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Adenocarcinoma of Uterine Cervix – Prognostic Significance of Clinicopathologic Parameters

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Aim To investigate prognostic significance of several clinicopathologic parameters in patients with adenocarcinoma of the uterine cervix.

Methods We retrospectively studied 36 patients treated at the Department of Gynecology and Obstetrics, Zagreb University School of Medicine, Croatia, in the period from 1978-1998. Cox proportional hazard analysis was performed to examine the prognostic significance of menstrual status, clinical stage, architectural grade, nuclear grade, DNA ploidy, proliferative activity, and mode of therapy.

Results The 5-year survival for this group of patients was 75%. The following parameters proved to be statistically significant in a univariate analysis: clinical stage ($P=0.042$), architectural grade ($P=0.009$), and nuclear grade ($P=0.002$). In the multivariate analysis, the nuclear grade ($P=0.007$) turned out to be the only statistically significant parameter. According to the nuclear grade, the five-year survival was 80% in the prognostically favorable and only 30% in the unfavorable group of patients.

Conclusion Our data showed that in patients with adenocarcinoma of the uterine cervix the nuclear grade, clinical stage, and architectural grade of the tumor represent the most important prognostic parameters. The analysis of DNA ploidy and proliferative activity had no prognostic significance.

Invasive carcinomas of the uterine cervix are among the most common neoplasms of the female genital tract. They are divided into squamous cell carcinomas and adenocarcinomas. The incidence of squamous cell carcinomas has markedly decreased recently, primarily due to early detection of the disease using the Pap smear test. On the other hand, the incidence of adenocarcinomas has increased over the last 30 years, probably reflecting both improved diagnostics and true increased frequency of this disease (1-5).

Cervical carcinoma with glandular differentiation has been broadly divided into two groups: adenosquamous carcinoma and pure adenocarcinoma. Pure adenocarcinoma can be further divided into several subgroups: mucinous adenocarcinoma, endometrioid adenocarcinoma, clear cell adenocarcinoma, minimal deviation adenocarcinoma (adenoma malignum), serous adenocarcinoma, mesonephric carcinoma, and well-differentiated villoglandular adenocarcinoma (3). Adenosquamous carcinomas were in some studies included in the adenocarcinoma group but, according to the modified World Health Organization (WHO) histological classification of invasive carcinoma of the uterine cervix, they represent a separate group of cervical cancers and they are not included in the pure adenocarcinoma group (3).

Because of the rising incidence of this disease, scientists have been trying to discover

prognostic parameters for patient survival. Different authors reported the influence of various parameters on patient survival such as clinical stage of disease (6-8), the largest diameter of the tumor (8-11), histologic subtype (9,10,12), architectural grade (7-10,13), nuclear grade (2,9,10), depth of invasion (12-15), presence of lymph node metastasis (15-17), mode of therapy (6,8,13,17), etc.

Ancillary methods such as DNA flow cytometric analysis, DNA ploidy, and proliferative activity have proven to be of prognostic significance in regard to overall patient survival and risk of recurrent disease in several malignant tumors, such as breast cancer (18), prostate cancer (19), non-Hodgkin lymphoma (20,21), and different gynecological cancers (22-24). However, these methods have not been applied extensively in the study of patients with adenocarcinoma of the cervix (9,16,17,25).

The aim of the present study was to investigate which clinical, histopathologic, and flow cytometric parameters of adenocarcinomas of the cervix are the best predictors of patient survival.

Patients and Methods

The medical records of patients with cervical adenocarcinoma who were treated from 1978 to 1998 at the Department of Gynecology and Obstetrics, Zagreb University School of Medicine, were retrospectively reviewed. Clinical information was recorded for each patient, including age at presentation, menstrual status, clinical stage of disease, and mode of therapy. Patients with incomplete clinical documentation, and those who received their initial treatment at another institution were excluded. Only the patients undergoing curettage of the uterus entered the further study. Clinical staging was done according to guidelines of the International Federation of Gynecology and Obstetrics (FIGO) (3). Stages I and IIA were considered favorable and stages IIB, III, and IV unfavorable.

Histopathology

Histopathologic diagnoses were reviewed by two pathologists, based on all hematoxylin and eosin-stained slides, without any knowledge of clinical outcomes. The following pathological features were assessed: histologic subtype, architectural grade, and nuclear grade of the tumor. Histologic subtyping was performed according to the generally accepted criteria (3). Cervical carcinomas showing glandular differentiation with either a malignant squamous component (10% or greater) or showing predominant glassy cell features were considered to be adenosquamous carcinomas and were excluded from the study.

Architectural and nuclear grades were determined separately. The architectural grade was based on the proportion of solid growth of the non-squamous component throughout all the histological material, as commonly used for endometrial cancer (3). If less than 10 percent of the tumor consisted of areas not forming glands or tubules, it was classified as well differentiated. If 10 to 50 percent of the tumor did not form glands or tubules, it was designated moderately differentiated. If the glands or tubules were not discernible in more than half of the tumor, it was considered to be poorly-differentiated. Well-differentiated tumors were considered to have a favorable prognosis, whereas moderate and poor tumor differentiation was considered unfavorable prognostic parameter.

Nuclear grade was based on the aspect of the nuclei and was evaluated in the most atypical area (2). Cells with oval nuclei, without prominent nucleoli, and with evenly dispersed chromatin were considered grade 1. Cells with markedly enlarged nuclei displaying irregular coarse chromatin and prominent nucleoli were considered grade 3, and those displaying features between grades 1 and 3 were designated grade 2. Nuclear grades 1 and 2 were designated as favorable and nuclear grade 3 as unfavorable prognostic parameters.

Flow Cytometry

Paraffin embedded tissue from adenocarcinomas of the uterine cervix was processed as described by Hedley et al (26). In addition to 3 sections 40-μm thick required for flow cytometry, 2 sections 4-μm thick were cut, one from the top and other from the back of the tissue. These were stained with hematoxylin and eosin, and the pathohistological parameters were evaluated. One to 2 tissue slices, dedicated for flow cytometric analysis, were deparaffinized in xylene and rehydrated in decreasing ethanol concentrations (100%, 95%, and 70%), followed by a final wash in distilled water. Tissue was then suspended in 0.5% pepsin (Sigma, St. Louis, MO, USA) in 0.9% sodium chloride (pH 1.5), and incubated in a shak-

ing water bath at 37 °C for 1 hour. Cells were washed in RPMI medium, filtered through a 42-µm nylon mesh and centrifuged 5 min at 800 G. The extracted nuclei were stained for DNA content after a modification of Vindelov et al (27) method. Ribonuclease S (Sigma), in a final concentration of 1 mg/ml, was added to approximately 1×10^6 nuclei and incubated in a water bath at 37 °C for 30 min. Afterwards, nuclei were resuspended in 50 µg/mL propidium iodide (Sigma) and incubated for 30 min in the dark at room temperature. Control tissues comprised normal tissue of the uterine cervix, which was always included in the sample as an control. Cellular DNA content and proliferative activity were analyzed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA), using a 15-milliwatt argon ion laser, producing an excitation wavelength of 488 nm. For each DNA content analysis, 20,000 nuclei were counted. DNA histograms were analyzed with ModFit LT program. The coefficient of variation was always less than 8%. A tumor was classified as DNA diploid if only one GO/G1 peak was observed in the expected region of the histogram. If more than one GO/G1 peak was seen, the tumor was considered DNA aneuploid (ie DNA index was not used for the definition of DNA aneuploidy). DNA tetraploid tumors were included in the DNA aneuploid group. Those tumors were separated from DNA diploid tumors, because the percentage of cells in the G2/M fraction was higher than 15% and their DNA index was 1.9-2.1. Proliferative activity was defined as the sum of the cell percentages in the S- and G2/M phases. Tumors with proliferative activity higher than 15% were considered prognostically unfavorable.

Therapy

The treatment was not uniform and many different therapies were used in this group of patients. Four principal modes of therapy could be recognized: operation only, radiation only, operation followed by radiation with or without chemotherapy, and radiation followed by operation with or without chemotherapy. For the purpose of the study, the patients were divided into two groups: the group that started treatment with a surgical operation, comprising 26 patients, and the group that started treatment with radiation, comprising 10 patients.

Exclusion Criteria

The patients for whom appropriate flow cytometric histograms could not be obtained were excluded from the study. Flow cytometric analysis and grading were not performed on material that had been subjected to radiation or chemotherapy. Only patients who had a follow-up of at least 30 months were included in the survival analysis. At the end, only 36 patients fulfilled all of the requirements and entered the study.

Statistical Analysis

Survival time was measured in months from the date of diagnosis to the date of death or last follow-up. Only the deaths caused by the tumor were considered as deaths in the survival analysis, and all others were excluded from the analysis. Actuarial survival probability curves were constructed using Kaplan-Meier method and compared using the log-rank nonparametric test. Categoric (nominal) parameters were analyzed as dummy variables in the case of a binary split, or were coded as ordinal (1, 2, etc.) when appropriate. The Cox proportional hazards regression model with forward stepwise variable selection was used to assess the relative effect of menstrual status, clinical stage, architectural grade, nuclear grade, DNA ploidy, proliferative activity, and therapy on survival prognosis. In all statistical analyses only probabilities lower than 5% ($P < 0.05$) were considered significant. Statistics was done with type II error $\beta < 0.2$, using MedCalc, version 7 (MedCalc, Mariakerke, Belgium) and Statistical Package for Social Scienes for Windows, version 7 (SPSS Inc., Chicago, IL, USA) software.

Results

Menstrual status, clinical stage, architectural grade, nuclear grade, DNA ploidy, proliferative activity, and mode of therapy for 36 patients are shown in Table 1. The median age of patients with invasive adenocarcinoma of the uterine cervix was 46 years (range 27-75). With regard to menstrual status, they were divided into two groups: the menstruating group included 24 patients and the postmenopausal group consisted of 12 patients. According to clinical stage, there were 22 patients in the favorable and 14 patients in the unfavorable group. With respect to histologic subtypes, there were 18 patients diagnosed with mucinous adenocarcinoma, 15 patients with endometrioid type, 2 patients with clear-cell adeno-

Table 1. Univariate and multivariate analysis of prognostic parameters in patients with adenocarcinoma of the uterine cervix (n=36)

Prognostic parameters	No. of patients		Statistical analysis	
	total	died	univariate	multivariate*
Menstrual status:				
menstruating	24	6	0.919	
postmenopause	12	3		
Clinical stage:				
I+IIA	22	3	0.042	0.070
IIB+III+IV	14	6		
Arhitectural grade:†				
WD	26	4	0.009	0.191
MD+PD	10	5		
Nuclear grade:‡				
G1+G2	31	6	0.002	0.007
G3	5	3		
DNA ploidy:				
diploid	13	2	0.415	
aneuploid	23	7		
Proliferative activity:§				
favorable	28	7	0.848	
unfavorable	8	2		
Mode of therapy:				
started with surgery	26	7	0.752	
started with radiation	10	2		

*Multivariate Cox regression analysis was performed only for parameters with significant ($P<0.05$) univariate impact.
†WD – well differentiated, MD – moderately differentiated, PD – poorly differentiated (3).
‡G1 – grade 1, G2 – grade 2, G3 – grade 3 (2).
§Proliferative activity: unfavorable findings were DNA diploidy with more than 15% of cells in the S+G2/M cell cycle phases.

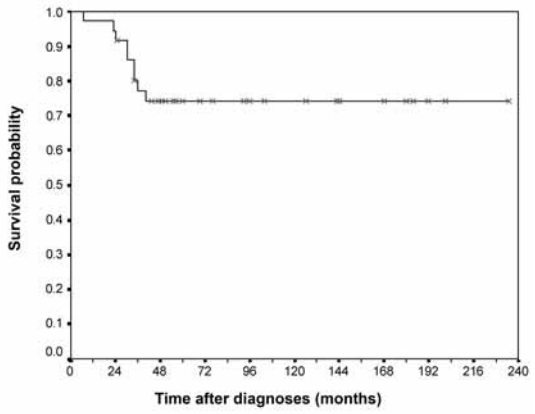

Figure 1. Overall survival in 36 patients with cervical adenocarcinoma (censored data denoted on lines).

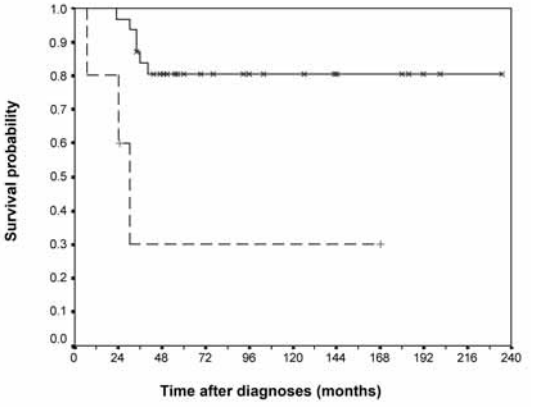

Figure 2. Overall survival in 36 patients with cervical adenocarcinoma according to the nuclear grade: favorable group (full line, n=31, mean survival time 195±15 months, 5-year survival 80.4±7.2%) and unfavorable group (dashed line, n=5), mean survival time 65±34 months, 5-year survival 30.0±23.9%) (censored data denoted on lines).

carcinoma, and 1 patient with minimal deviation adenocarcinoma (adenoma malignum). Twenty-six of 36 adenocarcinomas were well-differentiated and 10 were moderately or poorly-differentiated. There were 31 patients with tumors composed of cells with grade 1 or 2 nuclei (favorable group) and 5 patients with nuclear grade 3 tumors (unfavorable group). There were 13 patients in the DNA diploid and 23 patients in the DNA aneuploid group. Twenty eight patients had tumors with low proliferative activity and 8 patients had tumors with high proliferative activity. Five-year survival after diagnosis was 74.3% (Fig. 1).

Data in Table 1 show the results of univariate and multivariate analysis for clinical, histopathologic, and flow cytometric parameters. Univariate analysis revealed a significant association between patient survival and clinical stage ($P=0.042$), architectural grade ($P=0.009$), and nuclear grade ($P=0.002$) of the tumor. In multivariate analysis nuclear grade ($P=0.007$) was the only statistically significant parameter. With regard to the nuclear grade, mean survival time in the favorable group of patients was 195 ± 15 months, and 5-year survival was $80.4\pm7.2\%$. In the unfavorable group of patients, mean survival time was 65 ± 34 months and 5-year survival was $30.0\pm23.9\%$ (Fig. 2). Flow cytometric cell cycle parameters and mode of therapy had no impact on the overall survival.

Discussion

Invasive adenocarcinoma of the uterine cervix is not a common tumor. Studies where prognostic factors for survival were investigated in this particular group of patients are rare. In most studies, adenocarcinomas were analyzed together with adenosquamous carcinomas (6,8,10,12), ie with a group of tumors with more unfavorable

clinical outcome. Consequently, only a few studies have been published so far (7,9,11,17,28) where the importance of prognostic factors in the group of patients with pure adenocarcinomas was investigated. In our series of adenocarcinomas, we tried to identify a subgroup of high-risk patient population with unfavorable prognosis who may be candidates for further trials investigating the role of a more aggressive mode of treatment.

Our results show that nuclear grade, architectural grade, and clinical stage appear to be significant prognostic parameters for the survival of patients with cervical adenocarcinoma. In the multivariate analysis, however, nuclear grade was the only important prognostic parameter in predicting the survival of patients with pure cervical adenocarcinoma. Although the value of nuclear grade was mentioned in the past in the studies concerning cervical adenocarcinomas, some authors discovered the importance of nuclear grade in the group of patients with cervical adenocarcinoma, including the adenosquamous type (10).

Because of the strict criteria used for the diagnosis of pure adenocarcinoma in the current study, we were able to achieve a more precise determination of prognostic importance. In this study, the unfavorable group of patients regarding nuclear grade (G3) consisted of only 5 patients and 3 of them died of disease during the follow-up period. On the other hand, out of 31 patients with tumors that were classified as favorable regarding nuclear grade (G1 and G2), only 6 patients died of disease in the observed period of time. Moreover, for patients whose tumors were histopathologically classified as favorable regarding nuclear grade, 5-year survival rate reached 80%, whereas the other subgroup, with patients whose tumor nuclear grade was designated unfavorable, showed about 30% 5-year survival rate.

In addition to nuclear grade, architectural grade was evaluated as predictor of overall survival. The majority of the cervical adenocarcinomas are well to moderately differentiated and grading might be very important for prognosis of patients with this disease. In our study, architectural grade was not as important as a nuclear grade, so for the purpose of this study, it was combined with nuclear grade in a single parameter. Similar to endometrial carcinomas, the presence of grade 3 nuclear features in most neoplastic cells in architecturally well and moderately differentiated tumors raised an architectural grade 1 or grade 2 tumor by one. However, the combination of these two parameters did not prove to be statistically significant (data not shown).

A revision of all histological slides was performed to identify not only the grade of neoplasms but also to categorize pure adenocarcinomas of the uterine cervix according to modified World Health Organization histological classification (3). However, in our study histologic subtyping of adenocarcinomas had no independent impact on overall survival (data not shown). This finding is in agreement with the study of Alfsen et al (9) who concluded that no significant difference between major subtypes of adenocarcinoma existed and they favored a simplified classification for these tumors.

In some studies, FIGO stage was the only independent prognostic factor in the multivariate analysis (29). Adenocarcinoma of the cervix is confined to the cervix (stage I) or the parametrium/vagina (stage II) in 80% of women at the time of diagnosis (3), which was also true in our study. Our study confirmed the impact of clinical stage as a strong prognostic factor for patients with pure cervical adenocarcinoma in the univariate analysis, but that significance was not confirmed by the multivariate analysis.

It has been shown that flow cytometric analysis of tumor cells (DNA ploidy and proliferative activity) are of prognostic value for patients with adenocarcinomas of the uterine corpus (23,24). However, such studies investigating prognostic value of DNA content in patients with cervical adenocarcinoma have provided divergent results. Flow cytometric analysis did not show any prognostic significance with regard to survival in this study. These results support findings in other reported studies (9,16,17,25,29), suggesting that DNA ploidy does not affect the overall survival of patients with cervical adenocarcinoma. Leminen et al (16) reported a worse outcome of patients with DNA aneuploid tumors and high S-phase fraction, but the authors combined adenosquamous and pure adenocarcinomas, so the analysis of a separate group of pure adenocarcinomas of the cervix was not achieved. Magtibay et al (17) concluded that DNA ploidy did not predict a patient's risk for tumor recurrence, but they found that proliferative activity could potentially be of some value in determining recurrence risk in a

group of patients with cervical adenocarcinoma. In our study, the influence of proliferative activity determined by flow cytometry was not shown as a potentially significant predictor of outcome.

We evaluated these prognostic factors because we wanted to separate low and high-risk group of patients, and to suggest the best way of treatment planning. The undertreatment of high risk patients represents a great problem. On the other side, a considerable proportion of patients is subjected to unnecessarily aggressive treatment regimens that probably contributed to increased morbidity or even mortality in patients with cervical adenocarcinoma.

The mode of therapy primarily depends on the clinical stage. According to the literature, for stage IB and IIA carcinomas of the cervix, either radical hysterectomy and lymph node dissection or radiation therapy with cisplatin-based chemotherapy should be considered. Stages IIB and higher should be treated with external-beam and brachytherapy radiation and concurrent cisplatin-based chemotherapy. Our study suggests that mode of therapy had no impact on overall survival. We acknowledge that no conclusions can be drawn from this finding, given the small number of patients in each treatment group. We also realize that randomized controlled studies are needed to evaluate the best mode of treatment. Hopefully, in the future it will allow the selection of patients with favorable parameters, including nuclear grade of tumor cells, which would benefit from a less aggressive treatment.

In conclusion, the treatment of patients with cervical adenocarcinoma should be planned carefully, with special regards to the clinical stage, as well as histological parameters, including architectural grade and particularly nuclear grade. In our study, nuclear grade was, however, the only independent prognostic parameter for survival.

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