.6% (Figure 4).

Twenty-seven patients relapsed with a median of 15 months (range, 3–96 months) after hysterectomy. Site of initial failure is listed in Table 6. Site of failure was within the abdomen or pelvis in 21 patients whereas 9 patients had relapse outside the peritoneal cavity. Five of the recurrences were isolated initially at the vaginal vault, but none of the relapsed patients was cured; at the time of analysis, one relapsed patient was alive with disease and all others had died of their disease. We were able to retrieve initial hysterectomy slides in 65 cases and tissue for p53 immunostaining in 49 cases. These 65 cases were representative of the entire 78 patient cohort with no significant differences in stage distribution or overall survival. Thirty-eight out of 49 tumors (77.6%) showed p53 overexpression. Lymphatic invasion, depth of myometrial invasion, tumor grade, and patient age were not predictive of outcome on univariate analysis and not subjected to a subsequent multivariate analysis (Figures 5-7). Tumor p53 overexpression and percent of papillary serous component of the tumor (≤ 10% versus >10%) were not predictive of disease specific or overall survival (p= 0.91 and 0.17, respectively) (Figures 8 & 9).

Patients with FIGO Stage I tumors had a significantly improved disease specific survival compared to patients with FIGO Stage II tumors (p= 0.03) (Figure 10).
DISCUSSION

A great deal of work has gone into defining the clinical, microscopic, and, more recently, molecular characteristics of PSCE. The importance of defining patients who have PSCE and who are at significant risk for relapse and then delivering effective adjuvant therapy to them were recognized as important clinical problems by Hendrickson et al. However, these problems have been difficult to address because of the relative rarity of PSCE.

Virtually all authors have noted the association between PSCE and deep myometrial invasion, lymphovascular involvement, and positive peritoneal cytology, but the prognostic value of these pathologic findings in the setting of PSCE have yet to be validated as fully as they have been with endometrioid endometrial carcinoma. Several studies have documented the prognostic significance of myometrial invasion and lymphovascular involvement in endometrioid carcinoma; they have been shown to be an independent predictor of outcome for women with early stage tumors. The survival of patients who had Stage I–II PSCE has been reported to be 40–100% in various study series. Several authors have emphasized the importance of complete surgical staging including lymphadenectomy, as up to 58% of clinical Stage I PSCEs may be upstaged surgically. Gitsch et al. and Rosenberg et al. reported no relapses in 6 Stage I–II and 10 Stage I patients, respectively, who were surgically staged. In contrast, Chambers et al. found that fewer than half of 15 surgically Staged I–II PSCE patients survived 5 years. Unfortunately, the absence of extrauterine disease, even
with aggressive surgical staging, does not appear to predict reliably for freedom from relapse.

Intuitively, there may be some prognostic indicators that identify tumors with a low enough risk of recurrence that adjuvant therapy may not be necessary. In Hendrickson's original series, four patients who had no demonstrable myometrial invasion and no extrauterine disease on surgical staging did not relapse. In contrast, Carcangiu and Chambers reported that 19 patients whose hysterectomy specimens showed PSCE confined to an endometrial polyp or the endometrial mucosa did no better than Stage I patients who had myometrial invasion. However, more than half of these patients received preoperative radiotherapy, which might have affected the pathologic findings in the hysterectomy specimens. In another study by Carcangiu and Chambers the overall estimated survival for 13 surgically staged stage Ia was 83%. Two of these patients died of the disease with intraabdominal carcinomatosis at 10 and 14 months after presentation. Although their overall survival rate was relatively showing favorable prognosis, absence of myometrial invasion may be associated with recurrence and death even at short follow up. Similar findings were also found in a recent study by Gehrig et al. in which two of six patients with surgically staged stage Ia serous carcinoma had recurrence despite absence of lymphovascular invasion.

In the current study, we found that, overall, patients with Stage I tumors fared significantly better than those who had Stage II tumors, with a 5-year disease-specific survival of 72% versus 51% (p= 0.03). The subgroups of Stage I PSCE patients who have
minimal myometrial invasion or who have disease removed at curetting appear to do particularly well. Neither of our two patients, who were previously reported, with disease removed by uterine curetting and who had no residual disease at hysterectomy have relapsed. Seven of 10 patients who had Stage Ia PSCE remained free of recurrence in this study. These observations lend support to the use of the endometrial FIGO staging system for PSCE.

If tumor bulk is prognostically important, then we should expect that patients who have a small absolute volume of PSCE or only a small component of PSCE in their tumors may have a better than average outcome. To test this hypothesis, we quantified the percent of papillary serous component of the tumor as \( \leq 10\% \) or as \( >10\% \) in our 65 pathology reviews. Of 9 patients who had papillary serous component of \( \leq 10\% \), 6 received whole abdominal radiotherapy, and only 1 died of disease, whereas 1 other died of unrelated causes during observation period. Of 56 patients who had papillary serous component \( >10\% \), 41 received whole abdominal radiotherapy, 21 relapsed, and 2 died of intercurrent illness. Whereas there were inadequate numbers of patients to demonstrate a statistically significant outcome difference \( (p=0.17) \), the absolute difference in relapse rate between these two groups suggests that this parameter is worthy of further investigation.

Carcangiu and Chambers \(^{25}\) examined a similar concept by quantifying the endometrioid component in PSCE tumors. This measure, however, did not predict outcome in their study.
Overexpression of p53 has been widely reported to occur more frequently in PSCE than in endometrioid endometrial carcinoma\textsuperscript{85,189}. The frequency of p53 overexpression detected by using immunohistochemistry ranges from 71\% to 86\% of cases of PSCE. In this study p53 overexpression was seen in 77.6\% of tumors. It is unknown what proportion of the remaining non-overexpressors will harbour nonsense p53 mutations. Some authors even have suggested that a p53 mutation may be necessary for a diagnosis of PSCE. King \textit{et al}.\textsuperscript{85} and Bancher-Todesca \textit{et al}.\textsuperscript{17} have found p53 overexpression to be prognostically important in series of patients who had all stages of PSCE. Some authors have not shown a predictive value to p53. In our study, p53 overexpression was not predictive of outcome. This statistical result, as in many other studies, might have been related to the high incidence of p53 overexpression (38 of 49 patients) and the consequent paucity of events in patients with p53 negative tumors.
### Table 6. Site of PSCE relapse

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>Time to relapse</th>
<th>Relapse site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>13.7</td>
<td>ascites</td>
</tr>
<tr>
<td>Ia</td>
<td>27.8</td>
<td>vaginal vault</td>
</tr>
<tr>
<td>Ia</td>
<td>9.7</td>
<td>vaginal vault</td>
</tr>
<tr>
<td>Ib</td>
<td>41.4</td>
<td>groin</td>
</tr>
<tr>
<td>Ib</td>
<td>19.8</td>
<td>lung</td>
</tr>
<tr>
<td>Ib</td>
<td>19.2</td>
<td>pelvis/retroperitoneum</td>
</tr>
<tr>
<td>Ib</td>
<td>45.4</td>
<td>lung</td>
</tr>
<tr>
<td>Ib</td>
<td>8.1</td>
<td>ascites/pleural effusion</td>
</tr>
<tr>
<td>Ib</td>
<td>76</td>
<td>pleura</td>
</tr>
<tr>
<td>Ib</td>
<td>24.9</td>
<td>paraaortic nodes</td>
</tr>
<tr>
<td>Ic</td>
<td>8.1</td>
<td>vaginal vault/ascites</td>
</tr>
<tr>
<td>Ic</td>
<td>6.8</td>
<td>pericardial effusion</td>
</tr>
<tr>
<td>Ic</td>
<td>19.4</td>
<td>pelvic nodes/lung</td>
</tr>
<tr>
<td>Ic</td>
<td>13.3</td>
<td>vaginal vault</td>
</tr>
<tr>
<td>Ic</td>
<td>20.7</td>
<td>pelvis</td>
</tr>
<tr>
<td>Ic</td>
<td>12.3</td>
<td>pelvis/retroperitoneum/liver</td>
</tr>
<tr>
<td>Ic</td>
<td>96.3</td>
<td>suburethra/distal vagina</td>
</tr>
<tr>
<td>IIa</td>
<td>6.2</td>
<td>vaginal vault</td>
</tr>
<tr>
<td>IIa</td>
<td>12.4</td>
<td>pelvis</td>
</tr>
<tr>
<td>IIa</td>
<td>15</td>
<td>vaginal vault/paraortic nodes/omentum</td>
</tr>
<tr>
<td>IIa</td>
<td>5</td>
<td>bowel serosa</td>
</tr>
<tr>
<td>IIb</td>
<td>23</td>
<td>vaginal vault</td>
</tr>
<tr>
<td>IIb</td>
<td>14.8</td>
<td>serosa of duodenum</td>
</tr>
<tr>
<td>IIb</td>
<td>19.3</td>
<td>ascites</td>
</tr>
<tr>
<td>IIIa</td>
<td>3.1</td>
<td>ascites/liver</td>
</tr>
<tr>
<td>IIIa</td>
<td>72.2</td>
<td>RUQ abdomen/lung</td>
</tr>
<tr>
<td>IIIa</td>
<td>12.6</td>
<td>left supraclavicular node</td>
</tr>
</tbody>
</table>
Fig. 4 Disease specific survival of all cases of PSCE
Fig. 5 Disease specific survival by lymphovascular invasion.
Fig. 6 Disease specific survival by myometrial invasion.
Fig. 7 Disease specific survival by substaging of FIGO stage I.
Fig. 8 Disease Specific Survival of PSCE by p53 overexpression
Fig. 9 Disease Specific survival by percentage of papillary serous carcinoma component in the tumor.
Fig. 10 Disease Specific survival by FIGO stage.

p=0.03
CHAPTER 3

Markers of Proliferative Activity are Predictors of Patient Outcome for Low Grade Endometrioid Adenocarcinoma but not Papillary Serous Carcinoma of Endometrium
OBJECTIVE AND HYPOTHESIS

As described in the introduction, two types of endometrial carcinoma can be recognized. These two types differ with regard to their epidemiological risk factors, molecular events, morphological features, and pathogenesis. In this chapter the difference between these two types of tumors with regard to the tumor proliferative activity is examined.

We hypothesized that proliferative activity would be higher in type 2 than type 1 endometrial carcinomas, and that for both groups of tumors the proliferative index would be of prognostic significance.

Several methods exist to examine the tumor proliferative activity; among these mitotic and MIB-1 indices are very reliable and relatively easier to apply on archival materials. The proliferative activity of endometrial carcinoma was examined with these indices in this study, and correlation made to patient outcome.

p53 plays a major role in suppression of cell proliferation in the presence of DNA damages. Loss of this gene function as in case of gene mutation would be expected to be strongly correlated with tumor proliferative capacity. We hypothesized that p53 mutation, as detected by p53 overexpression, would be significantly correlated with tumor proliferative activity in both type 1 and type 2 endometrial carcinoma.
MATERIALS AND METHODS

Cases selection

The British Columbia Cancer Agency is a regionally based comprehensive cancer centre with a catchment population of approximately 4 million people. Cases were selected as follows: for low grade endometrioid adenocarcinoma, between January 1992 and August 1996, 406 patients with endometrioid endometrial adenocarcinoma limited to the uterine corpus with <50% myometrial invasion, no vascular invasion, and no grade 3 disease were identified. Grading was done according to the FIGO (1988) system. Tumors with any component of papillary serous or clear cell differentiation were excluded. Of these tumors 315 were found to be diploid by flow cytometry. These latter patients were considered to have “low risk” endometrial carcinoma, and received no further treatment. The 5-year disease specific survival for these patients was 95%. Fourteen patients in the “low risk” group developed recurrent disease. Eleven of the recurrent tumors and 28 stage and grade matched controls, who did not experience a relapse, chosen from this cohort of patients were selected for this study. For papillary serous carcinoma, between 1985 and 1995, 64 patients with papillary serous carcinoma of endometrium limited to the uterine corpus (stage I) were identified, and staging was done according to the FIGO (1988) system. The 5-year survival was 75%. Formalin fixed paraffin-blocks and slides were available for 23 cases. Clinical follow up information from 4-144 months (mean 58) was available for these patients. Six of these patients died of the disease, and 17 were alive with no disease during the follow up period. Treatment of these patients was variable, although
a significantly better outcome was observed for patients receiving postoperative whole abdominal radiotherapy\(^{103}\).

**Pathological features and immunohistochemistry**

The H&E slides for all cases selected were reviewed; the proliferative activity of these tumors was evaluated using mitotic and MIB-1 indices. The mitotic index was done on H&E sections by counting the number of mitoses in 5 sets of 10 randomly selected high power fields (HPF) (x400) of the tumor, and the highest count/10HPF was used. One paraffin block containing representative portions of the tumor was selected from each case for MIB-1 and p53 immunostaining. The avidin-biotin (ABC) method was used for immunostaining and applied to formalin-fixed and paraffin-embedded tissue. Serial sections were cut at 3\(\mu\)m thickness, deparaffinized with xylene, and rehydrated through a series of graded alcohols. The immunostaining procedure was performed using an automated stainer (Ventana, Tucson, AZ). The primary antibodies were used against Ki-67 protein (MIB 1, Immunotech, 1:200) and p53 (DO-7, Dako, 1:400). The anti-p53 antibody DO-7 recognizes both wild type and mutant p53. In contrast to wild-type p53, which is relatively unstable and expressed in small amounts, mutant p53 is overexpressed, and therefore remains longer in the nucleus. Sections were counterstained with light hematoxylin. p53 nuclear staining of <5\% of tumor nuclei was considered negative, staining of \(\geq 5\%\)-50\% of tumor nuclei was considered weak positive, while staining of > 50\% of tumor nuclei was considered strong positive.
MIB-1 index was assessed by counting 500 tumor cell nuclei in at least five fields under a grid at 400x magnification (10x ocular and 40x objective) and calculating the percentage of positive nuclei. All degrees of nuclear staining intensity were considered positive.

Statistical analysis

The Mann-Whitney U test was used for comparison of median mitotic and MIB-1 indices with patient outcome for both low risk endometrial carcinoma and PSCE. The Fisher exact test was used to analyse a 2x2 table comparing the subset of low grade endometrioid carcinoma patients with high proliferative indices and p53 overexpression vs. the rest of patients, with regard to recurrence rate. The same test was also used to analyse a 2x2 table comparing the subset of PSCE patients with low proliferative indices and p53 negativity vs the rest of patients, with regard to patient outcome. Scatter plots were used to assess the linear association and simple linear regression analysis between mitotic and MIB-1 indices in both tumors. Spearman’s rank correlation coefficient was used as an estimator of the linear correlation. Calculations and production of graphs were done using SPSS, version 11.0 software.
RESULTS

Thirty nine low grade endometrioid adenocarcinomas were studied, 11 from patients who experienced recurrence on follow up and 28 stage and grade matched controls who did not have a recurrence in the same follow up period. The mean mitotic index of these tumors was 5 mitoses/10HPF (median was 3 mitoses/10HPF), and more than 65% of the tumors had less than 6 mitoses/10HPF (Fig. 11). The mean MIB-1 index of these tumors was 27.5% (median was 27%). These markers of proliferative activity were found to be independent predictors of patient outcome in low grade endometrioid adenocarcinomas with p=0.004 in the case of mitotic index and p=0.018 in the case of MIB-1 index (Fig. 12 & 13). There is strong positive correlation between mitotic and MIB-1 indices, with a Spearman’s rank correlation coefficient ($r_s$) of 0.85 (Fig. 14). These proliferative indices were significantly correlated with p53 overexpression status in low risk endometrial carcinomas with p=0.01 in case of mitotic index vs p53 and p=0.006 in the case of MIB-1 index vs. p53 (Fig. 15).

We analysed 23 stage I papillary serous carcinomas of endometrium. Six of these patients died of disease during the follow up period, and 17 were alive with no disease. The mean mitotic index of these tumors was 31 mitoses/10HPF (median was 28 mitoses/10HPF), and more than 55% of the tumors had greater than 25 mitoses/10HPF (Fig. 11). The mean MIB-1 index of these tumors was 30.5% (median was 31.0%). We did not find these proliferative indices of prognostic significance in stage I PSCE (Fig. 12 & 13). The relationship between mitotic and MIB-1 indices in these tumors showed weak positive correlation, with a Spearman’s rank correlation coefficient ($r_s$)
of 0.62 (Fig. 11). These proliferative indices were also not significantly correlated with p53 overexpression status in PSCE tumors (Fig. 15).

Low grade endometrioid adenocarcinoma were stratified into two groups based on mitotic index as tumors with high proliferative indices (defined as greater than or equal to 6 mitoses/10HPF) and tumors with low proliferative indices (defined as less than 6 mitoses/10HPF). The patients of the former group experienced recurrence more frequently than the latter group, and the difference was significant (p=0.01) (Fig. 16). The tumors showing high proliferative indices and p53 overexpression were compared to the rest of the group of low grade endometrioid carcinoma (Table 7). There were relatively few patients with high proliferative index and p53 positive tumors. The outcome for patients whose tumors showed both increased mitotic activity and p53 overexpression was worse than that of patients whose tumors lacked these features, but this difference was not statistically significant (p=0.083). No difference in outcome was observed when papillary serous carcinomas with low proliferative indices and p53 negativity were compared to those papillary serous carcinomas lacking these features (data not shown).
DISCUSSION

Endometrial adenocarcinoma has been subdivided into two distinct clinico-pathological groups that differ not only with respect to their histology but also risk factors, natural history, outcome, and molecular events during tumorigenesis. In this study, we looked at these two groups of patients with endometrial carcinoma, to evaluate proliferative activity in their tumors.

The proliferative activity of a tumor has long been considered to bear a relationship to its clinical course, and recent reports indicate that measurement of tumor cell proliferation yields useful prognostic information. There are several methods to measure cell proliferation. These include counting mitotic figures, S-phase fraction assessed by $^3$H-thymidine uptake or by DNA flow cytometry, or immunostaining for the proliferating cell nuclear antigen (PCNA), or MIB-1/Ki-67 index. MIB-1 is a monoclonal antibody that recognizes a nuclear antigen that is expressed in all cell cycle phases except G0. MIB-1 monoclonal antibody is a reliable means of assessing the growth fraction of normal tissues and has been used to study the cell proliferation in various cancers.  

Salvesen et al.\(^{149}\) found MIB-1 immunostain performed on paraffin-embedded tumor tissue to be an independent prognostic indicator in 142 patients with endometrial carcinoma. In another study series by Geisler et al.\(^{60}\) one hundred and forty seven consecutive patients with endometrial carcinoma who had no systemic therapy were followed for at least 60 months, and the authors showed that MIB-1 index was an
independent prognostic indicator of 5-year survival, particularly in patients with stage I
disease. Giesler et al. 61 in an earlier study on a series of 39 patients had shown increased
MIB-1 immunoreactivity to be an indicator of increased risk of recurrence. Nordstrom et
al. 126 found MIB-1/Ki-67 expression and S-phase fraction were significantly related to
disease specific survival in patients with endometrial carcinoma. Despite the
methodological differences between these studies, all found MIB-1 to be an significant
prognostic indicator. Our study differs from these earlier studies in separately considering
low grade, low risk tumors and high grade papillary serous tumors. As expected, in our
study the latter group had a much higher proliferative rate. We found that the mitotic
index, which is relatively simple and applicable in routine pathology practice is also an
independent prognostic factor and is strongly correlated with MIB-1 index in low grade
endometrioid carcinoma.

In PSCE, MIB-1 or mitotic index did not correlate with outcome, and the correlation
between mitotic and MIB-1 indices in these tumors was weak. Although expression of
Ki-67 is consistently seen in normal proliferating cells, it may be lost in high grade tumor
cells. Mitotic index is simpler than immunostaining, and based on these results it is a
better measure of cell proliferation than MIB-1 staining in these tumors.

Mitotic index is used routinely in the grading of breast carcinoma and increasingly is
applied to the grading of ovarian carcinoma 113,157. Pirog et al. 136 have shown that a
significantly higher 5-year mortality rate was associated with a mitotic index of more
than 5 mitoses/10HPF in endometrial adenocarcinoma. Tornos et al. 171 identified 8
mitoses/10HPF or more as a statistically significant adverse prognostic factor in stage I, grade I endometrioid endometrial adenocarcinoma.

The International Federation of Gynecology and Obstetrics (FIGO) grading of uterine endometrial endometrioid carcinoma requires evaluation of histologic features, including recognition of the amounts of solid growth, distinction of squamous from nonsquamous solid growth, and assessment of degree of nuclear atypia. It does not include mitotic index. Assessment of mitotic index could easily be incorporated into the grading of endometrial carcinoma, as is done for breast and ovarian carcinoma, and this potentially could improve our prognostication for patients with these tumors.

Low grade tumors tend to have a mitotic index less than 10 mitoses/10HPF, and high grade tumors greater than 10 mitoses/10HPF. Our results suggest that endometrial carcinoma can be divided into three groups based on the mitotic index, with low, intermediate, and high risk of recurrence using the cut off ≤5, 6-10, and >10/10HPF respectively. It is possible that a grading system combining architecture, nuclear grade, and mitotic index could replace the current practice of subclassifying endometrial cancer based on cell type and, in the case of endometrioid carcinoma, tumor architecture. This possibility would have to be tested in a large series of patients with endometrial adenocarcinoma.

p53 overexpression is more frequently seen in papillary serous carcinoma than in endometrioid adenocarcinoma. We have shown in previous studies that p53 overexpression can be of prognostic significance in low risk endometrial carcinoma, but
not in papillary serous carcinoma\textsuperscript{103,104}. p53 overexpression in endometrial carcinoma was correlated with higher cell proliferative indices, such as MIB-1 index, as shown by Ioffe \textit{et al.}\textsuperscript{78}. In this study we looked at the correlation between p53 overexpression and proliferative activity markers. We found significant correlation between both markers of proliferative activity (mitotic, and MIB-1 indices) and p53 in low risk endometrial carcinoma (p=0.001, and p=0.006 respectively) but not in PSCE.

We hypothesized that a subset of tumors classified as low grade/low risk by conventional histopathological assessment would show immunophenotypic/cell proliferation features of high grade tumors. Accordingly, we compared low grade endometrioid carcinoma with increased mitotic index and p53 overexpression to tumors lacking these features. The difference in outcome for these patients approached but did not reach statistical significance (p=0.08) when we used the combination of mitotic index of more than 5/10HPF and p53 overexpression to define this subset of patients. A similar approach was also used for the patients with PSCE. In this group of patients we attempted to identify a subset of patients with low proliferative markers and lack p53 overexpression that behave less aggressively than the rest of PSCE patients. Identification of such a subset did not prove possible.

In summary, markers of proliferative activity, and particularly mitotic index, are good predictors of patient outcome in low grade endometrioid adenocarcinoma. Routine inclusion of mitotic index in assessment of tumor grade could improve prognostication for patients with these tumors.
TABLE 7. p53 overexpression and mitotic index in low grade endometrioid endometrial adenocarcinoma

<table>
<thead>
<tr>
<th></th>
<th>No recurrence</th>
<th>Recurrence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest of patients</td>
<td>25</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>P53 +ve and MI ≥ 6/10HPF</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>11</td>
<td>39</td>
</tr>
</tbody>
</table>

MI: mitotic index (mitotic figures/10 high power fields).
FIGURE 11. Mitotic index in low risk endometrial carcinoma and papillary serous carcinoma tumors. More than 65% of low risk tumors have a mitotic index less than 6 mitoses/10HPF, compared to more than 55% of PSCE tumors which have a mitotic index higher than 25 mitoses/10HPF.
FIGURE 12. Scatter plots showing the relationship between patient outcome and mitotic index of the tumor (A) in low risk endometrial carcinoma; p=0.004, (B) papillary serous carcinoma; p value is not significant. (DOD: dead of disease, AND: alive no disease).
FIGURE 13. Scatter plots showing the relationship between patient outcome and MIB-1 index of the tumor (A) in low risk endometrial carcinoma; p=0.018, (B) papillary serous carcinoma; p value is not significant. (DOD: dead of disease, AND: alive no disease).
FIGURE 14. Simple linear regression plots of mitotic and MIB-1 indices in (A) low risk endometrial carcinoma; $r_s=0.85 (r_s=\sqrt{R \text{ square}})$, (B) papillary serous carcinoma of endometrium; $r_s=0.62$. 
FIGURE 15. Scatter plots showing the relationship between proliferative indices and p53 overexpression in both tumors. (A) Mitotic index and p53 overexpression in low risk endometrial carcinoma; $p=0.01$. (B) Mitotic index and p53 overexpression in PSCE; $p$ value is not significant. (C) MIB-1 index and p53 overexpression in low risk endometrial carcinoma; $p=0.006$. (D) MIB-1 index and p53 overexpression in PSCE; $p$ value is not significant.
FIGURE 16. The disease free probability for the low grade endometrioid adenocarcinoma patients, comparing tumors with <6 mitoses/10HPF and tumors with ≥6 mitoses/10HPF, (p=0.01).
CHAPTER 4

Description of a Novel Grading System for
Endometrial Carcinoma
OBJECTIVE AND HYPOTHESIS

The most widely used system for grading of endometrial carcinoma is the FIGO grading system. This grading system requires evaluation of histologic features that can be difficult to assess, particularly distinction of squamous from nonsquamous solid growth, distinction of more than 5% from a lesser fraction of nonsquamous solid component of the tumor, and assessment of degree of nuclear atypia. These difficulties lower the reproducibility of this system; this is particularly true with respect to the assessment of nuclear grade.

A new grading system for endometrial carcinoma is proposed. This system takes into account three histological parameters that have been proven to be of prognostic significance in endometrial carcinoma; tumor architectural pattern, nuclear atypia, and mitotic index. We hypothesize that by using a grading system that takes into account architecture, nuclear grade and proliferative index we could better prognosticate in cases of endometrial carcinoma, independent of tumor stage or tumor cell type. Based on our proposed grading system, endometrial carcinomas can be divided into high and low grade. In the following study this new system is described and applied to 200 cases of endometrial carcinomas of different stages and histologic types retrieved from the archives of the Department of Pathology at Vancouver General Hospital. Each of the histological parameters that constitute the proposed grading system is tested for its ability to predict patient survival. The prognostic value of this system is then compared to two other grading systems used for endometrial carcinoma.
MATERIAL AND METHODS

_Tumors and Patients selection_

Two hundred cases of endometrial carcinoma, treated by hysterectomy, were retrieved from the archives of the Dept. of Pathology, Vancouver General Hospital, for the period 1983-1998. None of the included cases received preoperative radiotherapy or chemotherapy. 156 cases were of endometrioid (78%), 13 papillary serous (6.5%), 5 clear cell (2.5%), 4 small cell (2%), and 22 mixed (11.5%) subtypes (Fig. 14). Hematoxylin & eosin (H & E) slides and follow up data were available for all cases. For each case the slides were reviewed, diagnosis confirmed, and cell type assessed. The patients were staged according to 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria.

_Tumor grading_

Each tumor was graded using three grading systems: FIGO grading, binary grading, and the new grading system. The FIGO grading is performed as described in the revised 1988 FIGO criteria. For the binary grading system, devised by Lax et al., the tumors are classified into high and low grades based on the presence of greater than 50% solid growth, tumor cell necrosis, and diffusely infiltrative rather than pushing pattern of myometrial invasion. High grade tumors display at least two of these three features whereas low grade tumors show none or one.
The new grading system is similar to the widely used Nottingham grading system for breast carcinoma. This system incorporates the tumor architecture, nuclear grade, and mitotic count, regardless of the tumor cell type. Three architectural patterns were recognized, and we used score 1 for a predominantly glandular morphology, and 2 for predominantly papillary or solid growth. A two tiered nuclear scoring was used, assigning score 1 for mild or moderate atypia, and score 2 for severe nuclear atypia (grade 3 nuclei using a traditional three part nuclear grading system), using the criteria described by Zaino et al. Grade 3 nuclei are markedly enlarged and markedly pleomorphic, with coarse, clumped and sometimes smudged chromatin, and frequent prominent nucleoli. Nuclear features less than these are given a score of one. A minimum of 50 fields were assessed for mitotic index, consisting of five sets of 10 randomly selected high power fields (x400) of the tumour, and the highest count/10HPF was recorded. Mitotic counting was performed on a Zeiss microscope (10x wide eye piece, 40x objective) with field diameter of 0.35 mm and field area 0.096 mm². Tumors with mitotic index (MI) of less than 6 mitoses/10HPF were given score of 1, with a score of 2 assigned to tumors with MI of greater than or equal to 6 mitoses/10HPF. Tumors with a total score of 3-4 were considered low grade and those with a total score of 5-6 as high grade (Table 8). Put another way, the presence of at least two criteria out of these three, predominantly papillary or solid growth pattern, mitotic index ≥6/10HPF, or severe nuclear atypia, would qualify the tumor as high grade. Low grade tumors have at most one of these criteria.
Statistical analysis

The Kaplan-Meier method was used to construct survival curves for subgroups of patients based on all variables examined including, mitotic index, FIGO stage and grade, the binary grade and the new grade, cell type, and patient age. Comparison of curves was done using log-rank statistic. “Time to event” was defined as disease specific survival from date of hysterectomy to date of death due to endometrial carcinoma, with all others considered censored. When appropriate either Mann-Whitney or Kruskal-Wallis Test was used to analyse the correlation between mitotic index and patient’s outcome, tumor FIGO stage and grade. Either Fisher exact or chi-square tests were used to analyse 2x2 or 2x3 tables examining the correlation between variables. Multivariate analysis was performed using Cox’s proportional hazards method. For all analyses, two-sided tests of significance were used with $\alpha$ of 0.05. All analyses were performed using SPSS software version 11.0 (Chicago, USA)
RESULTS

The median follow-up was 77 months (range, 10 days – 224 months). Forty six patients died of endometrial carcinoma (23%), and 36 (18%) died of unrelated or unknown causes. The 10-year disease specific survival of all 200 patients was 77% (Fig. 20). The median patient age was 66 years (range, 28-95 years). There were 152 FIGO stage I (76%), 22 stage II (11%), 25 stage III (12.5%), and 1 stage IV (0.5%) tumors. Both patient’s age and FIGO stage were significant prognostic factors in endometrial carcinoma (p=0.009, and p<0.0001 respectively).

Grading results

Ninety nine tumours were FIGO grade 1 (49.5%), 27 grade 2 (37%), and 74 grade 3 (13.5%) (Fig. 21). FIGO grading was a significant predictor of patient outcome in univariate analysis (p<0.0001) (Fig. 22). On multivariate analysis, FIGO grading was an independent prognostic indicator when the patient’s survival was adjusted for patient age, tumor FIGO stage, and cell type (p=0.01). Of note, patients with FIGO grade 3 tumours had a significantly worse outcome than patients with either grade 1 or grade 2 tumors (p<0.0001, and p=0.001 respectively). There was no significant difference between the outcomes for patients with grade 1 and grade 2 tumours (p=0.85).

By binary grading, 110 tumors were low grade (55%) and 90 tumors were high grade (45%) (Fig. 23). There was a significant difference in patient survival between patients with low grade and high grade tumors (p<0.0001) by univariate analysis (Fig. 24). The
difference remained significant on multivariate analysis when patient survival was adjusted for patient age, tumor stage, and cell type (p=0.035).

Mitotic index was strongly correlated with patient outcome, tumor cell type, and FIGO grade and stage (p<0.0001 for each). The tumors were stratified into 2 groups based on MI: tumors with MI < 6 mitoses/10HPF, and tumors with MI ≥ 6 mitoses/10HP. The latter group had a significantly worse outcome compared to the former on univariate analysis and also on multivariate analysis, when patient survival was adjusted for patient age, tumor cell type, and FIGO stage (p<0.0001, and p= 0.028 respectively) (Fig. 25).

The tumor growth pattern was a significant predictor of patient outcome. Fig. 26 shows the Kaplan Meier survival curves of endometrial carcinoma patients divided based on their tumor growth pattern into predominantly glandular, papillary, and solid patterns (p<0.0001). Patients with predominantly glandular tumours had a significantly better outcome than patients with either predominantly papillary or solid tumors (p<0.0001 for both). There was no significant difference between the outcomes for patients with predominantly papillary and solid tumours (p=0.37).

Tumor nuclear grading divided the patients into three groups as shown in Fig.27, and was a significant prognostic factor (p<0.0001). No significant difference in the outcome between patients having tumors with mild nuclear atypia and moderate atypia was seen (p=0.5), but the outcome was significantly worse for patients having tumors with severe
nuclear atypia compared to either those with mild or moderate atypia (p<0.0001, and 
p=0.001 respectively).

The last three criteria were used to construct the new grading system. With this system 
117 tumors (58.5%) were classified as low grade and 83 tumors (41.5%) were high grade 
(Fig. 23). This system was a strong predictor of patient outcome in univariate and 
multivariate analyses when patient survival was adjusted for patient age, tumor cell type, 
and FIGO stage (p<0.0001, and p=0.003) (Fig. 28).

Table 9 summarises the proportional hazard analysis coefficient, relative hazard, 95% 
confidence interval, and p-values in univariate and multivariate analyses for all grading 
system used.
DISCUSSION

The prognostic and therapeutic value of histologic grading of endometrial carcinoma has been documented repeatedly, but an ideal system has not been unequivocally defined. Several methods have been proposed and used for grading of endometrial carcinoma.

Tumor architectural pattern was used early in grading of endometrial carcinoma. Although it was poorly defined, the initial FIGO grading of endometrial carcinoma in 1973 was based entirely on the tumor architecture. Tumors were subdivided into three grades: highly differentiated adenomatous carcinoma as grade 1, differentiated adenomatous carcinoma as grade 2, and predominantly solid or entirely undifferentiated carcinoma as grade 3. In 1988 the FIGO grading remained as architectural grading but was more precisely defined, based on the proportion of the nonsquamous solid component of the tumor (Table 3). In the revised version of 1988 FIGO grading, the tumor architecture still served as the basic grading parameter, but amended such that "nuclear atypia inappropriate for the architectural grade raises the grade by one." Several reports have confirmed the prognostic value and showed fair to an acceptable level of reproducibility of architectural grading of endometrial carcinoma.

The pure architectural grading system would under-grade some tumors since it does not take the nuclear grade into account. Zaino et al. compared the pure architectural grading of endometrial carcinoma with the revised 1988 FIGO grading, and defined notable nuclear atypia as grade 3 nuclear features (i.e. marked nuclear pleomorphism, coarse chromatin, and prominent nucleoli). In the latter system, tumors with architectural
grade 1 or 2 but with predominantly grade 3 nuclei were raised one grade. This change resulted in upgrading 44 tumors in their study. They found justification for this upgrading since the risk of recurrence and death of these upgraded tumors was similar to that of other tumors with grades corresponding the reassigned grades, based on notable nuclear atypia. This study emphasised the importance of nuclear grading and provided support for the FIGO modification of the previously used purely architectural grading system.

Another study by Takeshima et al.\textsuperscript{164} found that architectural grade 1 or 2 tumors with more than 25% grade 3 nuclei had a behaviour similar to those with more than 50% grade 3 nuclei.

FIGO grading requires differentiation as to whether the solid part of the tumor is squamous or nonsquamous and whether this nonsquamous solid component is truly less than or more than 5% to assign tumors to grade 1 or 2. This differentiation can be at times difficult and lowers the reproducibility of this system.

Taylor et al.\textsuperscript{169} have questioned the clinical relevance of designation of grade 2 and proposed a two-tiered grading system. Their system is based on the FIGO grading in which low grade tumors have 20% or less nonsquamous solid component, whereas high grade tumors have more than 20% nonsquamous solid area. They found that this system was less cumbersome, had less interobserver variation, and had the same or better prognostic significance than the three-tiered system. While it is undoubtedly true that the inter-pathologist variability would be decreased and reproducibility would be increased
with two rather three grades, it is not clear whether or not some important prognostic information would be lost by this classification.\textsuperscript{81}

In this study the tumors were divided into 3 architectural groups: predominantly glandular, predominantly papillary, and predominantly solid tumors. We found significant prognostic differences between the first and either of the latter two groups but not between the latter two in univariate analysis ($p=0.0001$, and $p<0.0001$ respectively). The prognostic significance of papillary architecture is lost in multivariate analysis when the patient’s survival was adjusted for tumor cell type and FIGO stage ($p=0.18$), but this was not the case for solid morphology ($p=0.001$ in multivariate analysis. It is undoubtedly true that at least part of aggressive behaviour of tumors with predominantly papillary morphology can be attributed to the inclusion of serous and clear cell tumors. Papillary endometrioid carcinoma may also be aggressive. Ambros et al.\textsuperscript{7} have examined the prognostic significance of papillary differentiation of endometrioid carcinoma. They found that endometrioid carcinoma displaying papillary differentiation in the myometrium were associated with higher frequency of vascular invasion, a higher rate of lymph node metastasis, and worse outcome compared with carcinomas showing myometrial invasion in form of glandular or solid patterns.

Another reason for inclusion of papillary architecture in this new grading and giving it the same score and importance as solid is our interest of describing a system that can be applied to all endometrial carcinoma regardless of cell type. The FIGO grading system for serous and clear cell carcinoma is based on nuclear atypia only and does not use the
tumor architecture. To avoid overgrading of endometrioid carcinoma with papillary morphology two more prognostic factors need to be examined: nuclear atypia and mitotic index of the tumor. In our system at least two unfavourable prognostic features need to be present in the tumor before calling it high grade, so a papillary endometrioid tumor with mild or moderate nuclear atypia and low mitotic index (less than 6/10HPF) will be called low grade in the new grading system.

The prognostic significance of nuclear atypia has also been demonstrated in multiple studies. Christopherson et al. have reported that nuclear grade of endometrial carcinoma is superior to architectural grade as a prognostic parameter for detection of patients with relapse and fatal outcome. Although different criteria were used to define nuclear grading, other reports have indicated the prognostic importance of nuclear grading. Mittal et al. found that nuclear and architectural grading provided similar prognostic information.

One of the major weaknesses of nuclear grading is its poor reproducibly. Nielsen et al. have examined the reproducibility of the revised FIGO grading and nuclear grading, and found poor reproducibility for nuclear grade (kappa value 0.55) compared to acceptable level for the revised FIGO grading (kappa value 0.65). This improvement in the reproducibility for the latter is a reflection of higher level of agreement for the architectural grade. They speculated that the absence of well defined criteria for nuclear grading resulted in diminished reproducibility. On the other hand Zaino et al. have
reported very similar reproducibility for nuclear and architectural grading systems (kappa values of 0.57 and 0.49 respectively).

To improve the reproducibility of nuclear grading, a quantitative and morphometric method has been suggested. Baak et al. 16 showed that the mean shortest nuclear axis of cells was the most significant factor discriminating between surviving and non surviving patients with stage I endometrial carcinomas. Hachisuga et al. 68 have proposed a semiquantitative system based on the proportion of nuclei with large shortest axis and examined its prognostic value. They also used this nuclear grading combined with architectural grading, and concluded there was improved prognostication of the combined method even when applied to tumors of all stages.

In the current study the tumors were divided into three groups based on their nuclear features. We did not find significant prognostic differences between nuclear grade 1 and 2 (p=0.42), but there were significant differences between grade 3 and either grade 1 and 2 (p<0.0001 and p=0.002 respectively). These significances were independent of either tumor cell type or FIGO stage when the patient's survival was adjusted for the latter two (p=0.003, and p=0.04 respectively). These findings provide support for the prognostic significance of nuclear grading of endometrial carcinoma, which is in agreement with previous studies. The current study shows the importance of recognising grade 3 nuclei, but not differentiation of nuclear grade 1 from 2. For this reason, we included grade 3 nuclear atypia in our proposed new grading as one of the prognostically unfavourable criteria that can contribute to designation of a tumor as high grade. It is likely that
identification of grade 3 vs. lower grade nuclei, rather than defining three nuclear grades, will increase the reproducibility of this grading system.

We have shown in the previous study that the proliferative activity of endometrial carcinoma as measured by either mitotic or MIB-1 indices has prognostic significance in low grade endometrioid carcinoma but not in papillary serous carcinomas. Other reports also have shown prognostic value for proliferative activity in endometrial carcinoma despite the methodological differences between them\textsuperscript{61,78,126}. In the current study mitotic index was a significant prognostic indicator for endometrial carcinoma in univariate and multivariate analyses (p<0.0001, and p=0.028). The latter was adjusted for patient's age, tumor FIGO stage and cell type. We performed the analysis initially on mitotic index as an interval variable and it showed a strong significance. Then the tumors were divided into two groups based on mitotic index by using 6 mitoses/10HPF as a cut-off. The mitotic index showed significant correlation with FIGO stage, and more importantly the cell type. In the previous study we have shown higher mitotic index median for papillary serous carcinoma compared to low grade endometrioid carcinoma (28 versus 3 mitoses/10HPF respectively). In this study the mitotic index showed prognostic significance independent of cell type which seems to be different from our earlier study results. Two possible reasons for this apparent discrepancy are that firstly, we used more cases in the current study and secondly, in Cox's regression the cell type was divided into endometrioid with mucinous carcinomas as one set versus others in another set. We did not find mitotic index of prognostic significance when it was examined in pure papillary serous carcinoma in this study, and this is in agreement with our previous finding.
We decided to include the mitotic index in this proposed grading for two reasons. The first is because of its prognostic value; this would improve the prognostication of the new grading. Moreover, it is theoretically true that adding mitotic index to the grading would improve the reproducibility of the grading system, as determination of the mitotic index has proven the most robust component of the Nottingham grading of breast carcinoma. This latter statement needs to be tested.

Binary grading is a new proposed grading for endometrioid subtype of endometrial carcinoma. In this study we applied it to all histological subtypes of endometrial carcinoma. We found this system to be applicable to all these histological subtypes, and it was a significant predictor of patient outcome independent of tumor FIGO stage, patient age, and moreover the cell type. It should be noted that this grading system is not truly independent of FIGO substage as stage Ia tumors by definition lack a diffusely infiltrative pattern of invasion. The p-value in multivariate analysis for this system was less than what we found for the new system. Furthermore, the relative hazard, which reflects the hazard of disease specific death of high grade as compared to low grades, is lower than the one for the new grade. These results suggest that the new grading has more prognostic power than the binary system proposed by Lax et al. One of the advantages of this grading system over the FIGO grading is that the binary system does not require differentiation between squamous and non squamous components of the tumor, and this should improve the reproducibility of grading. On the other hand, we found differentiation of diffusely infiltrative from expansive pattern of tumor growth is rather subjective and can be difficult to determine at times. This could lower the reproducibility.
of this system, and studies need to be done to compare the interobserver and intraobserver agreement of the different grading systems.

There are similarities and differences between the new system of grading and FIGO grading for endometrial carcinoma. The proposed grading system is not primarily based on architecture as is the case for FIGO grading. We also do not call for differentiation between squamous and non squamous solid growth of the tumor, which is simpler than FIGO grading. The observer does not need to decide the percentage of the solid or glandular components, but rather needs to decide what is the predominant component. This can be achieved by using low power microscopic examination in most cases. For nuclear grading, we adopted the Zaino et al.\textsuperscript{186} modification for FIGO grading where the observer needs only to recognize grade 3 nuclei. We did not find prognostic difference between tumors with grade 1 and 2 nuclei in our analysis that is why we do not propose such a distinction in this grading. This should improve the reproducibly of our system, since this distinction is the major factor that negatively affects the reproducibility of FIGO grading. We added an additional prognostic factor, the mitotic index, which we and others found to be a useful predictor of patient outcome in endometrial carcinomas. Finally, we have a grading system that is applied to tumors of all cell types, without modification.
In summary, we are proposing a new grading system that has more prognostic power than either the FIGO or binary system of Lax et al, and it has the potential to be more reproducible than either of these grading schemes. This system divides endometrial carcinomas, regardless their histological subtype, into two groups, high versus low grade, based on the architectural, nuclear, and mitotic index of the tumors. High grade tumor has two of these three features: predominantly papillary or solid architecture, grade 3 nuclei, or mitotic index $\geq 6$ mitoses/10 HPF. Low grade tumors have at most one of these criteria.
TABLE.8 Architectural, nuclear, and mitotic scoring for endometrial carcinoma. The final grading is given according to the total score.

<table>
<thead>
<tr>
<th>Architectural Scoring</th>
<th>Nuclear Scoring</th>
<th>Mitotic Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor predominant architecture</td>
<td>Score</td>
<td>Score</td>
</tr>
<tr>
<td>Glandular</td>
<td>1</td>
<td>Mild or</td>
</tr>
<tr>
<td>Papillary or Solid</td>
<td>2</td>
<td>Severe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Score</th>
<th>Final grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4</td>
<td>Low grade</td>
</tr>
<tr>
<td>4-6</td>
<td>High grade</td>
</tr>
</tbody>
</table>
TABLE 9 Summary of proportional hazard analysis coefficients, relative hazards, 95% confidence interval for relative hazard, and p-values in univariate and multivariate analyses for the three grading systems used

<table>
<thead>
<tr>
<th>Grading</th>
<th>Prognostic significance</th>
<th>Proportional hazard analysis coefficient</th>
<th>Relative Hazard</th>
<th>95% CI for relative hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate (p-value)</td>
<td>Multivariate (p-value)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGO</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>0.672</td>
<td>1.959</td>
</tr>
<tr>
<td>Binary</td>
<td>&lt;0.0001</td>
<td>0.027</td>
<td>0.842</td>
<td>2.321</td>
</tr>
<tr>
<td>New grading</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>1.179</td>
<td>3.25</td>
</tr>
</tbody>
</table>
Fig 17 Classification of study cases according to cell type.
Fig. 18 Architectural scoring, A: solid B: papillary, C: glandular
Fig. 19 Nuclear grading. A: grade 1, B: grade 2, C: grade 3
Fig. 20 Disease specific survival of all 200 patients with endometrial carcinoma.
Fig. 21 Classification of study cases according to FIGO grade and stage.
Fig. 22 Disease specific survival of endometrial carcinoma by FIGO grade.
Fig. 23 Classification of tumors according to the binary and the new grading.
Fig. 24 Disease specific survival of endometrial carcinoma by binary grading system.
Fig. 25 Disease specific survival of endometrial carcinoma by mitotic index (MI).
Fig. 26 Disease specific survival of endometrial carcinoma based on tumor growth pattern.

P<0.0001
Fig. 27 Disease specific survival of endometrial carcinoma based on tumor nuclear grade.
Fig. 28 Disease specific survival of endometrial carcinoma based on the new grading.
CHAPTER 5

FUTURE DIRECTIONS

In the first two studies we examined the prognostic values of several predictors of patient outcome in an attempt to identify additional factors that could help to stratify patients with endometrial cancer according to the aggressiveness of these tumors. This would help to reduce the potential for overtreating or undertreating patients. In the last part we proposed a modification to the FIGO grading of endometrial that can increase the prognostic power of endometrial carcinoma grading.

In the first part we found none of the examined predictors, with exception of FIGO grading, were of prognostic value. The percentage of papillary serous carcinoma shows a trend toward significance (p=0.17) as a prognostic indicator, and further examination of this factor with larger set of cases may be helpful. Variability of type of therapy given to the patients we examined compromises our ability to draw conclusions, so we would recommend also analysing a set of papillary serous carcinoma patients who were treated at more uniformly.

In the second part of our work, we observed that patients with low grade endometrioid tumors with p53 overexpression and high proliferative index (more than 5 mitoses/10HPF) showed a trend toward worse outcome (p=0.08) that warrants study of a larger set of cases. The observation suggests that building a prognostic model for endometrial carcinoma cases where several histopathological, clinical, and molecular markers are included, to clearly stratify the patients into prognostic groups that more
precisely predict their outcome. This can be also utilised in the future for building more personalised treatment for the patients. The molecular markers could include not only p53 but possibly PTEN, MSI, and hMLH1. Zaino et al.\textsuperscript{185} proposed pathologic models to estimate the absolute risk of tumor recurrence/disease specific death outcome for the women with endometrial carcinoma. These models did not include information about molecular markers, so a study examining these markers together with histological and clinical parameters would be helpful.

In the last part of our work, an essential aspect of the grading system we propose still needs to be tested. Reproducibility of any grading system is an integral part for its acceptance. For this reason we need to examine both the interobserver and intraobserver variability and calculate the kappa value for our new grading system.
REFERENCES


55. Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating
mutations for transformation by p53 produce a gene product that forms an hsc70-p53

Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the
National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer


58. Gallup DG, Stock RJ. Adenocarcinoma of the endometrium in women 40 years

59. Gehrig PA, Groben PA, Fowler WC Jr, Walton LA, Van Le L. Noninvasive

60. Geisler JP, Geisler HE, Miller GA, Wiemann MC, Zhou Z, Crabtree W. MIB-1 in
endometrial carcinoma: prognostic significance with 5-year follow-up. Gynecol


