Identification of high risk patients with endometrial carcinoma. Prognostic assessment of endometrial cancer.

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Summary

Objectives: To verify the importance of DNA ploidy on clinical outcome in endometrial carcinoma and to investigate whether the prognostic information obtained by this variable is independent from other clinical-pathologic features.

Materials and methods: Univariate and multivariate analysis of clinical and pathologic prognostic factors obtained from 203 consecutive cases of endometrial cancer, that had been surgically treated in our hospital, were performed.

Results: Significant prognostic factors according to the Kaplan-Meier method were age at the time of diagnosis, grade of differentiation, peritoneal cytology, node involvement, vascular invasion, myometrial infiltration and ploidy. At multivariate analysis only DNA ploidy resulted to be an independent variable.

Conclusions: In our analysis DNA content is the only parameter which preserved prognostic significance in multivariate analysis.

Key words: Endometrial cancer; Prognostic factors; Ploidy.

Introduction

Adenocarcinoma of the endometrium is the most common malignancy of the female genital tract and it is the fourth cause of death in women after breast, colorectal and lung carcinomas [1].

Endometrial cancer is usually considered to be a relatively curable cancer, with an overall 5-year survival rate approaching 75-80%. In 80% of cases endometrial cancer is diagnosed when malignancy is confined to the uterine fundus with a survival rate of 90-95% [2].

In the last decades, despite the fact that the annual incidence of new cases has remained relatively stable, the annual number of deaths for endometrial cancer has not decreased significantly: it seems to be due to the fact that a moderate percentage of patients with a good prognosis, contrary to all expectations, relapse or die of disease [3].

An accurate prognostic assessment is required to identify groups of patients who might benefit from more aggressive treatment. Stage, histologic type, age at the time of diagnosis, grade of differentiation, depth of myometrial invasion, peritoneal cytology and lymph node spread are well-known prognostic indicators for endometrial carcinoma, and they are routinely used in the clinical management of this neoplasm. However, none of these factors allows a very accurate stratification of patients. Such parameters have also been criticized for their poor reproducibility. More objective and more accurate prognostic parameters are clearly needed. Many efforts have been made to identify molecular and genetic variables of endometrial carcinoma that may be objectively measured and may facilitate the selection of patients whose cancer will be cured with hysterectomy only versus those who will require intensive adjuvant therapy. To date, one of the most promising techniques for determining molecular or cellular characteristics of endometrial cancer is flow cytometry. Using this technology, individual cellular characteristics such as DNA content (ploidy), cell cycle (S-phase fraction) and cell size can be rapidly and accurately determined using either fresh or paraffin-embedded tissue.

Different markers were investigated immunohistochemically on endometrial tissues. P53, MIB-1, HER-2/neu were studied in endometrial cancer and were associated with the presence of extra uterine disease. These biological markers were correlated with recurrence-free survival and disease-related survival [4].

The objective of our study was to verify the importance of DNA ploidy on clinical outcome in endometrial carcinoma and to investigate whether the prognostic information obtained by this variable is independent from the other clinical-pathologic features.

Materials and Methods

Two hundred and three consecutive cases of endometrial carcinoma, referred to the Department of Obstetrics and Gynecology, H. S. Raffaele, University of Milan, were studied.

Diagnosis was performed by D&C. The patients were submitted to chest X-ray, transvaginal ultrasonography, and abdominal echotomography, TAC or MRI.

All the patients underwent primary surgery: the operation has been established conforming to the age and clinical condition of each patient: 23 cases received vaginal hysterectomy; four patients were treated by laparoscopic-assisted vaginal hysterectomy and bilateral salpingo-oophorectomy; in the other cases

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total abdominal hysterectomy, bilateral salpingo-oophorectomy with or without pelvic and aortic lymphadenectomy, upon clinical judgement, were performed. Pelvic nodes, which included external iliac and retrocrural, internal iliac, obturator and common iliac, were biopsied in 119 subjects; aortic nodes were removed in seven cases.

Peritoneal washing was effected in all the patients submitted to laparotomy but in 151 cases only was it possible to perform cytological examination.

One hundred and twenty-three patients were treated by surgery alone; 23 received adjuvant chemotherapy, 57 were submitted to complementary radiotherapy. Radiotherapy was effected in stage IC, II and III; some patients were randomized in a large clinical trial comparing chemotherapy (cisplatin, doxorubicin and cyclophosphamide) versus radiotherapy [5].

Tumor cases considered in this study were in stage I, II, IIIA and IIIC, according to the FIGO classification.

Special histotypes, such as clear cell carcinomas, papillary serous carcinomas or undifferentiated carcinomas were excluded.

Histological grading was classified as follows: G1: high degree of differentiation; G2: medium degree of differentiation; G3: low degree of differentiation. Vascular invasion and depth of myometrial invasion were also histologically determined. Myometrial invasion was graded as follows: M0: carcinoma confined to the endometrium; M1: tumor involving inner half of the myometrium; M2: tumor involving outer half or the full thickness of the myometrium.

Cytofluorimetric analysis:

On the 4 μm-thick sections stained with haematoxylin and eosin and used for histologic diagnosis, we selected representative neoplastic areas of the lesion. Then, on the same paraffin blocks, we incised around them and cut two 40 μm-thick sections: the first section contained neoplasia while the second was composed of normal tissue. If normal tissue was not available, a normal lymph node obtained from the same patient during surgery was used as a diploid control. Tissue was treated according to Hedley [6] with minor modifications: sections were placed in 10 ml glass tubes, dew axed and rehydrated with 50% alcohol and 50% xylene, 100, 95, 70, 50% ethanol in sequence for 10 minutes each at room temperature, and placed in distilled water in a water-bath at 37°C for 1 hour. They were then placed in 0.5% pepsin in TRIS-HCl pH 1.5 for 1 hour at 37°C in a water-bath. After centrifugation at 300 g the specimens were stained with propidium iodide (5% in Na citrate 0.1%), 15 μl of nonidet p40 (1% in distilled water) and 5 U of ribonuclease at room temperature in darkness for 3-4 hours. The cell suspensions were then filtered through a 70 μm nylon mesh. The DNA cell content was measured using a FAC Scan analyser (Becton Dickinson. Mountain View, CA); 20,000 events were counted for each specimen.

Results with a coefficient variation (CV) lower than 8 were considered valid [7]; if the CV was higher than 8 the procedure was performed again, starting from the paraffin section. For each specimen a haematoxylin and eosin smear of the suspension was performed to evaluate the adequacy of the material in terms of number, desegregation and preservation of nuclei.

The DNA index was calculated as the ratio between the mode of the relative DNA content of G0/G1 cells of the sample and the mode of the relative DNA measurement of the diploid G0/G1 reference cells [6]. All the cases were divided into aneuploid (one or more abnormal peaks) and diploid groups. Among the aneuploid groups, cases with 0.8-1.2 DI were considered neardiploid, while cases with a 4N peak (higher than 20% of analyzed events) and an 8N peak were considered tetraploid [8].

Statistical analysis:

Overall survival was calculated from the day of operation to the date of death or last contact.

Survival rates and disease-free survival were determined by Kaplan-Meier curves [9].

Univariate analysis, according to the Log-rank test, was used to assess the effect of each prognostic variable on survival.

A multivariate analysis (Cox proportional hazards) was also performed to identify which of the parameters yield independent prognostic information. Age at the time of diagnosis, histologic grading, DNA content, depth of myometrial invasion, peritoneal cytology, vascular invasion and node involvement have been compared. Relative risks for each parameter, with the corresponding confidence interval of 95%, were calculated using the Cox model [10].

Results

Characteristics of the patients:

Median age was 63.9 (range 27-88 years). Median follow-up was 41.2 months (range 3-143 months). Final event, defined according to date of death or last contact, showed 170 patients still alive and free of disease, three alive with tumor and 30 dead of disease.

Histological findings:

Twenty-three of 203 (11.3%) patients were in stage IA, 74 (36.5%) in stage IB, 41 (20.1%) in stage IC; 9 (4.5%) were in stage IIA and 24 (11.8%) in stage IIB; 19 cases (9.4%) were in stage IIIA and 13 (6.4%) in stage IIIC.

Table 1 shows the percentages of survival and death found between subgroups within each stage.

According to grade of differentiation we observed 35% of G1, 44.3% of G2 and 20.7% of G3. Myometrial invasion was absent in 23 cases (11.3 %), lower than 50% of total thickness in 98 patients (48.3%) and higher than 50% in 82 (40.4%).

Distribution of survival and death for each subgroup is reported in Table 1.

Vascular invasion was observed in 62 histological specimens (30.5%) with a mortality of 27.5% (Table 1).

Sampling of pelvic and para-aortic lymph nodes was performed in 119 cases (median number of nodes removed: 15.5; range: 1 - 40); metastases were found in 13 (11%) patients. Among the 106 patients with negative nodes we counted eight deaths (7.5%); in presence of retroperitoneal node metastases the number of deaths increased to 30.8% (4/13) (Table 1).

Peritoneal cytology was effected in 151 cases: 13 resulted positive for the presence of malign tumoral cells and 138 were negative (Table 1).

Cytofluorimetric analysis of DNA content showed 152 diploid tumors (DI=1) (74.8%); 21 neardiploid tumors ($0.8 < DI < 1.2$) (10.4%) and 30 aneuploid tumors ($DI < 0.8$ or > 1.2) (14.8%). Considering together diploid and neardiploid tumors we observed 19/173 (10.9%) deaths; among the aneuploid tumors we counted 11/30 (36.7%) deaths (Table 1).

Univariate analysis showed a significant relation of survival with all the variables examined: age at time of diagnosis ($p = 0.0001$), stage ($p = 0.001$), grade of diffe-

Table 1. — *Univariate analysis of prognostic factors.*

	Alive	Dead	Total	P
Stage				**0.001**
IA	21	2	23 (11.3%)	
IB	70	4	74 (36.5%)	
IC	38	3	41 (20.1%)	
IIA	3	6	9 (4.5%)	
IIB	20	4	24 (11.8%)	
IIIA	12	7	19 (9.4%)	
IIIC	9	4	13 (6.4%)	
Grade				**0.003**
GI	69	2	71 (35%)	
G2	72	18	90 (44.3%)	
G3	32	10	42 (20.7%)	
M				
M0	21	2	23 (11.3%)	
M1	88	10	98 (48.3%)	
M2	64	18	82 (40.4%)	
Vascular invasion				**0.009**
Present	45	17	62 (30.5%)	
Absent	128	13	141 (69.5%)	
Nodes				**0.006**
Negative	98	8	106 (89%)	
Positive	9	4	13 (11%)	
Washing				**0.005**
Negative	121	17	138 (91.4%)	
Positive	7	6	13 (8.6%)	
Ploidy				**0.001**
Diploid	135	17	152 (74.8%)	
Neardiploid	19	2	21 (10.4%)	
Aneuploid	19	11	(14.8%)	

rentiation (p = 0.003), peritoneal cytology (p = 0.005), node involvement (p = 0.006), vascular invasion (p = 0.009), myometrial infiltration (p = 0.019), ploidy (p = 0.001) and relapse of disease (p = 0.0001).

The relation between node involvement and ploidy was also investigated: aneuploidy was found in 4.1% of the 102 patients with negative lymph nodes; in cases of positive nodes aneuploidy was observed in 35% of patients (p = 0.001).

Multivariate analysis was realized comparing the following parameters: age at the time of diagnosis, myometrial invasion, peritoneal cytology, node involvement, grade of differentiation, vascular invasion and ploidy. Only DNA ploidy resulted to be an independent variable (p = 0.0129) with a relative risk of 6.712 (Table 2).

Table 2. — *Multivariate analysis of prognostic factors.*

Prognostic factors	Risk ratio	95% confidence limits
Age	1.070	0.997 – 1.148
Peritoneal cytology	0.244	0.048 – 1.233
Lymph nodes	1.655	0.297 – 9.212
Grading	2.422	0.782 – 7.496
Ploidy	6.712	1.497 – 30.095
Myometrial invasion	1.778	0.379 – 8.347
Vascular invasion	1.151	0.245 – 5.398

Discussion

Many clinical and pathological studies have demonstrated that probability of relapse and death for endometrial cancer could be predicted by different histological parameters as histologic type, grade of differentiation, vascular invasion, peritoneal cytology and cervical infiltration [11, 12].

In this study we analyzed the most commonly used prognostic factors in the management of endometrial cancer, such as age at the time of diagnosis, staging, grade of differentiation, myometrial infiltration, vascular invasion, peritoneal cytology and node involvement. Besides these "traditional" parameters we examined DNA ploidy determined by cytofluorimetric analysis.

In univariate analysis age, staging, grade of differentiation, peritoneal cytology, node involvement, DNA content, myometrial infiltration and vascular invasion resulted to be significant. The relative importance of the prognostic variables considered above cannot be determined by univariate analysis because most of them could be inter-related and not independently significant. Many well-differentiated tumors are only superficially invasive, while poorly differentiated tumors are frequently deeply invasive. Creasman *et al.*[11] observed 87% of G1 tumors with superficial involvement of the myometrium, while 58% of G3 tumors showed deep myometrial invasion. In this analysis some important exceptions appeared: 7% of G3 tumors were confined to the endometrium, while 10% of G1 lesions showed deep myometrial infiltration. Mangioni *et al.* [12] reached the same conclusion: in 1,055 patients affected by endometrial carcinoma, well differentiated tumors (G1) more frequently showed absence of myometrial invasion (16%) or involvement of the internal third of the myometrium (54%). On the contrary undifferentiated tumors (G3) were associated in 62% of cases to involvement of the external third of the myometrium. Sometimes it is impossible to establish if the biological behavior of the tumor is determined by myometrial invasion or histologic grade or both parameters. To identify the independent variables we used multivariate analysis which allows the creation of prognostic models based on the determination of relative risk for each parameter to predict the probability of relapse or death from disease. The importance of defining new prognostic models for endometrial cancer is due to the great diffusion of the disease and, above all, to the presence of a moderate percentage of patients with a good prognosis that, contrary to all expectations, relapse or die from disease.

In multivariate analysis DNA ploidy was the only independent variable. Histologic grading, age at time of diagnosis, node involvement, myometrial infiltration, vascular invasion and peritoneal cytology were not significant.

Age at the time of diagnosis is considered as an important prognostic factor in many studies; Lindhal *et al.* [13] observed 5% of mortality for patients 40-49 years old, 3% for 50-59, 15% for 60-69 and 25% for patients older than 70 years (p = 0.01). In our analysis the median age was 65 years. The median age in living patients was 61.6;

in deceased patients it was 75. In multivariate analysis age lost its significance ($p = 0.058$). This result is similar to that of Susini *et al.* [14] and in contrast to the study of Abeler and Kjorstad [15]. Discordance of data in the literature can have a double meaning: age at the time of diagnosis could be related to a tendency in developing well-differentiated tumors in young women with minor myometrial infiltration; on the other hand this variable should be included among operative risk factors. Thus it is possible to argue that the more unfavorable prognosis observed in older patients is due to inadequate staging related to difficulties of effecting a more radical surgery.

Staging is considered by many authors [15, 16] as the most significant prognostic factor in endometrial cancer. The importance of correct surgical staging has frequently been discussed in the last decade. In our study all the patients underwent primary surgery: 23 cases were submitted to vaginal hysterectomy; four patients underwent laparo-assisted vaginal hysterectomy with bilateral salpingo-oophorectomy; in the other cases abdominal hysterectomy and bilateral salpingo-oophorectomy with or without pelvic and aortic lymphadenectomy were performed. Pelvic lymph nodes, including the external iliac and retrocrural, internal iliac, obturator and common iliac, were biopsed in 119 cases (median number of lymph nodes removed: 15; range: 1-40); aortic nodes were biopsed in seven cases. In our analysis lymph node status was significatively correlated to survival, in accordance with the literature which shows survival rates of 30-50% for pelvic and of 20-40% for aortic nodes. Despite these results the usefulness of lymphadenectomy in the management of endometrial carcinoma is still discussed. Many authors [12, 17] have underlined the need of utilizing extensive surgical staging, above all in the apparently stage I carcinomas of the endometrium. In Mangioni *et als'*. analysis [12] of 1,055 patients affected by endometrial cancer, clinical staging in 1971, contrary to surgical stagingin 1988, resulted to be inadequate in 16% of the cases. In patients in which tumor was confined to the uterus the frequency of retroperitoneal nodal metastasis was 4%, while in cases of extrauterine diffusion this percentage reached 17%. Retroperitoneal lymph node status and its eventual correlation to other prognostic factors has caught the attention of clinicians for many years. Frequency of pelvic and aortic lymph node metastasis is about 4% and 2%, respectively, for G1 tumors and about 30% and 17% for G3; the probability of node involvement is 1% if myometrial invasion is absent and 32% for pelvic and 16% for aortic nodes if myometrial infiltration is higher than 50%. Myometrial invasion seems to be more strictly connected to lymph node metastasis than grade of differentiation, as demonstrated in Creasman *et als'*. analysis [11]. Recently a Japanese analysis [18] of 684 patients affected by adenocarcinoma of the endometrium stage I (all patients had undergone pelvic lymphadenectomy) demonstrated that of 100 cases of tumor confined to the endometrium, four (4%) showed positive nodes and the histological grade was well differentiated. It is impossible to exclude node involvement even if myometrial invasion and histological differentiation are favorable. Pelvic lymphadenectomy is therefore recommended in all the cases of endometrial cancer except for the presence of operative risks such as advanced age, obesity, diabetes, hypertension, cardiopathies and other diseases.

In our study we also examined the correlation between node involvement and ploidy: of the 13 cases with positive lymph nodes nine (69.2%) presented aneuploid tumors. Other authors [19] reported similar results, observing a higher frequency of aneuploid tumors in patients with positive nodes. Of the 76 patients considered, 66 had negative lymph nodes. In this group the percentage of aneuploid tumors was 18%; when nodes were involved (10/76) frequency of aneuploidy was 60% ($p < 0.01$). These results are very important because DNA content can be determined before surgery from biopsies. The finding of a condition of aneuploidy could be useful for clinicians to effect a more radical surgery which would include systematic retroperitoneal lymphadenectomy [20].

The DNA content of tumor cells has been found to be a significant indicator of prognosis for many kinds of cancer, including ovarian cancer [21-23]. Studies on endometrial carcinoma have shown that DNA content correlates with tumor grade and clinical outcome [24-29] and it has been reported to be an independent prognostic factor [24, 28].

Moberger *et al.* [26], in a retrospective study with eight years follow-up, showed that 88% of deceased patients had aneuploid tumors, whereas living patients had aneuploid tumors in 24% of cases. In multivariate analysis ploidy resulted to be an independent prognostic factor.

Britton *et al.* [30] analyzed paraffin-embedded tissue from 203 cases of endometrial cancer stage I and observed 16% of aneuploid tumors representing 50% of relapses which occurred during the follow-up period. In multivariate analysis a comparison among histologic type, grade of differentiation and DNA ploidy, only DNA content and histologic type were demonstrated to be independent variables.

Newbury *et al.* [31] showed a correlation between aneuploidy, adverse histologic type, high grade and depth of myometrial invasion: a DNA index greater than 1.5 strongly predicted death from disease.

Ikeda *et al.* [19] and Melchiorri *et al.* [32] found DNA ploidy to be an independent variable characterized by the strongest prognostic significance.

During the last decade efforts have focused on attempting to identify cytokinetic or molecular variables that correlate with the malignant potential of endometrial cancer. Specifically, the expression of oncogenes and tumor suppressor genes (HER-2/neu oncogene, bcl-2 protein and p53 tumor suppressor gene) and the indicators of cell proliferation (S-phase fraction, MIB-1 proliferation marker and proliferating cell nuclear antigen - PCNA) have been evaluated [33]. Most of these parameters are still in process of evaluation and they are not clinically applicable yet. DNA ploidy, on the contrary, is an objective and reproducible prognostic factor, easy to determine and inexpensive. In our opinion DNA ploidy should be introduced routinely in the clinical management of this neoplasm.

Conclusions

Traditional prognostic factors currently available in preoperative endometrial biopsies are histologic grade and histologic type. Prognostic value derivable from the assessment of these elements is recognizably limited, while the subjective nature of their analysis prompts concern about reproducibility.

In our analysis DNA content was the only parameter which preserved prognostic significance in multivariate analysis.

Ploidy is a quantitative parameter and it could provide more objective and reproducible information than other prognostic factors like histologic grade.

Ploidy can be determined preoperatively on bioptical materials; thus it would be possible to establish preventively the type and extension of the surgical procedure.

After primary surgery DNA content could be used for selecting patients to submit to adjuvant therapy. The evaluation of ploidy may enable clinicians to stratify endometrial cancer patients into low and high-risk groups before starting definitive therapy.

References

[1] Genest P., Drouin P., Gerig L., Girard A., Stewart D., Prefontain M.: "Prognostic factors in early carcinoma of the endometrium". *Am. J. Clin. Oncol.*, 1987, *10 (1)*, 71.

[2] Morrow C. P., Bundy B. N., Kurman R. J.: "Relationship between surgical pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium". *Gynecol. Oncol.*, 1991, *40*, 55.

[3] Petterson F.: "Annual report on the results of treatment in gynecological cancer. Radimhemmet, Stockholm". *Int. J. Gynecol. Obstet.*, 1991, *36 (suppl.)*, 132.

[4] Silverman M. B., Roche P. C., Kho R. M., Keeney G. L., Li H., Podratz K. C.: "Molecular and cytokinetic pretreatment risk assessment in endometrial carcinoma". *Gynecol. Oncol.*, 2000, *77 (1)*, 1.

[5] Maggi R., Cagnazzo G., Atlante G., Marinaccio M.: "Risk groups and adjuvant therapy in surgical staged endometrial cancer patients. A randomized multicenter study comparing chemotherapy with radiation therapy". *Int. J. Gynecol. Cancer*, 1999, *9*, 85.

[6] Hedley D. W., Friedlander M. L., Taylor I. W., Rugg C. A., Musgrove E. A.: "Method for analysis of cellular DNA content of paraffin embedded pathological material using flow cytometry". *J. Histochem. Cytochem*, 1983, *31*, 1333.

[7] Shankey T. V., Rabinovitch P. S., Bagwell B., Bauer K. D., Duque R. E., Hedley D. W. *et al.*: "Guidelines for implementation of clinical DNA cytometry". *Cytometry*, 1993, *14*, 472.

[8] Joensun H., Klemi P. J.: "DNA aneuploidy in adenomas of endocrine organs". *Am. J. Pathol.*, 1988, *132*, 145.

[9] Kaplan E. I., Meier P.: "Nonparametric estimation from incomplete observation". *J. Am. Stat. Assoc.*, 1958, *53*, 457.

[10] Cox D. R.: "Regression models and life tables". *J. Res. Stat. Soc.*, 1972, *34*, 187.

[11] Creasman W. T., Morrow P. C., Bundy B. N. *et al.*: "Surgical pathologic spread patterns of endometrial cancer. A Gynecologic Oncologic Group study". *Cancer*, 1987, *60*, 2035.

[12] Mangioni C., De Palo G., Marubini E., Del Vecchio M.: "Surgical pathologic staging in apparent stage I endometrial carcinoma". *Gynecol. Cancer*, 1993, *3*, 373.

[13] Lindhal B., Raustam J., Willen R.: "Five year survival rate in endometrial carcinoma stage I-II: Influence of degree of tumor differentiation, age, myometrial invasion and DNA content". *British J. Obstet. Gynecol.*, 1994, *101*, 621.

[14] Susini T., Rapi S., Savino L., Baddi V., Berti P., Massi G.: "Prognostic value of flow cytometric deoxyribonucleic acid index in endometrial carcinoma: comparison with other clinical-pathologic parameters". *Am. J. Obstet. Gynecol.*, 1994, *170*, 527.

[15] Abeler V. M., Kjorstad K. E.: "Endometrial adenocarcinoma in Norway". *Cancer*, 1991, *67*, 3093.

[16] Maneschi M., Maneschi F., Geraci P. *et al.*: "Surgical pathological staging of endometrial carcinoma and results of treatment". *Eur. J. Gynecol. Oncol.*, 1992, *13 (suppl. 1)*, 30.

[17] Wolfson A. H., Sightler S. E., Markol AM. *et al.*: "The prognostic significance of surgical staging for carcinoma of the endometrium". *Gynecol. Oncol.*, 1992, *45*, 142.

[18] Takeshima N., Hirai Y., Tanaka N. *et al.*: "Pelvic lymph node metastasis in endometrial cancer with no myometrial invasion". *Obstet. & Gynecol.*, 1996, *88, 2*, 280.

[19] Ikeda M., Watanebe Y., Nanjoh T., Noda K.: "Evaluation of DNA ploidy in endometrial cancer". *Gynecol. Oncol.*, 1993, *50*, 25.

[20] Susini T., Rapi S., Massi D., Savino L., Amunni G., Taddei G. L., Massi G.: "Preoperative evaluation of tumor ploidy in endometrial carcinoma. An accurate tool to identify patients at risk for extrauterine disease and recurrence". *Cancer*, 1999, *86 (6)*, 1005.

[21] Rodenburg C. J., Cornelisse C. J., Heintz P. A., Herinans J., Fleuren G. J.: "Tumor ploidy as a major prognostic factor in advanced ovarian cancer". *Cancer*, 1987, *59*, 317.

[22] Friedlander M. L., Hedley D. W., Swanson C., Russel P.: "Prediction of long term survival by flow cytometric analysis of cellular DNA content in patients with advanced ovarian cancer". *J. Clin. Oncol.*, 1988, *6*, 282.

[23] Kallioniemi O., Punnonen R., Mattila J., Lehtinen M., Koivula T.: "Prognostic significance of DNA index, multiploidy, and S-phase fraction in ovarian cancer". *Cancer*, 1988, *61*, 334.

[24] Iversen O., Laerum O.: "Ploidy disturbances in endometrial and ovarian carcinomas. A review". *Anal. Quant. Cytol. Histol.*, 1985, *7*, 327.

[25] Atkin N.B.: "Prognostic significance of ploidy level in human tumors. Carcinoma of the uterus". *J. N. C. I.*, 1976, *56*, 909.

[26] Moberger B., Auer G., Forfslund G.: "The prognostic significance of DNA measurement in endometrial carcinoma". *Cytometry*, 1985, *5*, 430.

[27] Iversen O.: "Flow cytometric deoxyribonucleic acid index: A prognostic factor in endometrial carcinoma". *Am. J. Obstet. Gynecol.*, 1986, *155*, 770.

[28] Lindahl B., Alm P., Killander D., Langstrom E., Trope C.: "Flow cytometric DNA analysis of normal and cancerous human endometrium and cytological-histopathological correlations". *Anticancer Res.*, 1987, *7*, 781.

[29] Lindahl B., Alm P., Ferno M. *et al.*: "Prognostic value of flow cytometric DNA measurements in stage I-II endometrial carcinoma: Correlations with steroid receptor concentrations, tumor myometrial invasion, and degree of differentiation". *Anticancer Res.*, 1987, *7*, 791.

[30] Britton L. C., Wilson T. O., Gaffey T. A. *et al.*: "Flow cytometric DNA analysis of stage I endometrial carcinoma". *Gynecol. Oncol.*, 1989, *34*, 317.

[31] Newbury R., Schuerch C., Goodspeed N., Fanning J., Glidewell O., Evans M.: "DNA content as a prognostic factor in endometrial carcinoma". *Obstet. & Gynecol.*, 1990, *76, 2*, 251.

[32] Melchiorri C., Chieco P., Lisignoli G., Marabini A.: "Ploidy disturbances as an early indicator of intrinsic malignancy in endometrial carcinoma". *Cancer*, 1993, *72, 1*, 165.

[33] Mariani A., Sebo T. J., Katzmann J. A., Keeney G. L., Roche P. C., Lesnick T. J., Podratz K. C.: "Pretreatment assessment of prognostic indicators in endometrial cancer". *Am. J. Obstet. Gynecol.*, 2000, *182, 6*, 1535.

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