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Prognostic Factors in Surgical Stage I Endometrial Carcinoma

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The prognostic impact of DNA ploidy, MIB-1 and p53 was evaluated in relation to clinical and histopathological features in surgical stage I endometrial carcinoma ($n = 284$) and in the histopathological endometrioid subgroup ($n = 257$). Tumour material from 284 consecutive patients was analysed regarding image cytometric DNA ploidy and the immunohistochemical MIB-1 and p53 expression. Twenty-four tumours relapsed. In univariate analysis, histopathological subgroup (endometrioid vs. non-endometrioid), grade, DNA ploidy and p53 were highly significant prognostic factors ($p \leq 0.001$). MIB-1 was also significant ($p = 0.039$). In the endometrioid subgroup only DNA ploidy and p53 were significant ($p < 0.001$). In multivariate analysis of the entire material, ploidy and histopathological subgroup retained their significance ($p = 0.001$, $p = 0.004$), whereas only ploidy was significant in the endometrioid subgroup ($p = 0.001$). DNA ploidy was the strongest predictor of relapse-free survival and the only independent prognostic factor in the endometrioid subgroup.

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Endometrial carcinoma is the third most common malignancy in Swedish women. About 70% are in stage I at diagnosis (in both surgical and clinical stage I) (1). The prognosis is favourable in 87% of the surgical stage I patients (1) but it falls rapidly in more advanced stages. Despite the early stage diagnosis, most recurrences occur in stage I as this is the most common stage (1). Since 1988 surgical staging has been recommended as the standard management of endometrial cancer (2). Surgical staging includes histological or cytological verified tumour growth in suspicious, that is enlarged, lymph nodes. There is no agreement as to whether a systematic lymphadenectomy should be performed irrespective of any suspicious nodes. Centres in many countries like Sweden advocate only extirpation of enlarged nodes or fine-needle aspiration. There is considerable morbidity in this elderly population and routine lymphadenectomy has not been shown beyond doubt to improve survival. It is recognized that the histopathological subtype is an important risk factor; uterine papillary serous carcinoma (UPSC) and clear-cell tumours have an inferior prognosis compared to the common endometrioid adenocarcinoma. About 17% of endometrial carcinomas are of the non-endometrioid type in material world-wide (1). Histopathological parameters, such as myometrial infiltration, degree of differentiation and histopathological subtype, are widely used (3) to assess the risk of relapse and to provide support in adjuvant therapeutic decisions. It is well known that these variables are insufficient to detect women at risk of relapse. Thus, there is a need to find more objective parameters with better prognostic impact.

DNA ploidy has proven to add prognostic information in several studies (4–7). One large study found a relationship between DNA ploidy and relapses in surgical stage I (7), but two others did not (8, 9). Measurements of DNA ploidy are usually performed with flow cytometry (FCM), but only a few investigators have used image cytometry (ICM). Both methods have advantages and disadvantages. FCM analyses a large number of nuclei, but cannot separate tumour cells from numerous normal cells in the sample. In ICM, fewer cells are analysed but the tumour cells are selected in a microscope, which leads to minimal or no admixture of normal cells. Studies have shown that there is 80% agreement between the methods and that small aneuploid populations detected by ICM are missed by FCM (6, 10, 11).

There are several methods of estimating proliferative activity. In this study, the immunohistochemical expression

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of MIB-1 (Ki-67) is used; MIB-1 is a monoclonal antibody recognizing a nuclear antigen that reflects the growth fraction in all phases of the cell cycle (12). In previous studies, there are conflicting data concerning the prognostic impact of MIB-1. Some authors, for example Geisler et al. (13), found a prognostic impact on survival in contrast to others, such as Nordström et al. (14).

Mutation of the p53 suppressor gene is one of the most frequently encountered alterations in human neoplasms. The normal p53 protein has a protective roll in cell division as it prevents DNA-damaged cells from dividing. In cells with mutated p53 this guardian function is lost. Mutation of the p53 gene might lead to overexpression of p53 protein, which can be detected immunohistochemically. Mutation can also result in a deficient protein that cannot be recognized by immunohistochemical methods, or no protein at all. It has been suggested that immunohistochemistry preferentially detects protein from the mutated p53 gene because of its prolonged half-life compared with the wild type (15). Overexpression of p53 protein occurs in approximately 20% of endometrial carcinomas and in 9% of those in stage I (16, 17). Several studies, for example Geisler et al. (18), indicate that immunohistochemical overexpression of p53 is of prognostic importance in endometrial carcinoma, while others do not agree (19).

The aim was to study the impact of DNA ploidy with image cytometry, MIB-1 and p53 in relation to clinical and histopathological features on the prognosis of endometrial carcinoma surgical stage I both in the entire material (endometrioid/non-endometrioid) and in the purely endometrioid adenocarcinoma.

MATERIAL AND METHODS

Patients

In a previous study cohort comprising 376 women with endometrial carcinoma stages I–IV (5), 86% (309 patients) had stage I disease (both surgical and clinical staging). This study included all of the 284 surgical stage I patients in the Stockholm–Gotland region consecutively referred to the Department of Gynaecologic Oncology at Radiumhemmet, Karolinska University Hospital, Stockholm, Sweden, between January 1994 and December 1995. The median age at diagnosis was 69 years (range 44–90 years). Four per cent were premenopausal at diagnosis. Median menopausal age was 50 years (range 40–64 years) and median age at menarche was 13 years (range 9–18 years).

Treatment

All women underwent hysterectomy and bilateral salpingoophorectomy. As is commonly the case in Sweden, no routine sampling of lymph nodes was performed. Only a few had cytological sampling. After surgery, patients were divided into a high-risk group and a low-risk group. The high-risk group consisted of myometrial invasion $> 50\%$ and/or adenocarcinoma grade 3 or UPSC or clear-cell carcinoma. The low-risk group consisted of myometrial invasion $< 50\%$ and adenocarcinoma grades 1 or 2. The high-risk group was treated with external pelvic radiotherapy 39.6 Gy (1.8 Gy fractions, 5 days/week) + vaginal brachytherapy 5 Gy $\times$ 2. The low-risk group was treated with vaginal brachytherapy 3 Gy $\times$ 6. In the high-risk group a few patients also had chemotherapy and one patient received only chemotherapy because she was unfit for radiotherapy. Altogether 39 patients had no adjuvant therapy, the majority because of very low-risk tumours and five patients because of their own request or severe health problems. One patient had only adjuvant chemotherapy. A total of 125 patients (44%) were treated with brachytherapy and 119 patients (42%) with pelvic radiotherapy + brachytherapy.

Follow-up

The mean observation time was 77 months (65–91 months). The causes of deaths were taken from clinical records and autopsy reports.

Stage

All the patients had surgical stage I endometrial cancer. Subgroups of stage I (i.e. depth of myometrial invasion) are presented in Table 1. Ten per cent of the patients were in stage Ia (no myometrial invasion), 54% in stage Ib (myometrial invasion $< 50\%$) and 36% in stage Ic (myometrial invasion $> 50\%$).

Table 1

Histopathological variables, DNA ploidy and p53 in surgical stage I endometrial carcinoma

Variable	Number	%
Stage (n = 284)		
1a (no MI)	29	10
1b (< 50% MI)	154	54
1c (> 50% MI)	101	36
Histopathology (n = 284)		
Endometrioid AC	257	90
Non-endometrioid	27	10
Degree of differentiation (n = 282)		
Well	139	49
Moderate	109	39
Poor	34	12
DNA (n = 270)		
Diploid	184	68
Aneuploid	86	32
p53 (n = 257)		
No overexpression	219	85
Overexpression	38	15

Abbreviations: MI = myometrial invasion; AC = adenocarcinoma.

Histopathology

There were 90% endometrioid and 10% non-endometrioid cancers diagnosed (Table 1). The primary diagnose was performed by one pathologist according to the FIGO grading system (1) for histopathological subtype, degree of differentiation and myometrial infiltration. A second pathologist specially trained in endometrial cancer performed a re-examination. If there was a discrepancy between the first and the re-examining pathologist the statement of the second was applied to get as uniform an estimation as possible. The discrepancies concerned degree of differentiation, myometrial invasion (usually close to the 50% border of depth of myometrial invasion) and, more seldom, subtype. In all, there were 10–15% discrepancies.

Image DNA cytometry

Of the 284 specimens, 270 were analysed for DNA ploidy. It was not possible to analyse the remaining specimens because of the sparse number of tumour cells or for other technical reasons. DNA ploidy analysis was performed on Feulgen-stained histopathological sections (8 μm) using image cytometry. The staining procedure, internal standardization and tumour cell selection were based on methods described previously (20) and DNA histograms were sampled from at least 100 interface nuclei of each specimen. All DNA values were expressed in relation to the corresponding staining controls, which were given the value 2c, denoting diploid DNA content. The computer software was ACAS 6.0, Ahrens ICM Cytometry System.

Interpretations of DNA histograms

In order to be able to compare the DNA values of various tumours DNA content was expressed in c-units, 2c being defined from the median value (P_{50}) of the control cells (normal human epithelial cells and normal human lymphocytes). An interval of 1.8c–2.2c was selected empirically as comprising the c values of the majority ($>98\%$) of the thousands of non-proliferating diploid control cells. Analogously, an interval of 3.8c–4.2c was selected as the 'tetraploid' region. The classification of the DNA profiles was carried out according to Auer et al. (21). According to this classification, the DNA histograms obtained by image cytometry were subdivided into four types. Type I ('diploid') showed a single peak in the 'diploid' or 'near diploid' region (1.8c–2.2c) of normal cells. Type II ('diploid–tetraploid') was characterized by a single peak in the 'tetraploid' region (3.8c–4.2c) or a peak in both the 'diploid' and the 'tetraploid' regions ($>20\%$ of the total cell population). The total number of cells with DNA-values between the 'diploid' and 'tetraploid' regions and those exceeding the 'tetraploid' region was $<5\%$. Type III ('proliferating diploid') showed a distribution pattern comparable with that of proliferating normal populations, that is a main peak in the 'diploid' region, a substantial number of cells in the S-phase region ($>5\%$) and a minor peak in the 'tetraploid' region ($<20\%$). Type IV was characterized by scattered DNA-values significantly exceeding the 'tetraploid' region. In this material, types I–III were considered as diploid, and type IV as aneuploid.

MIB-1 (Ki-67) immunohistochemistry was performed with the Avidin-Biotin-Peroxidase complex technique. The monoclonal antibody MIB-1 (Immunotech S.A., Marseille, France) was used. Paraffin-embedded, formalin-fixed tumour tissue samples of thickness 4 μm were used for this study. The preparation procedure was described earlier (5). Only cells with distinct brown staining confined to the nuclei were regarded as immunoreactive. The number of positive cells was determined by counting approximately 2000 cells in the various lesions. The evaluation was expressed as a percentage of the immunoreactive cells in relation to the total number of cells. A malignant tumour with known MIB-1 expression served as a positive control and internal negative controls were normal tissues in each specimen.

p53 immunohistochemistry was performed with the same technique as MIB-1 immunohistochemistry. p53 expression was detected using the DO-1 antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA; diluted 1:100 in 1% BSA). The detailed procedure was described earlier (5). Only cells with distinct brown staining confined to the nuclei were regarded as immunoreactive. Approximately 2000 cells per specimen were counted and the percentage of stained cells was noted. A malignant tumour with known p53 expression served as a positive control, whereas normal tissue in each specimen served as the negative control. If p53 expression was $>50\%$ of the tumour cells, p53 was assessed as overexpressed. In 166 of the specimens p21 expression as detected by means of a WAF1 antibody (22) (Oncogene Research Products, Boston, MA, USA; diluted 1:50) was analysed. In these cases p53 was evaluated as overexpressed if p53 was >80–90% and p21 <10–20%.

Statistical analysis

Distribution of the DNA ploidy factor on the other histopathological factors was compared using the χ^2 exact test for trend and heterogeneity. The effect of different categorical variables on predicting tumour-free survival was studied using the life table method, and tests were performed with Gehan–Wilcoxon statistics. Continuous factors were evaluated with the Cox regression as well as multivariate analysis.

RESULTS

Relapses in surgical stage I endometrial carcinoma

Twenty-four (8.4%) of 284 stage I patients had relapsed during the more than 5 years observation time. Fourteen relapses occurred in the irradiated tissue while 10 relapsed

Table 2

Relapses according to location in or outside irradiated tissue in relation to ploidy (n = 24)

Variable	Relapse in irradiated tissue	Relapse outside irradiated tissue
Diploid (n = 6)	2	4
Aneuploid (n = 16)	11	5
Ploidy not performed (n = 2)	1	1

Three of the patients with relapse in irradiated tissue also had relapse outside irradiated tissue.

outside the irradiated tissue (Table 2). Three of the relapses in the irradiated tissue also had tumours outside the irradiated area. Relapses in the irradiated area occurred after 17 months (range 9–57 months) and those outside the irradiated tissue after 25 months (range 9–57 months). Nine patients (38%) had local failure in the vagina, four (17%) presented with tumour in the pelvis and 11 (46%) had distant failures. Twenty of the patients died from their endometrial carcinoma. Four of them are still alive, three with active disease (one with recurrence in fossa Douglasii and two with vaginal failure) and one without evidence of disease (vaginal failure). The median time to relapse was 21 months (range 4–57 months). In 22 of the 24 relapses DNA analysis was performed. There were 12 relapses in the endometrioid subgroup (5%). A presentation of histopathological variables in relation to ploidy in this group is shown in Table 3.

Clinical and histopathological variables with respect to relapse-free survival Univariate analysis

Non-endometrioid and endometrioid surgical stage I endometrial carcinoma (n = 284). The stage I subgroups, that is depth of myometrial invasion (Ia with no myometrial invasion, Ib < 50% myometrial invasion and Ic > 50% myometrial invasion), had no impact on the risk of relapse. The three relapses in stage Ia consisted of one endometrioid and two seropapillary tumours (Table 4). A significantly higher proportion of relapses was found in non-endometrioid tumours compared with endometrioid tumours, $p < 0.001$ (Table 4). The grade of differentiation of the tumour (well-, moderately or poorly differentiated) was also a significant factor, $p = 0.001$ (Table 4). Whether or not the patient had been on hormone replacement therapy had no impact (Table 4). With increasing age the risk of relapse increased by about 70% per decade, $p = 0.034$ (Table 5).

DNA ploidy revealed significantly less risk of relapse for diploid tumours than for aneuploid ones, $p < 0.001$ (Fig. 1) (Table 4). Sixty-eight percent of all stage I tumours were diploid whereas 32% were aneuploid (Table 1). MIB-1 as a continuous factor showed a 20% higher risk of relapse per 10% rise, which was significant, $p = 0.039$ (Table 5). We found that only one patient with MIB-1 $\leq$ 20% relapsed. Overexpression of p53 was strongly associated with increased risk of relapse, $p < 0.001$ (Tables 1 and 4).

The relapses divided into diploid and aneuploid groups are shown in relation to other variables in Table 3. Only 2/6 in the diploid population were endometrioid tumours. None of the diploid relapsing tumours had a proliferation rate below 50% as estimated by MIB-1 (only 2/6 analysed). Sixty-two percent (10/16) of the aneuploid relapsing tumours were also overexpressing p53, whereas none of the diploid overexpressed p53 (only 4/6 analysed).

Endometrioid surgical stage I endometrial carcinoma (n = 257). As it is already well known that UPSC and clear-cell carcinomas (non-endometrioid tumours) have an inferior prognosis compared with the endometrioid tumours, we conducted a separate statistical analysis of the 257 specimens of the latter. We found that the degree of differentiation lost its significance ($p = 0.055$) whereas the observed results for myometrial invasion (NS), DNA ($p < 0.001$) and p53 ($p < 0.001$) were unchanged, that is in line with the data found in the entire material (Table 6). Age was borderline significant ($p = 0.050$) and MIB-1 lost its prognostic significance (Table 7).

Multivariate analysis

Non-endometrioid and endometrioid surgical stage I endometrial carcinoma (n = 284). When the significant variables in the univariate analysis, i.e. histopathology, degree of differentiation, age, DNA ploidy, MIB-1 and p53, were

Table 3

Histopathological variables, MIB-1, p53 and site of relapse in relation to DNA ploidy in women with relapse in surgical stage I endometrial carcinoma (n = 22)

Variable	Diploid n = 6	Aneuploid n = 16	$\chi^{2\,1}$	p
Surgical stage			1.3	0.319
1a	2	1		
1b	2	7		
1c	2	8		
Histopathology			1.5	0.348
Endometrioid AC	2	10		
Non-endometrioid AC	4	6		
Degree of differentiation			0.8	0.673
Well	1	1		
Medium	3	10		
Poor	1	5		
Not graded	1	0		
MIB-1			1.4	0.530
< 50%	0	4		
≥ 50%	4	11		
Not performed	2	1		
p53			5.0	0.087
No overexpression	4	6		
Overexpression	0	10		
Not performed	2	0		

Abbreviation: AC = adenocarcinoma.
[1] χ^2 exact test (for trend and heterogeneity, respectively).

Table 4

Univariate analysis with respect to relapse-free survival in surgical stage I endometrial carcinoma. Categorical factors

Variables	Total number	Relapses n = 24	% Relapse	$\chi^{2\ 1}$	p
Stage (n = 284)				1.6	0.44 (NS)
1a	29	3	10.3		
1b	154	10	6.5		
1c	101	11	10.9		
Histopathology (n = 284)				30.5	< 0.001
Endometrioid AC	257	14	5.4		
Only or partly UPSC, clear cell, undifferentiated	27	10	37.0		
Degree of differentiation (n = 282)				13.5	0.001
Well	139	3	2.2		
Moderate	109	14	12.8		
Poor	34	6	17.6		
Previous HRT (n = 243)				2.0	0.16 (NS)
Yes	106	12	11.3		
No	137	9	6.6		
DNA (n = 270)				18.9	< 0.001
Diploid	184	6	3.3		
Aneuploid	86	16	18.6		
p53 (n = 257)				16.6	< 0.001
No overexpression	219	9	5.0		
Overexpression	38	11	23.7		

[1]Gehan–Wilcoxon statistics.
Abbreviations: NS = non-significant; AC = adenocarcinoma; UPSC = uterine papillary serous carcinoma; HRT = hormone replacement therapy.

submitted to multivariate analysis only DNA ploidy (p = 0.001) and histopathological subtype (p = 0.004) retained their significance (Table 8). Altogether 238 patients with complete observations of DNA ploidy, MIB-1 and p53 were available for the analysis. This group included 18 patients (seven non-endometrioid) with relapse.

Endometrioid surgical stage I endometrial carcinoma (n = 257). Two hundred and twenty-five patients, including 11 relapses, had complete observations in all variables and could enter the analysis. The multivariate analysis included p53 expression, DNA ploidy, degree of differentiation and age. DNA ploidy was the only factor with preserved significance, p = 0.001 (Table 9).

DISCUSSION

In this study comprising 284 consecutive endometrial cancer surgical stage I patients with more than a 5-year follow-up, DNA ploidy with image cytometry was the strongest predictor of relapse-free survival (RFS), followed by histopathology (endometrioid/non-endometrioid tumour). In this study we had a relapse rate of 8.4%, which is lower than in similar material world-wide (1). This might be due to a lower frequency of non-endometrioid tumour in our material (10%), compared with the FIGO results, where 17% are non-endometrioid (1).

The importance of ploidy is well in line with the study by Britton et al. (7) on 203 surgical stage I patients, but neither Pfisterer et al. (8) nor Konski et al. (9), with 162 and 171 women in surgical stage I, respectively, could identify DNA ploidy as an independent predictor of survival (9) or RFS

Table 5

Cox univariate regression analysis with regard to time to relapse in surgical stage I endometrial carcinoma. Continuous factors

Variable	RH	CI	p
Age (10) n = 284	1.70	1.04–2.77	0.034
MIB-1 (10) n = 248	1.20	1.01–1.41	0.039

Abbreviation: RH = relative hazard.
10: per 10 units increment.

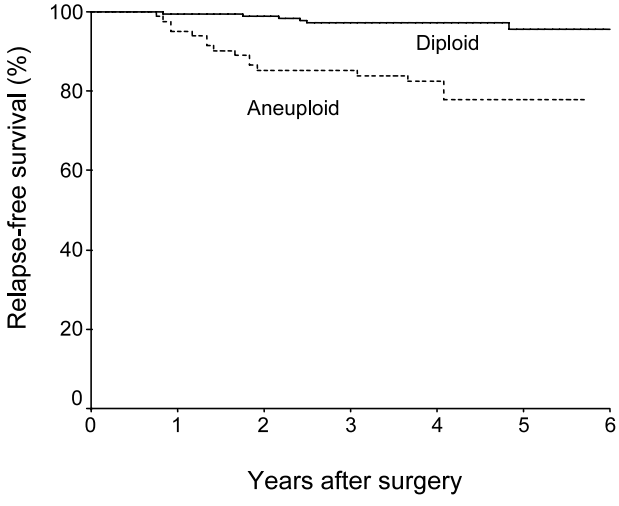

Fig. 1. Relapse-free survival as a function of aneuploidy and diploidy in endometrial carcinoma surgical stage I.

Table 6

Univariate analysis with respect to relapse-free survival in the endometroid subgroup of surgical stage I endometrial carcinoma. Categorical factors

Variable	Total number	Relapses n = 14	% Relapse	$\chi^{2\,1}$	p
Stage (n = 257)				1.3	0.52 (NS)
1a	24	1	4.2		
1b	142	6	4.2		
1c	91	7	7.7		
Degree of differentiation (n = 256)				13.5	0.055 (NS)
Well	137	3	2.2		
Moderate	95	9	9.5		
Poor	24	2	8.3		
DNA (n = 243)				19.6	< 0.001
Diploid	176	2	1.1		
Aneuploid	67	10	14.9		
p53 (n = 232)				18.7	< 0.001
No overexpression	206	5	2.4		
Overexpression	26	6	23.1		

[1]Gehan–Wilcoxon statistics.
Abbreviation: NS = non-significant.

(7, 8). The diverging importance of DNA ploidy could be attributed to varying study design (selection of patients), different methods of evaluation and number of relapses, i.e. statistical events. To achieve valid statistical figures in a disease with a favourable prognosis, such as surgical stage I endometrial carcinoma, requires a large number of patients to obtain enough statistical events. With the ratio of 86/186 (= 0.46) for the numbers of patients in the 'aneuploid' and 'diploid' groups and the 5-year relapse rates of 3.3 and 18.6%, respectively, the required number of patients (to detect such a difference) would be 111 plus 51 (total n = 162). A two-sided 'two-groups continue [G1] corrected χ^2-test of equal proportions (unequal n values)' with 80% power and 5% significance level was assumed. The aneuploidy rate varies from 14% in Pfisterer's study, to 16% in Britton's and to 32% in Konski's, as was also found in the present study. In our study, DNA ploidy was analysed with image cytometry whereas the other three studies used flow cytometry. The differences in aneuploidy rate could be attributed to varying definitions of ploidy status or methods of analysis. In Sweden, as in many other European countries, surgical staging of endometrial cancer does not include routine lymphadenectomy or sampling of unenlarged lymph nodes, which has been advocated as a criterion for complete surgical staging in some countries, e.g. the USA. When dissection/sampling of macroscopically normal lymph nodes is not performed, as in our study, some high-stage tumours might be misclassified as stage I, thus causing higher aneuploidy rates and a poorer prognosis. In particular, the non-endometrioid tumours are known to set node metastases early when the uterine tumour is still small. Thus the surgical staging method might influence the ploidy rates and RFS. In Britton's (7) and Konski's (9) studies there are no statements on nodal sampling, whereas in Pfisterer's (8) study 47 patients had pelvic lymphadenectomy if they exhibited risk factors. This does not seem to account for the differences in aneuploidy rate.

Of particular interest in this study is the finding that in cases of diploid tumour of the endometrioid type, the risk of relapse is extremely low: only 2/177 (1%) relapsed. The risk of relapse increases rapidly if the tumour is aneuploid; however, more than 80% of patients with aneuploid tumours remained relapse free. This may reflect a generous definition of aneuploidy. In our study, we were faced with the question of whether there were some aneuploid tumours that were genetically stable or whether adjuvant radio-

Table 7

Cox univariate regression analysis with regard to time to relapse in the endometroid subgroup of surgical stage I endometrial carcinoma. Continuous factors

Variable	RH	CI	p
Age (10) n = 284	1.96	1.00–3.84	0.050
MIB-1 (10) n = 225	1.02		0.16 (NS)

Abbreviation: RH = relative hazard.
(10): per 10 units increment.

Table 8

Cox multivariate stepwise regression analysis with respect to time to relapse in surgical stage I endometrial carcinoma. Relative hazards (RH) and 95% confidence intervals (CI); n = 238; relapses n = 18

Variable	Wald	RH	CI	p
Histopathology	8.1	4.09	1.55–10.8	0.004
DNA	10.3	8.31	2.28–30.3	0.001

Competing non-significant factors were age, degree of differentiation, MIB-1 and p53.

Table 9

Cox multivariate stepwise regression analysis with respect to time to relapse in the endometrioid subgroup of stage I endometrial carcinoma. Relative hazards (RH) and 95% confidence intervals (CI); n = 225; relapses n = 11

Variables	Wald	RH	CI	p
DNA	10.9	32.0	4.08–100	0.001

Competing non-significant factors were age, degree of differentiation and p53.

therapy might prevent a substantial number of relapses. There is evidence that endometrial carcinoma is more than one entity. Endometrioid and non-endometrioid tumours are known to have different backgrounds. Furthermore, recent molecular-based evidence has revealed two possible pathways for carcinogenesis of the endometrioid adenocarcinoma (23). The large number of aneuploid tumours, where only a small part relapsed, might reflect such a pathogenetic difference.

The factor reflecting proliferation, MIB-1, was significant in univariate analysis; there was a 21% higher risk of relapse with each 10% increment in MIB-1 staining and only one patient with MIB-1 $\leq$ 20% relapsed. MIB-1 loses its prognostic impact in multivariate analysis when compared with strong factors such as DNA ploidy and histopathology. Other studies on MIB-1 contain more advanced stages and most of them have not identified MIB-1 as an independently prognostic factor of survival, e.g. Nordström et al. (14), even if a few do, e.g. Geisler et al. (13).

p53 was a highly significant prognostic factor in univariate analysis but failed to retain its significance in multivariate analysis. Kohlberger et al. studied p53 in surgical stage I where p53 was significant in multivariate analysis, although it was not compared with DNA ploidy (17). There are numerous studies on p53, where some find it significant in univariate analysis but not in multivariate, e.g. Inoue et al. (19). Many others, including Geisler et al. (18), find p53 to be an independent factor for survival in multivariate analysis. In these studies, immunohistochemical expression of p53 is assumed to reflect the state of p53 mutation, but it is well known that there are both false-positive and false-negative cases, which should call for some caution in interpretation. Another possible source of error is the problem of null mutations, that is mutations in the p53 gene without protein expression or expression of altered proteins not recognizable by the antibody. The kinds of variables that are entered into multivariate analysis might also influence the result. If a strong factor, such as DNA, is included in the multivariate analysis, other co-varying factors are less likely to retain their significance.

Myometrial invasion has been considered an important factor in postoperative risk evaluation and treatment decisions (24). It is interesting to note that in our study of surgical stage I, the substages, that is myometrial invasion, were not significantly related to RFS. Two studies, however, have shown that the depth of myometrial invasion in patients treated with radiotherapy (25, 26) is not a significant factor for RFS. In these two studies, patients treated with radiotherapy because of high-risk factors were chosen from a larger group of patients. Most patients received pelvic irradiation and a few received only vaginal brachytherapy. In our present study, 44% received only vaginal brachytherapy and 42% pelvic radiotherapy in combination with vaginal brachytherapy. The lack of prognostic significance of myometrial invasion might reflect a preventive effect of radiotherapy on RFS.

CONCLUSION

Myometrial invasion is usually one of the pillars in deciding adjuvant therapy for the individual patient. In our study, myometrial invasion was not significantly related to RFS and could not support its importance in patients treated with adjuvant radiotherapy. Instead it might reflect a preventive effect of adjuvant pelvic radiotherapy that evens out the difference in survival between superficial and deep myometrial invasion. Thus no conclusion on the importance of myometrial invasion could be drawn from the study.

In this clinical study comprising 284 women consecutively staged with endometrial carcinoma, surgical stage I, DNA ploidy and the histopathological subgroup (endometrioid vs. non-endometrioid) were found to be the only two independent significant risk factors with regard to relapse-free survival. In the purely endometrioid group, where we have the greatest difficulty in estimating the risk of relapse, only DNA ploidy independently predicted RFS. It is noteworthy that only two out of 177 (1%) women with endometrioid diploid tumours relapsed.

This study clearly demonstrates that DNA ploidy is a useful tool in clinical practice when decisions concerning adjuvant therapy are made.

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