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Evaluation of prognostic factors following flow-cytometric DNA analysis after cytokeratin labelling: II. Cervical and endometrial cancer

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Abstract. In gynecologic oncology valid prognostic factors are necessary to define biologically similar subgroups for analysis of therapeutic efficacy. This study is the first published prospective study concerning prognostic significance of DNA ploidy and S-phase fraction in cervical and endometrial cancer following enrichment of tumor cells by cytokeratin labelling. Epithelial cells were labeled by FITC-conjugated cytokeratin antibody (CK 5, 6, 8, and CK 17) prior to flow cytometric cell cycle analysis in 91 specimens of cervical cancer and 73 samples of endometrial cancer.

In cervical cancer neither DNA-ploidy nor S-phase fraction were relevant prognostic parameters. But CV of the G_0G_1-peak showed prognostic relevance in cervical cancer cells, even in multivariate analysis. This interesting observation, however, seems to have no therapeutic consequence due to the small discrimination capacity of CV.

In endometrial carcinoma, gross DNA-aneuploidy (DNA-index > 1.3) and a high percentage of proliferating cells ($>$75th percentile) were univariate and multivariate highly significant prognostic factors for recurrence-free survival. Especially DNA-aneuploidy (DI > 1.3) is one of the most important independent molecular biological prognostic factors. While diagnostic curettage we could identify risk patients even preoperatively by determination of the prognostic factors like histologic tumor type, grading, cervical involvement and DNA-ploidy. Thereby these patients could be treated primarily in an oncologic center.

In conclusion, our investigations showed that the determination of DNA-ploidy should be done in endometrial carcinoma. In cervical cancer no clinical significance for determination of DNA-parameters was found.

1. Introduction

Prognostic factors are an important basis for optimal choice of therapeutic strategy. Prognostic factors are important to decide if additional adjuvant therapeutic modalities are necessary. They render an individual care for patients with gynecologic malignancies.

Alteration of genetic factors is often accompanied by quantitative changes of DNA content such as the p53 gene alteration, which is associated with DNA aneuploidy [1].

Chromosomal aberrations seem to occur in aneuploid tumor cell lines [2]. It has been shown for different carcinomas that cytokeratin staining is able to detect epithelial tumor cells [3,4].

Apart from clinico-pathological parameters, DNA-ploidy and S-phase fraction as parameters for changes in genetic information and proliferation behaviour have been intensively studied in tumors of the female

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Table 1
Distribution of clinical-histopathological parameters of patients with primary cervical cancer ($n = 91$). Distribution of patients with prospective analysis were documented in brackets $\{n = 69\}$

FIGO-stage	Ib	IIa	IIb	III	IV	nk
number (n)	40{35}	11{9}	22{20}	4{2}	3{3}	11{0}
Lymph node involvement		pN0		pN1		nk
number (n)		46{41}		34{28}		11{0}
Grading		1	2	3		4
number (n)		2{2}	31{25}	57{41}		1{1}
Lymphangiosis		no		yes		nk
number (n)		64{54}		16{13}		11{0}
Hemangiosis		no		yes		nk
number (n)		74{63}		6{6}		11{0}

genital tract [5]. However, in spite of a great quantity of data no clear conclusion can be drawn from the available data.

Concerning cervical [6–8] and endometrial cancer [9] conflicting results were reported with respect to clinical significance of determining DNA-ploidy and S-phase fraction.

Methodical problems in determination of DNA-parameters may responsible for the discrepancy of results. To evaluate the prognostic significance of cell cycle analysis based on cytokeratin labelling, a prospective study was initiated.

The presented prospective study is the first published study concerning prognostic significance of DNA ploidy and S-phase fraction in cervical and endometrial cancer following enrichment of tumor cells by cytokeratin labelling.

2. Materials and methods

Fresh tumor tissue of carcinomas of 91 cervical cancers and 73 endometrial carcinomas were dissociated by combined mechanical/enzymatic method [10] as described in detail [11]. The detailed method for evaluation of DNA parameters by DNA flow cytometry was shown elsewhere [12].

2.1. Patients

2.1.1. Cervical cancer

91 patients with cervical carcinomas were investigated. Thereby 81 squamous epithelial carcinoma, 7 adenocarcinomas, one adenosquamous and one mucoepidermoid carcinoma and also one carcinoid were found. The following cases were excluded: 7 patients with secondary malignancy, 3 patients with primary metastasis and 12 patients with primary combined radiation therapy with or without a former staging laparatomy. Thus, 69 patients were analyzed with primary radical hysterectomy and pelvic and in some cases also paraaortic lymphadenectomy. The distribution of clinical and histopathological parameters were described in Table 1. Numbers of prospective analysis were noticed in brackets. The subsequent therapy was described in Table 2. The median observation time after operation was 1073 days (25th percentile 528). From 68 of 69 patients a complete follow-up was achieved, one patient was lost of follow-up after 1 year. Thereby 19 patients developed relapse and 15 patients died, 13 of them due to cervical cancer.

Table 2
Distribution of therapy modalities in patients with primary cervical cancer. Only patients were mentioned, that could be included in prospective analysis ($n = 69$). HE = hysterectomy, LNE = Lymphonodectomy, pelv. = pelvic, pa. = paraaortic

Therapy of cervical cancer, $n = 69$	Number (n)
Radical HE with pelv. LNE	42
Radical HE with pelv./pa. LNE	25
Radical HE with pelv./pa. LNE + exenteration	2
Adjuvant radiation (vaginal afterloading)	24
Adjuvant radiation (combined internal/external)	8
Adjuvant chemotherapy	9

2.1.2. Endometrial carcinoma

73 patients were analyzed, hereby 65 were endometrioid, 2 adenosquamous, 3 clear cell and 3 mullerian tumors. Tumors of all patients were analyzed following cytokeratin labelling. 10 patients with secondary malignancy, 2 with primary metastasis and 8 patients with primary combined radiation were excluded. That means that 52 patients with primary rad-

Table 3
Distribution of clinical-histopathological parameters of patients wit primary endometrial cancer ($n = 73$). Distribution of patients with prospective analysis were documented in brackets $\{n = 52\}$

FIGO-stage	Ia	Ib	Ic	II	III	IV
number (n)	5{5}	32{22}	13{12}	12{7}	7{4}	1{2}
Lymph node involvement		pN0		pN1		nk
number (n)		38{32}		5{4}		33{16}
Grading		1		2		3
number (n)		19{14}		37{27}		17{11}
Myometrane infiltration		no	<50%	>50%		serosa
number (n)		5{4}	24{24}	37{23}		3{1}

Table 4
Distribution of therapy modalities in patients with primary endometrial cancer. Only patients were mentioned, that could be included in prospective analysis ($n = 52$). Abd. = abdominal, rad. = radical, HE = hysterectomy, LNE = lymphonodectomy, pelv. = pelvine, pa. = paraaortic

Therapy endometrial cancer, $n = 52$	Number (n)
Abd. HE with adnectomy	16
Abd. HE with adnectomy and pelv. LNE	29
Abd. HE with adnectomy and pelv./pa. LNE	5
Restricted rad. HE with adnectomy and pelv./pa. LNE	2
Adjuvant radiation (vaginal afterloading)	28
Adjuvant radiation (combined internal/external)	4

ical hysterectomy and pelvic and in some cases also paraaortic lymphadenectomy were analyzed. 28 patients had a postoperative adjuvant irradiation, i.e., vaginal afterloading, and 4 patients had a combined vaginal and abdominal radiation. The distribution of clinical histopathological parameters of both collectives were shown in Table 3 and the therapies in Table 4. The median observation time after operation was 1301 days (25th percentile 746/75th percentile 1779 days). The median age of patients was 68.4 years with a range of 29 to 91.2 years. We performed a complete follow-up of 51 patients. 8 patients showed metastasis or recurrence and 15 patients died, but only 7 patients due to endometrial carcinoma.

2.1.3. Statistics

The experimental data were analyzed with using SAS (SAS Institute Inc., Cary, NC) on a UNIX work station. The different proportions of DNA-aneuploid tumors found with gating for cytokeratin positive cells compared to those without gating were described using a two-by-two table. As no definitive reference test for determination of ploidy was available, the results obtained with gating could only be described in relation to our results without gating. The McNemar test was used to test the null hypothesis that the results of the two methods are distinguishable. In order to compare proportions from cell counts obtained by cell cycle analysis with the statistical test they were transformed to the angular scale using an arcsin transformation. The transformed proportions were compared with the use of a paired t-test [13]. Calculation of recurrence free survival (RFS) and tumor dependent overall survival (CSS) was estimated according to the Kaplan–Meier method. The independent prognostic significance of each factor was evaluated by Cox regression model. Two-tailed p-values are given. P-values less than 0.05 were considered statistically significant.

3. Results

Cervical cancer

3.1. Results of DNA-cell cycle analysis in dependence on cytokeratin labelling

3.1.1. Detection of DNA-aneuploid subpopulations

Tumor specimens of 91 patients with primary cervical cancer were examined. The analysis of total cell suspension found 60% DNA-aneuploid tumors. After cytokeratin labelling a rate of 80% DNA-aneuploid tumors were detected (see in detail in [11]).

3.1.2. Distribution of DNA-indices of cytokeratin-positive subpopulations

In 91 cervical carcinomas 18 tumors were DNA-diploid after cytokeratin labelling; in 73 DNA-aneuploid tumors 121 DNA-aneuploid subpopulations were found. The distribution of DNA-indices were published elsewhere [11].

3.1.3. Correlation of DNA-parameters with clinical histopathological prognostic factors

The parameter DNA-ploidy, DNA-index, S-phase fraction, G_2M-phase fraction and CV of tumor cells were examined for correlation to the parameters FIGO-stage, pT-stage, pelvic lymph node status, grading and histologic subtype by Chi-square-test. Worse differentiated tumors have significantly more frequently DNA-index > 1.3 ($p = 0.04$), adenocarcinoma have a lower CV in contrast to squamous carcinoma ($p = 0.05$), whereas tumors of higher FIGO-stages have higher CV-values ($p = 0.05$). The other parameters did not correlate to each other.

3.2. Prognostic significance of DNA-ploidy and S-phase fraction in cervical cancer in comparison to classic prognostic factors: univariate analysis

3.2.1. Prognostic significance of classic clinical histopathologic prognostic factors

In univariate analysis of clinical histopathological prognostic factors, the influence of age of patients, tumor histology, tumor stage, pelvic lymph node involvement, grading, lymphangiosis and hemangiosis carcinomatosis on RFS and CSS were examined. The strongest influence on prognosis had the age of patients. RFS was 81% for patients $\leqslant$60 years and for patients older than 60 years RFS was 40% ($p = 0.0006$). CSS decreased in the same way from 90 to 53.3% ($p = 0.005$). Significance was also present for influence of tumor stage with 88.6% (pT1) and 55.2% for pT2-tumors (RFS; $p = 0.005$) respectively 91.4% (pT1) and 69.0% for pT2-tumors (CSS; $p = 0.10$). All patients with pT4-stage ($n = 3$) were locally advanced and got an exenteration. All 3 patients survived the observation time recurrence-free. This explains the absent significance of tumor stage for CSS. The absent significance of the other factors may be present because of the small collective. Only for pelvic lymph node involvement we found a borderline significant decrease of RFS from 80.5 to 60.7% ($p = 0.06$).

For better estimation of time course and relation of censored patients to patients with relapse or tumor dependent death Kaplan–Meier curves were shown for the parameters age, tumor stage (pT), grading and pelvic lymph node involvement (pN) (data not shown).

3.3. Prognostic significance of DNA-ploidy, DNA-index and CV of G_0G_1 peak

The univariate analysis of DNA-ploidy, DNA-index and CV of G_0G_1 peak showed no significant influence on RFS or CSS. Only a trend for worse prognosis for DNA-diploid tumors (not significant) and for tumors with high CV (RFS; $p = 0.05$) was recognizable.

For better estimation of time course and relation of censored patients to patients with relapse or tumor dependent death, Kaplan–Meier curves were shown for DNA-ploidy with and without cytokeratin labelling, for CV and for S-phase fraction (Fig. 1). The difference in course of DNA-diploid and DNA-aneuploid tumors was higher after cytokeratin labelling. Only the CV is of borderline significance for RFS.

3.4. Prognostic significance of S-phase fraction, G_2M phase fraction with and without identification of tumor cells by cytokeratin labelling

For the parameters S-phase fraction and G_2M phase fraction no significant influence on RFS or CSS was found after univariate analysis with and without cytokeratin labelling.

3.5. Prognostic significance of DNA-ploidy and S-phase fraction in cervical cancer in comparison to classic prognostic factors: multivariate analysis

The univariate significant prognostic factors tumor stage (pT), age and the borderline significant parameter pelvic lymph node stage (pN) and CV were examined by Cox-multivariate analysis (Table 5). Multivariate analysis detected a significance for age of patients concerning RFS (RR 2.8; $p = 0.008$) and for CV (RR 2.7; $p = 0.04$). For CSS only the age of patients was prognostic relevant (RR 2.9; $p = 0.01$). The other parameters offered no further information.

To avoid, that the 3 tumors with adenosquamous, mucoepidermoid and carcinoid histology have a relevant influence on results, a multivariate analysis with exclusion of these tumors was performed. Only marginal changes were found.

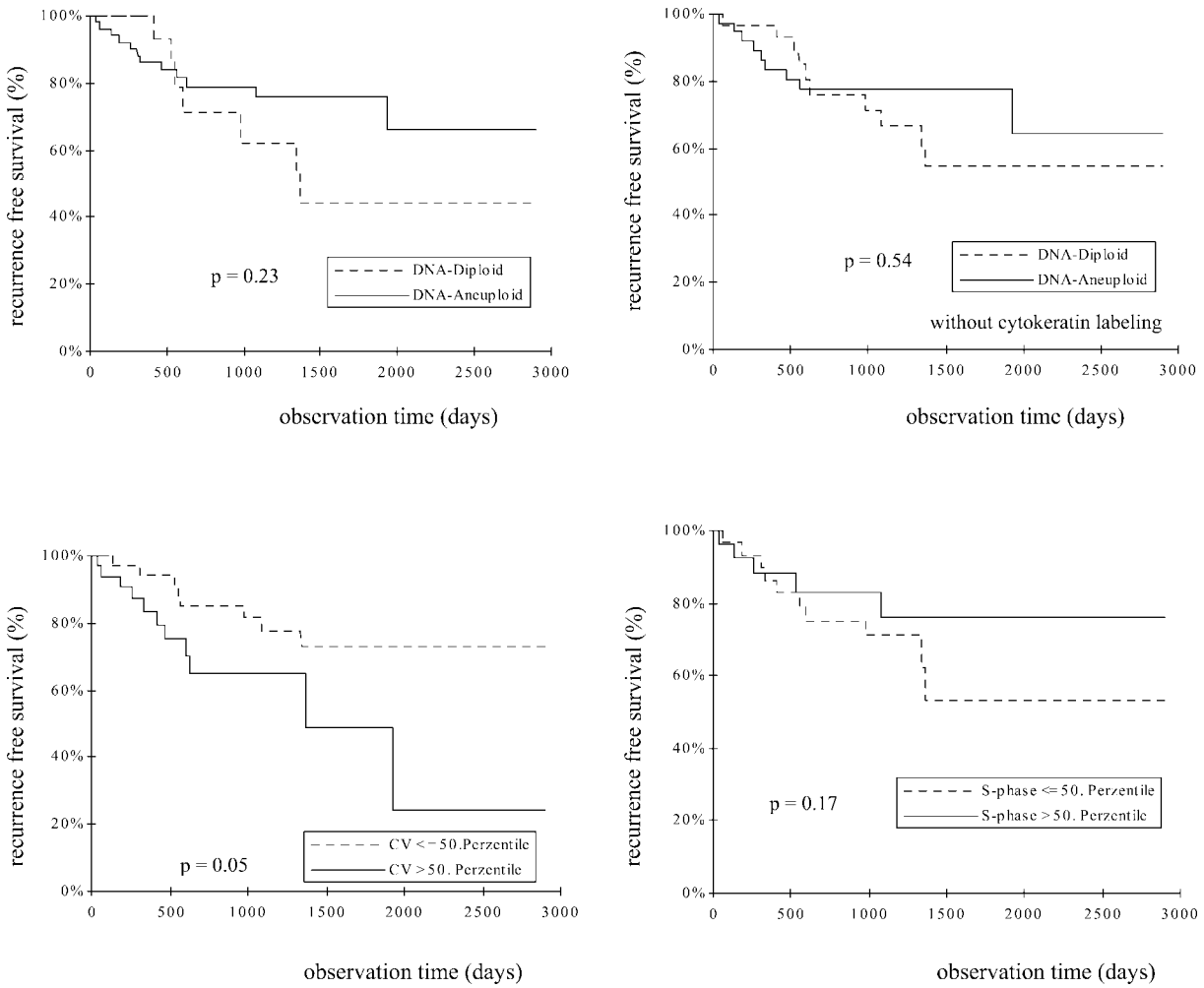

Fig. 1. Cervical cancer ($n = 69$). Kaplan–Meier curves for RFS in dependence on DNA-ploidy (with and without cytokeratin labelling), CV and S-phase fraction were performed. The significance level (p) was determined by univariate analysis with the log rank test.

Table 5

Primary cervical cancer ($n = 68$). Cox-multivariate analysis of univariate prognostic relevant parameters for recurrence-free survival (RFS) and tumor-dependent overall survival (CSS). For all parameters multivariate analysis were performed. For all parameters the value of effect of covariable estimation (β), standard deviation (σ), relative risk (RR) and the belonging 95% confidence interval (95%CI) was determined

Cervical cancer,	RFS					CSS				
$n = 68$	β	σ	p	RR	95% CI	β	σ	p	RR	95% CI
Age	1.02	0.38	0.008	2.8	1.45–5.31	1.07	0.44	0.01	2.9	1.39–6.09
Tumor stage	0.51	0.37	0.17			−0.13	0.52	0.81		
Lymphnode stage	0.09	0.52	0.86			0.26	0.60	0.66		
CV G0/G1-phase fraction	0.99	0.48	0.04	2.7	1.20–6.07	0.87	0.58	0.13		

Endometrial carcinoma

3.6. Results of DNA cell cycle analysis in dependence on cytokeratin labelling

3.6.1. Detection of DNA-aneuploid subpopulations

Tumor specimens of 73 patients with primary endometrial carcinoma were analyzed. Each cell suspension consisted of at least 1 cytokeratin-negative and 1 cytokeratin-positive subpopulation. 30% of specimens were DNA-aneuploid without cytokeratin labelling and after cytokeratin labelling 53% were identified as DNA-aneuploid.

The distribution of DNA-indices after cytokeratin labelling and DNA-cell cycle distribution were published elsewhere [11].

3.7. Correlation of DNA-ploidy, DNA-index, CV and cell cycle phase fraction in endometrial carcinoma with classic clinical histopathologic prognostic factors

The correlation of DNA-ploidy, DNA-index, CV and cell cycle phase fraction in endometrial carcinoma with classic clinical histopathologic prognostic factors like FIGO-stage, pT-stage, pN-stage, grading, steroid receptors and lymphangiosis carcinomatosa were analyzed with the Chi-square-test concerning statistic significance. No significant correlation between DNA-parameters and clinical histopathologic prognostic factors was found.

3.8. Prognostic significance of DNA-ploidy and S-phase fraction in endometrial carcinoma in comparison to classic prognostic factors: univariate analysis

3.8.1. Prognostic significance of classic clinical histopathologic prognostic factors

Univariate analysis was performed to investigate the influence of age of patients, tumor histology, tumor stage (pT), pelvic lymph node involvement, infiltration depth, grading, lymphangiosis carcinomatosa, estrogen- and progestagen receptor expression on RFS and CSS.

Whereas patients with pT1 tumor showed a RFS of 94.1% and a CSS of 97.4%, CSS and RFS decreased in pT2 stage from 71 to 50% in stage pT3/4 ($p = 0.006$, respectively 0.001). RFS and CSS correlated highly significant with pelvic lymph node involvement (93.8% versus 50%; $p = 0.005$) and with lymphangiosis carcinomatosa (89.6%/91.7% versus 50%; $p = 0.01$, respectively 0.003).

For better estimation of time course and relation of censored patients to patients with relapse or tumor dependent death Kaplan–Meier curves were performed for the parameters tumor stage (pT), pelvic lymph node involvement (pN), grading and age. Especially, tumor stage ($p = 0.006$) and pelvic lymph node involvement ($p = 0.005$) were not significant prognostic factors.

3.9. Prognostic significance of DNA-ploidy, DNA-index and CV in endometrial carcinoma

The analysis of prognostic significance of DNA-ploidy, DNA-index and CV found a trend for worse overall survival in DNA-aneuploid tumors and tumors with high CV, that was not significant. For DNA-index slightly hyperdiploid tumors were attached to DNA-diploid ones. A clear advantage in overall survival for patients with a DNA-index up to 1.3 was noticed. RFS and CSS decreased from 93.2 to 50% ($p = 0.0007$) respectively 62.5% ($p = 0.01$) for values of DNA-index > 1.3.

In Kaplan–Meier curves of RFS (Fig. 2) and CSS (data not shown) a paradox effect was shown, that the estimation of prognosis was better without cytokeratin labelling than with cytokeratin labelling. The greatest percentage of additionally diagnosed DNA-aneuploid subpopulations had a DNA-index of $\leqslant 1.3$. For S-phase fraction no prognostic relevant difference for low and high proliferating tumors was detected.

3.10. Prognostic significance of S-phase fraction, G_2M phase fraction with and without identification of tumor cells by cytokeratin labelling

For the parameters S-phase-fraction, G_2M phase fraction and their sum we found in univariate analysis a clear trend for worse prognosis in high proliferating tumors. But this trend was not statistically significant at cut off at median. But if we separately analyzed patients with high proliferating tumors (upper quartile), a significant worse prognosis was shown concerning S-phase fraction and also for proliferating fraction (sum of of S-phase fraction and G_2M phase). RFS and CSS decreased from 97.1 to 66.7%, respectively, 75% in patients with high proliferating tumors ($p = 0.003$ and 0.018). In cell cycle parameters, which were determined without cytokeratin la-

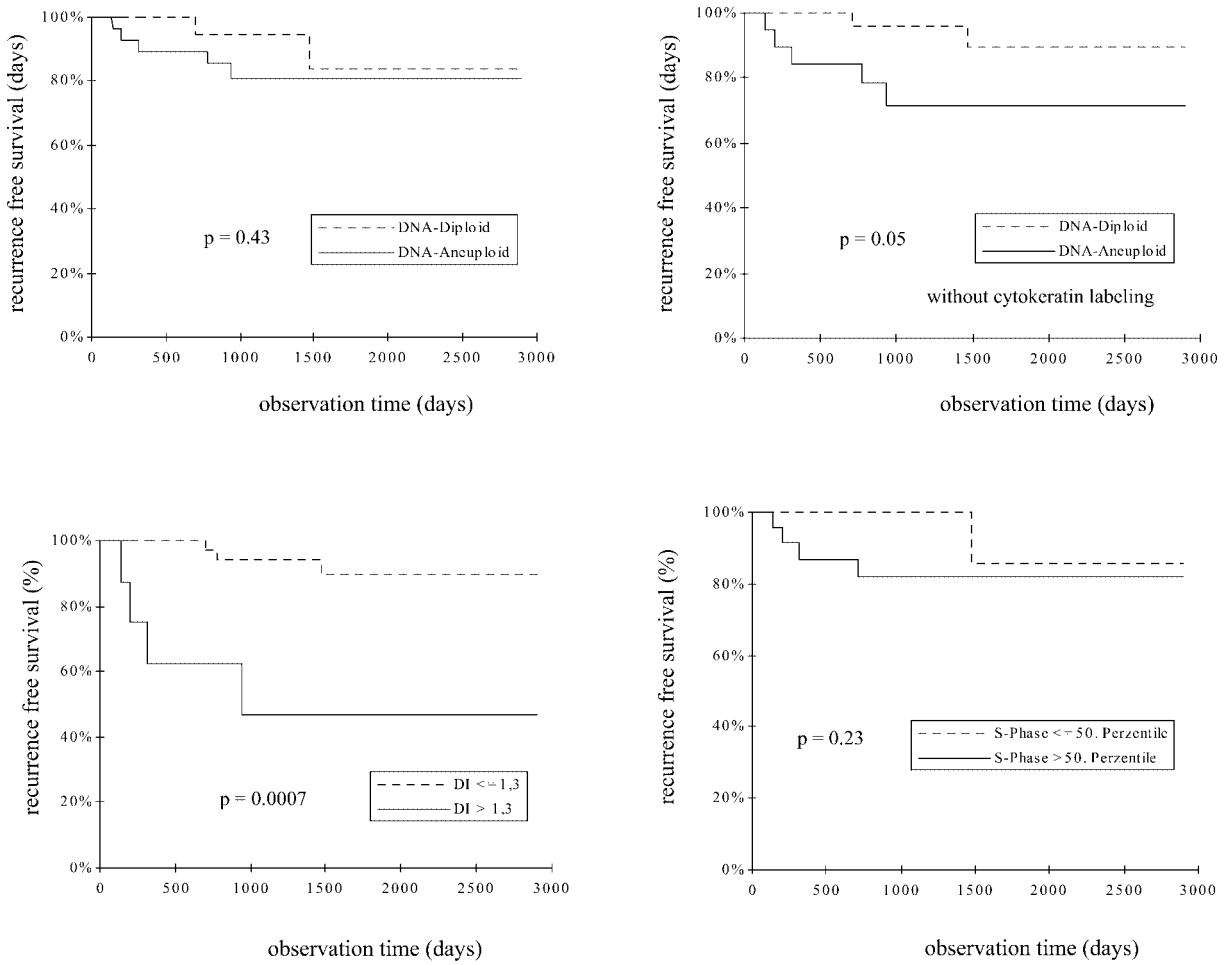

Fig. 2. Endometrial cancer ($n = 52$). Kaplan–Meier curves for RFS in dependence on DNA-ploidy (with and without cytokeratin labelling), DNA-index and S-phase fraction were performed. The significance level (p) was determined by univariate analysis with the log rank test.

belling, these correlations could be demonstrated in spite of the small spot check. For estimation of the different prognostic statement of S-phase fraction dependent on the cut-off, Kaplan–Meier curves were demonstrated splitted for S-phase fraction at median respectively at uppermost quartile for RFS and CSS (data not shown).

3.11. Prognostic significance of DNA-ploidy and S-phase fraction in endometrial cancer in comparison to classic prognostic factors: multivariate analysis

Multivariate analysis of 46 patients with endometrial cancer showed only borderline efficacy. This is expressed in extremely varying 95% confidence intervals. In multivariate analysis the parameters tumor stage (pT) (RR 6.7; $p = 0.05$), DNA-index (RR 32; $p = 0.02$) and the at the uppermost quartile splitted S-phase fraction (RR 19; $p = 0.02$) remained their significant prognostic significance for RFS. For CSS only the tumor stage (pT, RR 8.1; $p = 0.01$) and the at the uppermost quartile splitted S-phase fraction (RR 0 13; $p = 0.04$) were prognostic relevant (Table 6). But because of small patient number these results could only be a hint for prognostic significance of DNA-index and S-phase fraction in multivariate analysis. A second multivariate analysis was performed exclusively for endometrioid adenocarcinomas ($n = 41$) to avoid, that tumors without endometrioid differentiation take influence on the results. For RFS the DNA-index was the strongest factor with an RR of 12.0 ($p = 0.01$), followed by the S-phase fraction with RR 12.4 ($p = 0.05$).

Table 6

Primary endometrial cancer ($n = 46$). Cox-multivariate analysis of univariate prognostic relevant parameters for recurrence-free survival (RFS) and tumor-dependent overall survival (CSS). For all parameters multivariate analysis were performed. For all parameters the value of effect of covariable estimation (β), standard deviation (σ), relative risk (RR) and the corresponding 95% confidence interval (95%CI) was determined

Endometrial cancer,	RFS					CSS				
$n = 46$	β	σ	p	RR	95% CI	β	σ	p	RR	95% CI
Tumor stage (pT)	1.91	0.97	0.05	6.7	1.31–34.5	2.09	0.83	0.01	8.1	1.97–32.9
Lymphangiosis	0.60	1.65	0.71			1.23	1.74	0.48		
DNA-index	3.47	1.46	0.02	32.1	2.70–378	2.01	1.41	0.16		
S-phase fraction	2.97	1.32	0.02	19.4	2.06–33.0	2.61	1.25	0.04	13.6	1.66–111

4. Discussion

4.1. Cervical cancer

4.1.1. Prognosis of cervical cancer

Prognosis of cervical cancer is good. Patients with stage Ia survive five years in about 97%, in stage Ib 84%, in stage II 65 to 75%. In advanced stage survival decreases to about 30% to 40% in stage III and only 10% in stage IV [14]. In the past decades incidence of invasive cervical cancer decreased because of effective screening, but nevertheless despite of early diagnosis some patients relapse. Classic histopathologic criteria like tumor histology and tumor differentiation do not support in estimation of prognosis [15]. Some authors describe a worse outcome for adenocarcinoma compared to the squamous cell carcinoma [16]. Multivariate analysis concerning prognostic significance of clinical-histopathologic prognostic factors of squamous cervical carcinoma ($n = 3761$) showed significance for tumor size, lymph node involvement, invasion of parametrium and lymphatic vessel invasion, but only a slight significance for depth of stroma invasion, and no significance for age and tumor grading [17–19]. Similar results were detected for adenocarcinoma ($n = 577$), where tumor size and lymph node involvement showed high significance for clinical outcome, whereas depth of stroma invasion and tumor grading showed a questionable significance and age and adenosquamous histologic type were not significant [18,20]. The risk for relapse could be estimated somewhat better with the classic clinical histopathologic prognostic factors, but in case of stage I and II a clear cut calculation of prognosis is not possible. This could be seen in a collective of patients with cervical cancer ($n = 235$) with stage Ib/Iia. Multivariate analysis shows a low risk group ($n = 8$) with 100% of 10 years survive by the criteria tumor volume, lymphangiosis carcinomatosa, lymph node involvement and pregnancy, but differentiation of risk of remaining subgroups was not convincing [21]. Thus further objective prognostic factors are necessary for a more exact risk stratification.

4.1.2. Etiology of cervical cancer and molecular biologic aspects

Cervical cancer is the classic virus-induced and virus-associated neoplasia, especially the human papilloma virus (HPV) is of relevant significance [22]. Especially the subtypes 16 [23] and 18 [24] are the most relevant subtypes for development of cervical cancer. *In vitro* and also *in vivo* analysis showed in case of virus-induced cells or in case of cervical dysplasia a rate of DNA-aneuploidy in 38–100% in contrast to normal cervical cells [25,26], and HPV 16 and HPV 18-DNA could be detected in DNA-aneuploid cells of cervical dysplasia by *in situ* hybridization [27]. In these cells neither *in vitro* nor *in vivo* invasiveness or ability to form metastases was detected. In combination with the virus infection the early detection of DNA-aneuploidy was present, this seems to be due to genetic changes, that lead to immortalization. The HPV-detection is no significant help for estimation of prognosis of cervical cancer [17], but in future the expression of viral oncogenes E6 and E7 could be important for estimation of tumor aggressivity [28]. The detection of DNA-aneuploidy is an early event in carcinogenesis, and it leads to immortalization of cells, but not compelling in biologic malignant behaviour.

4.1.3. Prognostic significance of DNA-parameter in comparison to clinical histopathologic prognostic factors

The examination of DNA-ploidy and S-phase fraction as prognosis parameters lead to different results. Whereas some authors identified the DNA ploidy as independent prognostic factors [6–8], others could not confirm this observations [29–31]. The greatest, prospective study ($n = 465$) could not detect any significance of DNA-ploidy [32]. In studies that found a prognostic significance of DNA-ploidy, DNA-

aneuploid tumors [8] but also DNA-diploid ones [33] were identified to be unfavourable. In most studies tumors with low S-phase fraction showed good prognosis in comparison to high proliferating tumors [30,34], but this could not be confirmed in early invasive cervical cancers [31].

The detection of S-phase fraction is possible for nearly all cases of DNA-diploid tumors, but with the risk of wrong detection by admixture of non-tumorable elements.

In our collective all DNA-diploid tumors with and without cytokeratin labelling could be analyzed, the rate of analysis of DNA-aneuploid tumors were increased from 60% without cytokeratin labelling to 80% with cytokeratin labelling. In spite of optimization of flow-cytometric DNA analysis, we could not find any prognostic significance for recurrence-free and tumor-specific survival in univariate analysis for any examined DNA parameter. Only stage, tumor size and age of patients were prognostic significant with $p < 0.05$ in log rank test. The histopathologic grading had no influence. The age of patients was of prognostic significance in multivariate analysis. The detected significance for CV for RFS had no relevance for clinical decisions, because the ability of discrimination of this factor was low.

4.1.4. Clinical relevance of detection of DNA-ploidy and S-phase fraction for estimation of prognosis in cervical cancer

Controversial results of prognostic significance of DNA-ploidy could be due to methodological differences of study conditions. The largest prospective study ($n = 465$ patients) concerning cervical cancer found no prognostic relevance with respect to S-phase fraction [32]. Our prospective study using tumor cell labelling detected neither in univariate nor multivariate analysis any prognostic significance for DNA-ploidy and S-phase fraction. The multivariate prognostic significance of the variation coefficient of G_0G_1-peak of tumor cells is a highly interesting observation, however, has no clinical impact due to its small discrimination potential.

With respect to radiation therapy determination of DNA-ploidy was also dissappointing. The radiosensitivity seemed to be better for DNA-aneuploid tumors, but following 15 months of observation no advantage for estimation of prognosis could be detected any longer [35]. 10 years ago it was suggested that cell cycle analysis had a great impact on planning radiation therapy for synchronization of cells in radiosensitive G_2-phase [36].

4.1.5. Conclusion for cervical cancer

No parameter of DNA-analysis, like DNA-ploidy, DNA-index, S-phase fraction or variation coefficient of G_0G_1-peak of tumor cells show a reproducible, relevant significance for estimation of prognosis. Attempts to use DNA-parameter for planning and monitoring of radiation showed no success. However, this is in accordance to findings of molecular biology with respect to cancerogenesis of cervical cancer, especially in HPV-positive tumors, since prognostic significance of DNA-ploidy may not be expected. Therefore there is no indication in clinical routine for determination of DNA-parameters in cervical cancer.

4.2. Endometrial carcinoma

Endometrial carcinoma is the most common malignancy of the female genital tract with 44% [14]. The incidence is about 25 per 100,000 women per year and shows low specific mortality of 10.6%. Although the 5-year survival rate is good with 80 to 85% and the majority of the women have at primary diagnosis a tumor stage confined on the uterus, nevertheless 50% of deaths caused by endometrial carcinoma were observed in stage I disease [37]. Due to the overall good prognosis it is very important, to identify patients with high risk for relapse to perform additional adjuvant therapy and a close-meshed follow up of these patients.

Univariate analysis found important prognostic factors like age of the patients, FIGO-stage, histologic grading, depth of myometran invasion and lymph node involvement; in addition to histologic subtype, peritoneal cytology and expression of progestagen receptor could be of prognostic significance [14]. However, unfortunately, also patients with no risk factors relapse.

The flow cytometric evaluation of individual cellular characteristics like DNA-content and S-phase fraction and G_2M phase are also available preoperatively and for patients with primary irradiation. In high differentiated tumors between 9 and 20% DNA-aneuploid subpopulations were found, whereas worse differentiated tumors show between 20 and 85% DNA-aneuploid subpopulations [9,38]. With a median of 30% DNA-aneuploid tumors for endometrial carcinoma, the rate is significantly lower than for breast-, cervical- and ovarian cancer.

Some studies evaluated S-phase fraction as the most important prognostic factor [39], whereas other studies declared the DNA-ploidy as the most important factor [40]. A retrospective study of Pasini et al. showed that DNA ploidy does not seem to be positively cor-

related with any traditional histopathological analysis [41]. Other investigators found both parameters statistically significant correlated with recurrence-free and overall survival [9]. In our study multivariate analysis for recurrence-free survival identified tumor stage (pT), DNA-index and the high S-phase fraction as independent prognostic factors.

With respect to the literature and our data, DNA-ploidy and to a lower degree the S-phase fraction can be identified as important independent prognostic factors for the course of disease in endometrial cancer. We recommend therefore, to introduce determination of DNA-parameters for clinical routine in endometrial cancer because of rapid, simple and cheap determination on the one hand and pretherapeutical availability on the other hand. The preoperative knowledge of these parameters allows in context to the established preoperative detectable prognostic factors an identification of patients at risk, who should be treated in oncologic cancer centers. There it will be possible to treat these patients prospectively in multicenter trials to optimize success of treatment with respect to surgery and the setting of adjuvant therapy.

5. Summary

This is the first investigation in uterine malignancies concerning the influence of tumor cell enrichment by cytokeratin labelling concerning prognostic significance of DNA-parameters.

In cervical cancer, neither DNA-ploidy nor S-phase fraction were detected as prognostic relevant parameters. Only the variation coefficient of G_0G_1-peak (CV) of tumor cells showed prognostic significance in multivariate analysis. Probably this could be a result of genetic instability of stem cell lines with multiple small changes of the DNA-content in cells. Cause of the small prognostic discrimination capacity of the CV, this has no relevance for therapeutic decisions. There is no indication for determination of known DNA-parameters for estimation of clinical prognosis.

In endometrial carcinoma, gross DNA-aneuploidy (DNA-index > 1.3) and a high percentage of proliferating cells ($<$75th percentile) were found to be highly significant prognostic factors for recurrence-free survival in univariate and multivariate analysis. In spite of our small numbers it can be stated with respect to results of other authors that especially DNA-aneuploidy (DI > 1.3) is one of the most important independent molecular biological prognostic factors. By determination of prognostic factors like histological tumor type, grading, cervical involvement and DNA-ploidy during diagnostic curettage an identification of patients at risk is possible preoperatively. Therefore these could be admitted to an oncologic center for treatment. In addition DNA-ploidy could contribute to identify similar groups concerning tumor biology of patients for adjuvant therapeutic studies.

In conclusion, the determination of DNA-ploidy in gynecologic oncology should be performed in endometrial cancer and in ovarian cancer also [42]. In cervical cancer a DNA-analysis for prognostic purposes should not be performed, because neither a prognostic significance for DNA-parameters could be assessed, nor a clinical consequence will result.

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