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CLINICAL ARTICLE

Prognostic significance of DNA quantification by flow cytometry in ovarian tumors

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Abstract

Objective: The objective of our study is to evaluate prognostic significance of DNA quantification by flow cytometry in ovarian tumor. *Methods*: A prospective analysis was performed on 56 ovarian tumor patients treated in the Yonsei Medical Center from Feb. 2000 to Jan. 2003. *Results*: Regarding the association between tumor grade and the DNA quantitative analysis, as tumor grade increased, the quantity of aneuploid cells and S-phase fraction (SPF) increased. In addition, SPF was increased significantly in the advanced staged patients (P=0.04) and SPF was significantly increased in aneuploid tumors (P=0.03). The overall survival rate was poor for patients with aneuploid tumors and for patients with tumors showing over SPF (10%). *Conclusion*: Our data demonstrated that the prognosis was poor for patients with aneuploid cancers or increased SPF. Therefore, DNA quantification by flow cytometry may provide important information for predicting the prognosis of the disease.

1. Introduction

Ovarian cancer is currently a major cause of death in gynecological cancer. Of the several established prognostic factors for ovarian cancers, the major independent prognostic factors are stage, histologic grade, serum CA-125 level [1,2]. Rustin et al. have reported that, for ovarian cancer, the CA-125 level is a useful prognostic factor for the progression of cancer, the response to treatment and its recurrence [3]. The prognosis was predicted by evaluating the half-life of CA-125 and the prognostic score index

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was assessed by applying a scoring system or evaluating the CA-125 level after three cycles of adjuvant chemotherapy [4—6].

Considering the different tumor factors other than CA-125, several investigators have examined the tumor cell itself as a prognostic factor. Based on the truth of the assumption that "tumor cells proliferate vigorously", the proliferation capacity of tumor cells was quantitated [7]. Garcia and Gown have predicted the progression of cancer by quantitating tumor cell proliferation [8]. Examples of techniques being applied to assess cell proliferation are the various methods such as [^{3}H]thymidine uptake assay, Halo-pyrimidine bromodeoxyuridine (BrdU) assay, Ag-NOR assay, anti-Ki-67 antibody assay, the proliferation cell nuclear antigen (PCNA) assay and the DNA analysis by flow cytometry.

Friedlander et al. reported that the prognosis of patients with aneuploid tumors is worse than for patients with diploid tumors [9]. In contrast, other researchers have reported that the DNA ploidy does not correlate with the prognosis [10]. Meyer et al. have reported that the most accurate method of assessing the proliferation capacity of tumor cells is to measure the S-phase fraction (SPF) of the cell cycle by flow cytometry [11].

In this study, the correlation of the DNA ploidy and SPF with clinicopathological prognostic factors was evaluated for patients with ovarian carcinoma. In addition, by assessing the correlation of CA-125, the DNA ploidy and SPF with the 2-year overall survival rate, the prognostic value of the serum CA-125 level, the DNA ploidy and SPF was also investigated.

2. Materials and methods

2.1. Patients

A prospective analysis was performed on 56 ovarian cancer patients treated at the Department of Obstetrics and Gynecology at the Yonsei University College of Medicine from the time of Feb. 2000 to Jan. 2003. For 56 patients, 7 cases were benign ovarian tumor, 9 cases borderline ovarian tumor and 40 cases malignant ovarian cancer. For patients with benign and borderline tumors, the tumors were removed by exploratory laparotomy. Those patients with malignant disease were treated by staging laparotomy followed by postoperative adjuvant chemotherapy to debulk the tumors, as well as to determine the disease stage. The chemotherapy regimen was *paclitaxel* 175 mg/m^2 plus *carboplatin* AUC 6 for 6 cycles.

2.2. Flow cytometric analysis

Flow cytometry was used to analyze the DNA quantitatively. Fresh tissue specimens obtained during exploratory laparotomy were used. 0.2 g specimens were stored at −70 °C, fixed according to Vindelov's technique [12]. Up to 25,000 nuclei were analyzed on a FACScan flow cytometer (Becton-Dickinson, Sunnyvale, CA). Cell cycle evaluation of the DNA histograms including DNA index, ploidy and SPF was performed with a Modfit computerized software program (Verity Software, Inc., Topsham, ME). Samples were interpreted as DNA diploid when a single peak on the DNA histogram occupied the diploid position with a DNA index of 0.96—1.04. Aneuploidy was defined by any additional G0/G1 peak not falling into the range for discrete multiples of haploid DNA content. The accepted value of the coefficient variation (CV) for the G0/G1 peak was less than 8%.

2.3. CA-125

The serum CA-125 level determined after 6 cycles of adjuvant chemotherapy was measured by a radioimmunoassay. The serum CA-125 level was divided into two groups, the elevated group and the normal group, by applying 35 U/ml as the cut-off point.

2.4. Statistical analysis

Data was analyzed using parametric and non-parametric statistics, SPSS 10.0 (Chicago, IL). Descriptive statistics are summarized as means and standard deviations. Continuous variables were examined for a normal distribution (Kolmogorov—Smirnov test) before adopting parametric statistics. Differences between continuous variables were evaluated by a Student's *t*-test and a one-way ANOVA for normally distributed variables and by a chi-square test and the Kruskal—Wallis test for variables that were not normally distributed. The prognosis of the patients was determined by the overall survival after treatment. The overall patient survival was calculated from the time of the pathological diagnosis to the time of death of the last event. Estimates of the survival probability were obtained using the Kaplan—Meier non-parametric method. Differen-

Table 1 Clinical characteristics for malignant ovarian cancer

	No.	(%)
Age (years)		
Median	46	
Range	19~72	
Histopathology		
Serous	24	(60.0)
Mucinous	7	(17.5)
Endometrioid	1	(2.5)
Clear cell	1	(2.5)
Undifferentiated	7	(17.5)
Grade		
1	4	(10.5)
2	12	(29.0)
3	24	(60.5)
Stage		
Early (I+II)	15	(37.8)
Advanced (III+IV)	25	(62.2)
Residium		
<2 cm	28	(70.0)
≥2 cm	12	(30.0)

ces were considered significant when the probability of the error was below 5% ($P<0.05$).

3. Results

Of the 56 ovarian tumor cases examined, 7 cases were benign, 9 cases were borderline and 40 cases were malignant. The aneuploidy rate was 14.3% in the benign cases, 11.1% in the borderline cases and 40% in the malignant cases.

The characteristics of 40 patients with malignant tumors are shown in Table 1. The correlation of the DNA ploidy and the clinical prognostic factors was not statistically significant. However, as tumor grade increased or as the disease stage increased, more aneuploid tumor cells were then detected. Similarly, more aneuploid cells were detected in patients with the CA-125 level higher than 35 U/ml, as was measured after 6 cycles of adjuvant chemotherapy ($P=0.05$) (Table 2).

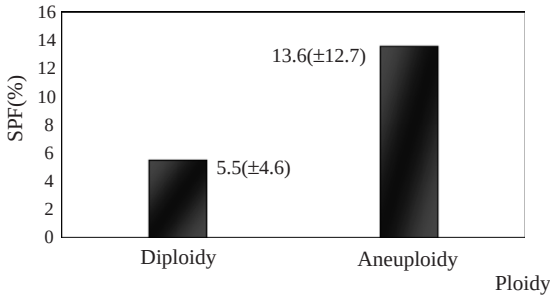

Figure 1 S-phase fraction (SPF) according to DNA ploidy. SPF in the aneuploid tumors was significantly higher than for the diploid tumors ($P=0.03$).

Although the correlation of SPF and the clinical prognostic factors was not statistically significant, SPF increased as the tumor grade increased ($P=0.08$). SPF was increased in the advanced stage tumors and this was statistically significant ($P=0.04$). SPF was increased in those patients with the CA-125 higher than 35 U/ml level after 6 cycles of adjuvant therapy, and this increase was statistically significant ($P=0.04$) (Table 2).

When we examined the correlation of the DNA ploidy and SPF, we detected that SPF in the aneuploid tumors was significantly higher than for the diploid tumors ($P=0.03$) (Fig. 1).

Comparing the 2-year overall survival rate, this rate was lower for patients with the CA-125 level higher than 35 U/ml than for those patients with the CA-125 level lower than 35 U/ml ($P=0.04$) (Fig. 2). Similarly, the survival rate for patients with a SPF higher than 10% was significantly lower than

Table 2 Correlation between clinical prognostic factors and DNA ploidy and S-phase fraction (SPF)

	Diploidy		Aneuploidy			SPF (%)	
	No.	(%)	No.	(%)	*P*	(Mean±S.D.)*	*P*
Grade					NS		NS
1	4	(100)	0	(0)		2.5 ±2.3	
2	8	(66.7)	4	(33.3)		6.5±5.7	
3	11	(46.0)	13	(54.0)		8.9±7.4	
Stage					NS		0.04
Early (I+II)	9	(60.0)	6	(40.0)		9.8±7.3	
Advanced (III+IV)	10	(40.0)	11	(60.0)		16.9±6.1	
CA-125 (U/ml)					0.05		0.05
≤35	18	(64.3)	10	(35.7)		6.9±5.4	
>35	2	(16.7)	10	(83.3)		13.8±9.9	

NS, not significant.
* Mean (±standard deviation).

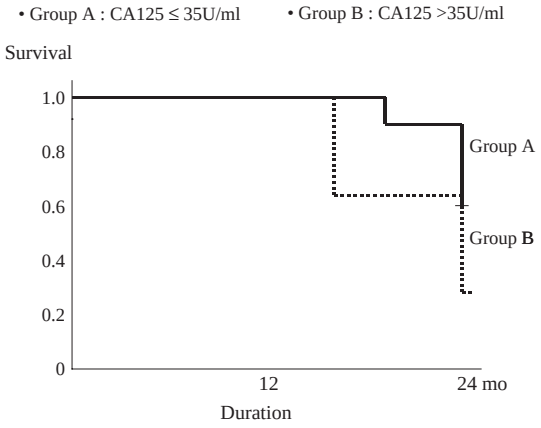

Figure 2 Overall survival curves according to CA-125 after the 6th course of postoperative adjuvant chemotherapy. The survival time in patients with the CA-125 level higher than 35U/ml (n=28, dotted line) was significantly lower than that in patients with the CA-125 level lower than 35 U/ml (n=12, thin line; P=0.04).

that for patients with a SPF lower than 10% (P=0.04) (Fig. 3).

4. Discussion

According to the annual Korean cancer registry report by the Department of Health and Welfare, ovarian cancer is one of 10 most frequent cancers and it is a major cause of death in gynecological cancer [13]. The mainstay of treatment of ovarian cancer is surgery to determine the disease stage as well as to debulk tumors in those patients with advanced disease, and this surgery is followed by a course of postoperative adjuvant chemotherapy. However, ovarian cancer is still considered very difficult to treat and it has a high recurrence rate.

Prognostic factors play an important role in the physician's selection of the therapeutic modalities. Several factors including the stage of disease, tumor grade and serum CA-125 level have been established as the prognostic factors of ovarian cancer [1,2]. Despite the establishment of such prognostic factors, more objective measures for predicting the progression of the disease are required. The assessment of the proliferation capacity of tumors has been reported to be an objective means to predict the malignant potential of tumor cells. Therefore, numerous efforts have been made to quantitate the proliferation potential of the cancer cells. Particularly, many studies that have focused on analyzing DNA quantitatively by flow cytometry have been reported.

Flow cytometry, in contrast to immunohistochemical techniques, can analyze a large number of cells in a relatively short time [14]. It has been reported that the tumor grade and the disease stage of aneuploid tumors is higher than that of the diploid tumors and the prognosis of aneuploid tumors is worse than for diploid tumors [15]. In our study, we observed that 1 patient out of 7 with benign tumor was aneuploid and 1 patient out of 10 with borderline tumor was aneuploid. In the malignant cases, however, 42% of the cases (13/31 cases) were aneuploid. Our data are in agreement with previous reports. Moreover, we have observed that, in the malignant cases, the tumor grade as well as the disease stage of the aneuploid tumors was higher than for the diploid tumors. CA-125 level higher than 35 U/ml (as measured after 6 cycles adjuvant chemotherapy) was detected more frequently for aneuploid tumors than for diploid tumors, and the 2-year survival rate for patients with aneuploid tumors was lower than for patients with diploid tumors.

Strang et al. have reported that for the relation between the DNA ploidy and SPF, SPF in the diploid tumors was lower than in the aneuploid tumors. They also reported that tumors with the same grade and stage might be subclassified according to their SPF [16]. In our study, we also observed that SPF in the aneuploid tumors was higher than in the diploid tumors and the difference was statistically significant.

SPF determined by flow cytometry is an accurate method for detecting the proportion of tumors undergoing proliferation. In general, it has been reported that tumors with high SPF are more aggressive [17]. Friedlander et al. have reported that the SPF in aneuploid tumors is higher than for diploid tumors in ovarian cancer [15]. In addition to ovarian cancer, SPF has been established as a useful prognostic factor for breast cancer, colorectal

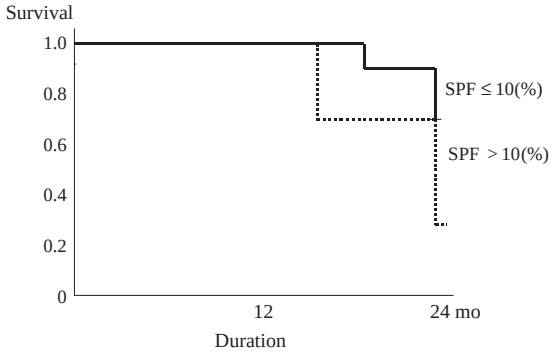

Figure 3 Overall survival curves according to S-phase fraction (SPF). The survival rate for patients with a SPF higher than 10% (n=11, dotted line) was significantly lower than that for patients with a SPF lower than 10% (n=29, thin line; P=0.04).

cancer, lung cancer and other solid tumors [18]. Our data has also demonstrated that SPF is increased in high grade tumors as well as in advanced stage tumors. The 2-year survival rate for patients with an SPF over 10% is poor, and the prognosis becomes worse as tumor grade increased and the disease stage advanced. Thus, our data suggest that as the prognosis becomes poor and, as tumor grade increases and the disease stage advances, there is a poor prognosis available for those patients with high SPF tumors and the increased aneuploid cells.

CA-125 is a prognostic factor for detecting ovarian cancer progression, its response to treatments and its recurrence [3]. Our data showed that, after 6 cycles of adjuvant therapy, a CA-125 level higher than 35 U/ml was detected more frequently in patients with aneuploid tumors and the SPF was increased. So it may be concluded that for patients with aneuploid tumor cells or high SPF, the response to adjuvant chemotherapy will be poor, tumor recurrence high and the prognosis for these patients will be poor.

Although the correlation between DNA ploidy and the clinical prognostic factors has been reported in numerous studies, most of these studies analyzed paraffin-embedded tissues. Hedley et al. reported the application of flow cytometry technique to paraffin-embedded tissues [19]. Schutte et al. reported that the results obtained from paraffin-embedded tissues and fresh tissue specimens were identical [20]. On the other hand, Stephenson et al. reported the inferior quality for the DNA histogram of flow cytometry using paraffin-embedded tissues as compared to fresh tissue specimens [21]. Similarly, Jacobsen et al. and Deitch et al. reported that for experiments using paraffin-embedded tissues, the coefficient of variance for the diploid peak was high. The aneuploid peak in their experiments appeared as small and unclear for sections, and the measurement accuracy of paraffin-embedded tissues was lower than for the fresh tissue specimens [22,23]. Nyvang et al. examined the correlation of CA-125, tumor grade, the disease stage and DNA index by utilizing fresh tissue specimens from ovarian cancer patients [24]. Except for some limited cases, those studies performed using fresh tissue specimens are rarely reported. In our study, in contrast to most other studies, we analyzed fresh tissue specimens for all patients (56 cases). Analyses were performed for the 2-year survival time, which was relatively short due to our experiment's use of fresh tissue specimen.

In conclusion, our data suggest that, for ovarian cancer, the determination of the DNA ploidy and SPF by analyzing DNA quantitatively with the use of flow cytometry may provide clinically important information for predicting the progression of the disease.

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