

Quantitative Pathologic Variables as Prognostic Factors in Epithelial Ovarian Cancer

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= 국문초록 =

상피성 난소암의 예후인자로서 정량적 병리 지표의 의의

김종혁 · 허주령* · 고창원 · 나준희 · 김봉희* · 김용만 · 김영탁 · 남주현 · 목정은

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상피성 난소암에서 예후에 영향을 주는 임상병리학적 변수에 관한 많은 보고가 있으나, 이들은 대부분 객관성과 재현성이 낮아 연구자들마다 서로 다른 결과를 보고하여 왔다. 최근의 연구들에 의하면 mitotic activity index(MAI), volume percent index(VPE), mean nuclear area(MNA)가 병리학적 특성을 정량적으로 표시할 수 있는 객관적이고 재현성이 우수한 지표로서 상피성 난소암 환자의 예후를 예측할 수 있는 가능성을 가지고 있다고 보고되고 있으나 아직은 논란이 있는 실정이다. 저자 등은 1989년 6월부터 1995년 12월까지 울산대학교 의과대학 서울중앙병원 산부인과에 내원하여 종양 축소수술 및 항암화학요법을 받고 최소한 6개월 이상 추적조사가 가능하였으며 파라핀 포매피가 잘 보존된 74명의 상피성 난소암 환자를 대상으로 이러한 정량적 병리 지표가 예후인자로서의 의미가 있는지 규명하고자 본 연구를 시행하였다. 종양 조직은 4 μ m 두께로 파라핀 포매피에서 자른 후 진단과 정량적인 검사를 위하여 H/E 염색을 시행하고 가장 분화가 나쁘면서 상피세포와 세포분열이 가장 많은 양상을 보이는 부위를 찾은 다음, 분명하게 확인이 가능한 세포분열이 있는 경우의 총 수를 MAI를 측정하기 위하여 계산하였다. 또한 조직 중 상피세포 체적의 백분율을 VPE로 하였으며 nuclear morphometry는 비디오 카메라가 장착된 현미경을 이용하여 시행하였는데 MNA를 측정하기 위한 최종 확대 배율은 3000배이었다. 이러한 정량적 병리 지표와 연령, FIGO 병기, 복수의 양, 잔류 종양의 크기, 조직학적 아형, 종양의 분화도, 수술 후 2차 항암화학요법의 혈청 CA 125치 등의 임상병리학적 예후인자의 상관관계를 분석하였으며 각 인자별 생존율을 계산하였다. 정량적 병리 지표 세 가지 중 VPE가 조직학적 아형과 상관관계가 있었으며 MAI 및 MNA는 종양의 분화도와 상관관계가 확인되었다. 생존율 분석에서는 FIGO 병기, 복수의 양, 수술 후 잔여량의 크기, 조직학적 아형,

수술 후 2차 항암화학요법 후 혈청 CA 125치는 생존율에 유의한 영향을 미치는 것으로 확인되었으나 정량적 병리 지표는 생존율과 유의한 관계가 없었다. 결론적으로 상피성 난소암에서 정량적 병리 지표는 종양의 아형 또는 분화도와 관계가 있으나 환자의 생존율에는 영향을 주지 못하였다.

Keywords: Epithelial ovarian cancer, Morphometry, Prognosis

I. Introduction

Ovarian cancer is the most common gynecologic cancer in Western countries and in Korea, it is the second most common gynecologic malignancy only next to uterine cervical cancer and accounts for 2.9% of all cancers in Korean women.^{1,2)} Of all gynecologic malignancies, cancer of the ovary ranks first as a cause of cancer-related death and in US, it ranks the fourth cause of all cancer-related death in women.³⁾ The main reason for the poor prognostic outcome is that most women present with already advanced disease at time of diagnosis.⁴⁾ With this significant difference in prognosis between early and advanced stages and limitations of present therapies, much attention has been recently focused on screening or early detection of ovarian cancers. Other important characteristic feature of ovarian cancer is their inherent heterogeneity with respect to biological behavior ranging from the relatively indolent nature of borderline malignancies to highly aggressive and rapidly fatal carcinomas. This wide clinical spectrum is partly reflected in a variety of clinicopathological variables and ongoing attempts would be made to refine the definitions of these variables and to determine the degree of benefit achieved by treatment in each.^{5,6)}

Well-known prognostic factors with regard to overall survival in epithelial ovarian cancer are FIGO stage, the performance status at diagnosis, the amount of residual tumor after first laparotomy, absence or presence of ascites, patient age, tumor grade and histiotype.⁷⁻¹³⁾ However, most of these are

based on subjective poorly reproducible criteria and their prognostic relevance may therefore vary between different populations and evaluators.¹⁴⁻¹⁶⁾

Recent some studies have shown that quantitative pathologic features such as mitotic activity index (MAI), volume percent epithelium (VPE) or mean nuclear area (MNA) are objective and give well reproducible values that have important predictive value for survival of patients with epithelial ovarian cancer, although in others, none of these features reached significance concerning survival.¹⁷⁻²⁵⁾ Possible explanations for the lack of prognostic value were sampling differences at the macroscopic level, fixation induced variations, differences in the selection of patients and geographic differences in the degree of malignancy of advanced ovarian cancer in those countries studied.

In this retrospective study, we evaluated the correlations between these features and known clinicopathological prognostic factors and their relevance to survival of patients with epithelial ovarian cancer in Korea.

II. Materials and methods

1. Patients

The charts from Department of obstetrics & Gynecology of Asan Medical Center, University of Ulsan in Seoul have been examined for patients with the diagnosis of ovarian cancer. Retrospectively, the charts were reviewed and certain patient and tumor characteristics were examined. The study group was narrowed by excluding nonepithelial

cancers, borderline grades, and those received prior chemotherapy before surgery. Seventy-four patients with ovarian carcinoma of the common epithelial type were included in this study. All patients were diagnosed between June 1989 and December 1995 and underwent exploratory laparotomy. The stage of the disease was assigned based on surgical-pathologic findings according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for carcinoma of the ovary. The patient characteristics are summarized in Table 1. The patients were treated by debulking surgery followed by combination chemotherapies with regimens based on cisplatin or taxol such as those consisting of cyclophosphamide and cisplatin (CP), or cyclophosphamide, adriamycin and cisplatin (CAP), or cyclophosphamide and carboplatin (CC), or taxol and cisplatin (TP), or taxol and carboplatin (TC) for from three to eighteen cycles. In case of advanced disease, second-look procedures were performed after 6 to 9 cycles of chemotherapy in patients with no clinical evidence of disease.

2. Qualitative and quantitative methods

Tumor material was routinely processed according to standard procedures and 4 μ m thick section were cut from the paraffin blocks and stained with H&E for diagnosis and quantitative assessments. The subjectively most poorly differentiated area was demarcated of for measuring, a so-called measurement area (approximately 0.5 \times 0.5cm). This area was the most epithelium rich and had the subjectively highest number of mitotic figures; areas with necrosis, inflammation or calcification were avoided as much as possible. The total number of mitotic figures was counted in 25 contiguous fields of vision with a conventional light microscope (final magnification \times 400, field diameter 450 μ m). Once having focused, no further adjustment was allowed. The total number of clearly identifiable mitotic figures was counted and taken as the mitotic activity index (MAI). The volume percentage of

Table 1. Patient characteristics

Characteristics	No. of patients
No. of patients studied	74
Age(mean \pm S. D.)	51.5 \pm 12.3
(range)	17 - 75
FIGO stage	
I	15(25.9%)
II	8(10.8%)
III	41(55.4%)
IV	10(13.5%)
Histologic subtype	
Serous	52(70.3%)
Mucinous	9(12.2%)
Endometrioid	12(16.2%)
Undifferentiated	1(1.4%)
Histologic grade	
Grade 1	23(31.1%)
Grade 2	13(17.6%)
Grade 3	38(51.4%)
Ascites volume	
< 500cc	16(24.3%)
> 500cc	56(75.7%)
Residual tumor size	
< 2cm	42(56.7%)
\geq 2cm	32(43.3%)
Serum CA 125 level after 2nd chemotherapy	
< 35 U/ml	59(79.7%)
\geq 35 U/ml	17(20.3%)

epithelium (VPE) was estimated (final magnification \times 200) with a 42 points parallel Weibel grid, assuming that the tissue was isotropic uniform randomly oriented. At least 300 points covering epithelial or stromal cells were counted in each tumor section. The percentage of points overlying epithelial cells was taken as the VPE.

Nuclear morphometry was performed with a video camera mounted on a microscope. A final magnification on screen of approximately \times 3000 (objective \times 100) was used for assessment of the MNA.

3. Data evaluation

The differences in quantitative features for various clinicopathologic prognostic factors such as age (younger or older than 56 years), FIGO stage, volume of ascites ($\geq 500\text{ml}$, or $< 500\text{ml}$), residual tumor size ($\geq 2\text{cm}$, or $< 2\text{cm}$), histologic subtype, tumor grade and serum CA 125 level after second chemotherapy ($\geq 35\text{U/ml}$, or $< 35\text{U/ml}$) were assessed using chi-square test. The level of significance was set to $P < 0.05$.

For survival analysis, the time from diagnosis to death of disease or last date of follow-up was used as the overall survival time. Cut-off points for the quantitative variables were chosen according to the value reported by Brinkhuis et al.²⁵⁾ for the MAI and VPE or to form four group of equal size for MNA. Kaplan-Meier curves were plotted and the differences between the curves were tested for significance using Log-rank test. We used Cox proportional hazards model in multivariate analysis to find out independent prognostic variables.

III. Results

All quantitative pathologic features were not significantly different according to age of patient, FIGO stage, volume of ascites, residual tumor size after cytoreductive surgery and serum CA 125 level after second chemotherapy. However, the VPE was significantly correlated with histologic subtype, hence undifferentiated histologic subtype showed higher prevalence of large volume percentage of epithelium than serous as well as mucinous tumor. The MAI and MNA were significantly correlated with tumor grade, hence the more differentiated the tumor was, the lower mitotic activity index and the smaller mean nuclear area the tumor had (Table 2).

The mean follow-up duration was 35 months with range of 8 to 86 months. At the end of follow-up 38 of the 74 patients (51.4%) were alive, 28 cases were known to be died of the disease and

Table 2. Results of chi-square test to evaluate the correlation between quantitative pathologic variables and other clinicopathological parameters in epithelial ovarian cancer

Clinicopathological variables	Quantitative pathologic variables		
	MAI	VPE	MNA
Age	0.352	0.146	0.369
FIGO stage	0.457	0.407	0.716
Volume of ascites	0.106	0.163	0.504
Residual tumor size	0.216	0.111	0.298
Histologic subtype	0.784	0.043	0.508
Tumor grade	0.005	0.108	0.022
Serum CA 125 level after 2nd chemotherapy	0.703	0.330	0.085

8 patients were lost during follow-up.

In survival analysis, the overall 5-year survival rate of all patients was 37.9% and most of clinicopathologic variables tested including such as age, FIGO stage, volume of ascites, residual tumor size after debulking surgery, histologic subtype and serum CA 125 level after second chemotherapy except tumor grade had significant influence on the clinical outcome (Table 3). Patients with age over 56 had poorer prognosis than younger group (5-year survival rate of 20.1% vs 52.4%). The more advanced the disease was, the poorer the prognosis was, hence 5-year survival rate of patients with stage I disease was 85.7%; 4-year survival rate of patients with stage II disease was 60.9%; 4.5-year survival rate of patients with stage III disease was 27.0%; and 3.5-year survival rate of patients with stage IV disease was 19.7%. Large amount of ascites (equal to or more than 500cc) render the patients to have poor prognosis (survival rate of 23.0% (5year) vs 62.2% (4.5year) and the patients with tumor of undifferentiated histologic subtype had the poorest prognosis (5-year survival rate of 22.2%) in comparison with those of serous as well as mucinous histology. Patients with small residual

Table 3. Actuarial survival rates of patients according to various clinicopathological variables in epithelial ovarian cancer

Variables	No. of patients	Survival rate(year)	p value
Age			
≤ 56	48	52.4 (5yr)	0.037
> 56	26	20.1 (5yr)	
FIGO stage			
I	15	85.7 (5yr)	0.0022
II	8	60.9 (4yr)	
III	41	27.0 (4.5yr)	
IV	10	19.7 (3.5yr)	
Ascites volume			
< 500cc	18	62.2 (4.5yr)	0.0299
≥ 500cc	56	23.0 (5yr)	
Histologic subtype			
Serous	52	38.7 (5yr)	0.0170
Mucinous	12	55.5 (4yr)	
Endometrioid	1	0.0	
Undifferentiated	9	22.2 (3.5yr)	
Tumor grade			
Grade 1	23	52.3 (5yr)	0.0991
Grade 2	13	37.6 (3.5yr)	
Grade 3	38	28.8 (4.5yr)	
Residual tumor size			
< 2cm	42	62.9 (5yr)	0.0001
≥ 2cm	32	21.5 (5yr)	
Serum CA 125 level after 2nd chemo.			
<35U/ml	59	46.0 (5yr)	0.0006
≥ 35U/ml	15	13.3 (3.5yr)	

by Kaplan-Meier method and Log-rank test

disease (less than 2cm, n=42) after debulking surgery had a good prognosis (5-year survival rate of 62.9%). However, when disease after debulking laparotomy was found with large gross investigation (equal to or larger than 2cm, n=32), only 21.5% of patients could survive for 5 years. The serum level of CA 125 after second chemotherapy showed important prognostic significance. Most of patients (86.7%) revealed to have a serum level of CA 125 after second chemotherapy equal to or more than

35U/ml had died of the disease within 3.5years. Tumor grade only was the variable that had no prognostic significance in our study.

Of the quantitative pathologic features we analysed, none had prognostic value (Table 4). There was no significant difference in survival according to the MAI when the classical cut-off points of 10, 30 and 50 mitoses counted in 25 high power fields were used (Fig. 1) (p=0.087). The patients with the tumor that had large volume percentage epithelium

Table 4. Actuarial survival rates of patients according to quantitative pathologic variables in epithelial ovarian cancer

Variables	No. of patients	Survival rate(year)	p value
Mitotic activity index			
≤ 10	52	42.6 (5yr)	0.0865
11-30	12	45.1 (4yr)	
31-50	1	25.0 (3.5yr)	
> 50	9	45.1 (4.5yr)	
Volume % epithelium			
≤ 83%	52	40.4 (5yr)	0.0920
> 83%	22	30.6 (5yr)	
Mean nuclear area			
≤ 260 μm^2	52	28.5 (4.5yr)	0.7890
261-300 μm^2	12	31.7 (4yr)	
301-385 μm^2	1	56.2 (3.5yr)	
> 385 μm^2	9	39.3 (5yr)	

by Kaplan-Meier method and Log-rank test

(VPE, larger than 83%) were tend to have shorter survival (5-year survival rate of 30.6% vs 40.4%), but no significant statistical difference was noted (Fig. 2) ($p=0.92$). The survival rates of the patients according to the mean nuclear area (MNA) were quite variable when the cut-off point of 260, 300 and 385 μm^2 were used, and did not show any trend for significance (Fig. 3) ($p=0.79$).

In multivariate analysis with the variables those had prognostic significance, the residual tumor size and the serum level of CA 125 after second chemotherapy were revealed to be independent prognostic factors with the relative risk of 2.67 and 3.45 respectively.

IV. Discussion

Several studies have shown that quantitative pathologic features are objective and give well reproducible values that have important predictive value for survival of cisplatin treated patients with advanced epithelial ovarian cancer. These features

include mitotic activity index (MAI), volume percent epithelium (VPE), mean nuclear area (MNA) and standard deviation of nuclear area (SDNA).¹⁷⁻²⁵⁾ However, in a recent study of 45 Danish patients with advanced ovarian cancer, none of these features reached significance concerning survival.²⁴⁾ More recently, the same group of authors have concluded that morphometric variables have important value in predicting prognosis in patients with FIGO stage III ovarian cancer according to their results of the study with 112 patients.²⁵⁾ In the present study the quantitative pathological features MAI, VPE and MNA did not have any prognostic value in 74 patients with epithelial ovarian cancer with all stages. Possible explanations for the lack of prognostic value in the former study were sampling differences at the macroscopic level, fixation induced variations, differences in the selection of patients and geographic differences in the degree of malignancy of advanced ovarian cancer in those countries studied.²⁴⁾ As to the latter, Danish ovarian cancers are more often poorly differentiated, and the same trend was found for tumors with high MAI

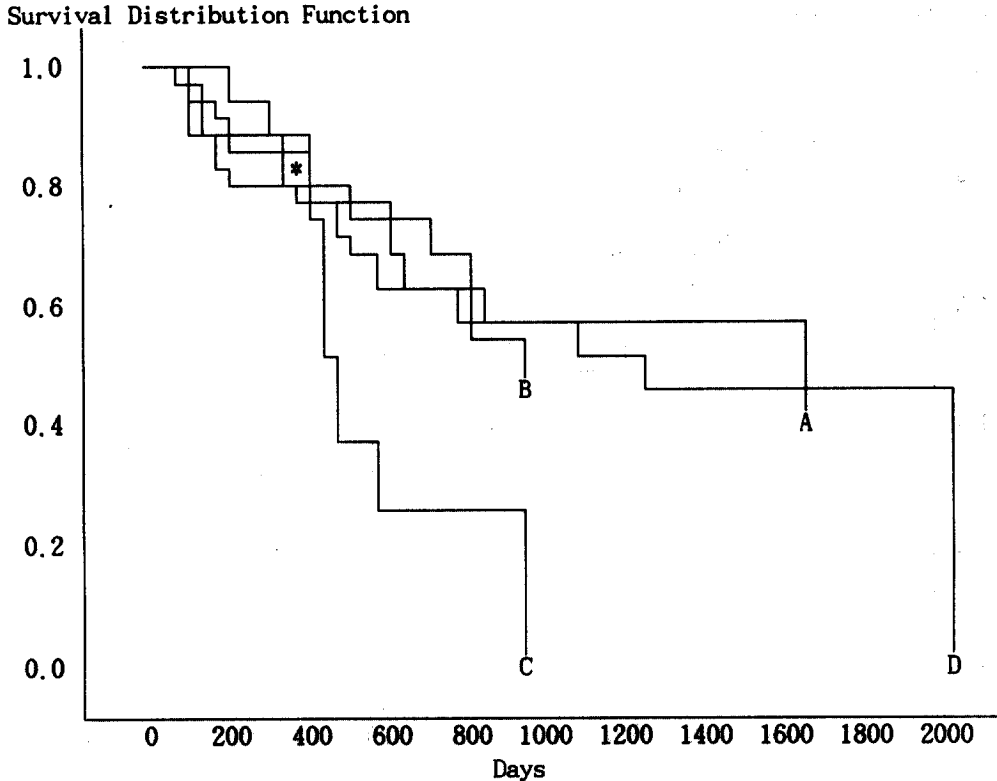


Fig. 1. Kaplan-Meier survival curves for 74 patients with epithelial ovarian cancer according to MAI (mitotic activity index), A: ≤ 10 , B: 11-30, C: 31-50, D: > 50 . Log-rank test showed no significant difference among four groups ($p=0.087$)

or high VPE. To minimize the possible variations in study, we used the methods same as the latter study, hence the most poorly differentiated area was demarcated of for measuring, which was the most epithelium rich and had the subjectively highest number of mitotic figures and areas with necrosis, inflammation or calcification were avoided as much as possible. And to avoid the selection bias, the pathologist did not get any information about the clinical features of the patients. The distributions of differentiation and histologic subtype of our study were comparable to the Danish studies although mucinous subtype was more common in our patients in comparison with the Danish. Therefore, the lack of prognostic significance of quantitative pathological features in the present study can not be

explained by technical or geographic differences in the degree of malignancy of ovarian cancer between Danish and Korean patients.

In the latter Danish study, the all quantitative pathological features are correlated with Broders grade of tumor although the former one had shown that the MAI only had a significant correlation with the grade.^{24,25} In our study, the MAI and MNA were significantly associated with the tumor grade whereas the VPE did not. Unexpectedly our study showed that the VPE was related with the histologic subtype. In the Danish studies, the VPE, the MNA or the SDNA had significant correlation with the well known prognostic variables such as residual disease, performance status of patient and age of patient.^{24,25} However, our study showed that

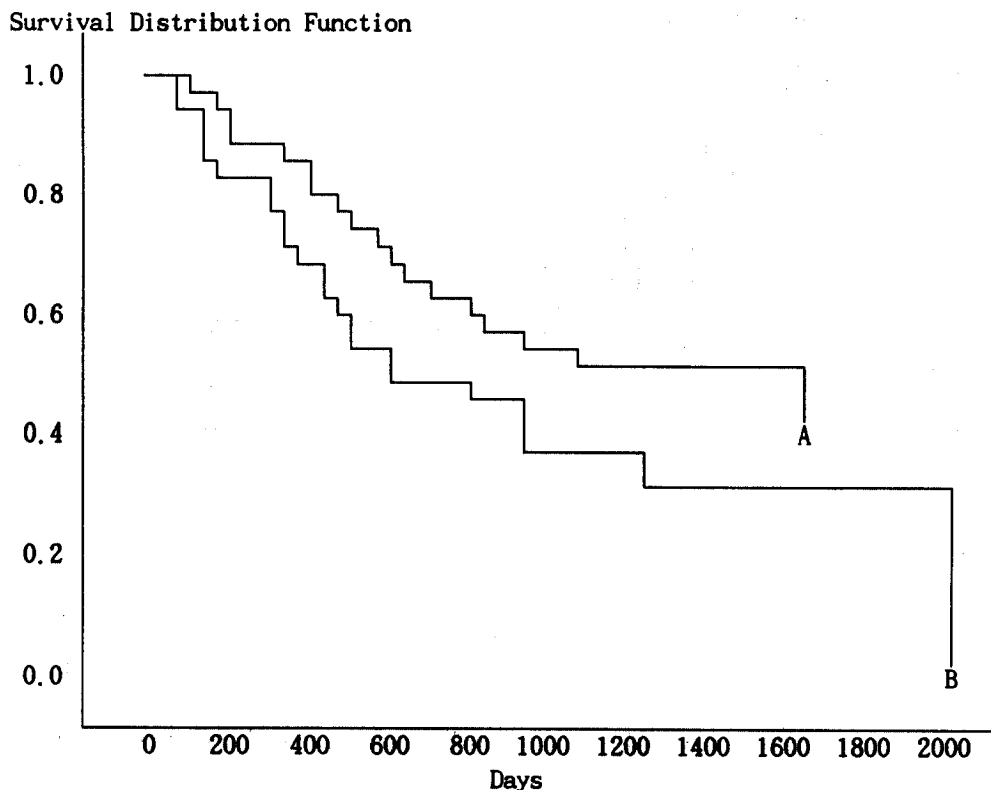


Fig. 2. Kaplan-Meier survival curves for 74 patients with epithelial ovarian cancer according to VPE(volume percentage epithelium), A: $\leq 83\%$, B: $> 83\%$. Log-rank test showed no significant difference between two groups($p=0.092$)

none of the quantitative pathological features had significant correlation with the prognostic variables including FIGO stage, residual tumor size and serum level of CA 125 after second chemotherapy which were previously studied with our patients.^{7,8,10)}

In our study, residual disease status and serum level of CA 125 after second chemotherapy were independent prognostic factor, which is in agreement with many other prognostic studies in ovarian cancer.^{10,11,14,26,27)} Moreover, it is biologically understandable that small tumor nodes are more vulnerable to chemotherapy because their tumor cells may be more accessible for the cytotoxic agents due to a better vascularization and oxygenation and that serum level of CA 125 after chemotherapy reflects the responsiveness to the chemotherapy.^{28,29)}

However, in the former study on Danish patients in which quantitative pathological features lacked prognostic significance, residual disease also failed to reach prognostic significance.²⁴⁾ In agreement with the previous two Danish investigations are the significant differences of the MNA and SDNA between patients with residual disease size smaller and larger than 2cm after debulking laparotomy.^{24,25)} Therefore, they thought that an association between nuclear size and the success of optimal debulking seems to exist, in such a way that tumors harboring large tumor cells are less easy surgically removable and that the association between MNA, SDNA and residual disease status was found in both investigations means that sampling differences at the macroscopic level of the tumor specimens or fix-

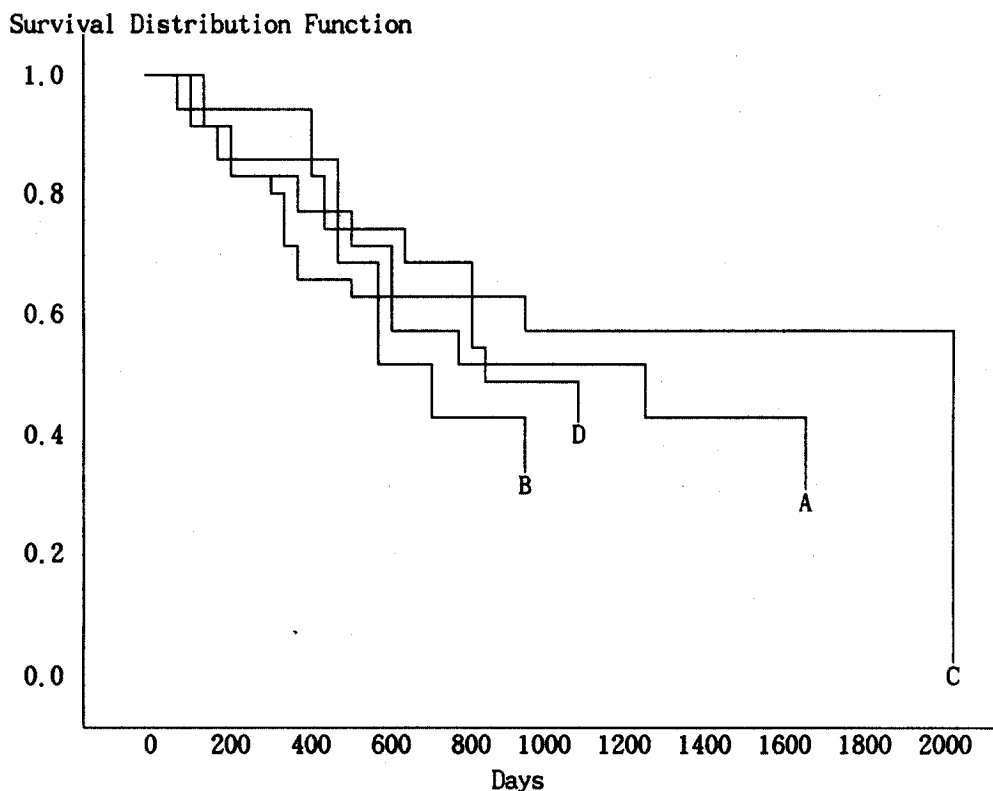


Fig. 3. Kaplan-Meier survival curves for 74 patients with epithelial ovarian cancer according to MNA(mean nuclear area), A: $\leq 260 \mu\text{m}^2$, B: $261-300 \mu\text{m}^2$, C: $301-385 \mu\text{m}^2$, D: $> 385 \mu\text{m}^2$. Log-rank test showed no significant difference among four groups($p=0.79$)

tion induced variations did not have an important impact on the values obtained for the MNA and SDNA.²⁵⁾ Their explanation for the lack of prognostic value of the quantitative pathological features in the former Danish investigation was that at some level selection of patients or material has taken place and an alternative hypothesis was that patients in the former Danish investigation could have biologically completely different tumors. However, in our study this seems less likely to us as mentioned above.

In the latter Danish study, the MNA and SDNA were the strongest prognostic quantitative variables.²⁵⁾ Their prognostic value might be partly explained by their association with residual disease status, but in multivariate regression analysis the

MNA was independently selected after residual disease status, indication that its independent prognostic value has also to be based on other as yet unknown control mechanisms of neoplastic growth. Associations were also found between the MNA, SDNA and histologic grade indicating an association between the size of tumor nuclei and tumor architectural organization. Performance status and age were also prognostically significant indicators of survival in the latter Danish study and were also associated with the MNA and SDNA, hence, younger patients with a good performance status had less aggressive disease marked by smaller tumor cell nuclei in their study. However, in this study we could not find out these association as mentioned above.

Because performance status is based on subjective criteria of both the patient and physician and tumor grade is also susceptible to intra and interobserver variation between the diagnosing pathologists, their prognostic relevance may vary between different populations and evaluator.¹⁴⁻¹⁶⁾ In order to be able to compare the results of different investigations it is mandatory that they are based on objective reproducible variables which should be easy to assess. The investigated quantitative pathological features in these studies including ours fulfil these criteria. Although we could not find the significant prognostic value of these quantitative pathological features, the attempt to find out the objective pathologic variables might be warranted for closer understanding of tumor growth in ovarian cancer and possible application in therapeutic approach to this serious disease.

V. Summary

In this retrospective study, we evaluated the correlations between these features and known clinicopathologic prognostic factors and their relevance to survival of seventy-four patients with epithelial ovarian cancer treated at Asan Medical Center from June, 1989 to December, 1995. Tumor material was routinely processed according to standard procedures and 4 μ m thick sections were cut from the paraffin blocks and stained with H&E for diagnosis and quantitative assessments. The subjectively most poorly differentiated area was demarcated as measurement area (0.5 \times 0.5cm), which was most epithelium-rich and had the subjectively highest number of mitotic figures. The total number of clearly identifiable mitotic figures was counted (final magnification \times 400, field diameter 450 μ m) and taken as MAI. The VPE was estimated (final magnification \times 200, objective \times 20) with a 42 points parallel Weibel grid, and at least 300 points covering epithelium were counted in each tumor section.

The percentage of points overlying epithelial cells were taken as the VPE. Nuclear morphometry was performed with a video camera mounted on a microscope. A final magnification on screen of approximately \times 3000 (objective \times 100) was used for assessment of the MNA. The differences in quantitative features for various clinicopathologic prognostic factors such as age, FIGO stage, volume of ascites, residual tumor size, histologic subtype, tumor grade and serum CA 125 level after second chemotherapy were assessed using chi-square test and for survival analysis, Kaplan-Meier curves were plotted and the differences between the curves were tested for significance using Log-rank test. Of three quantitative features, the VPE was associated with histologic subtype and the MAI and MNA were correlated with tumor grade. In survival analysis age, FIGO stage, volume of ascites, residual tumor size, histologic subtype and serum CA 125 level after second chemotherapy had significant influence on overall survival, but any of the quantitative pathologic variables did not. It is concluded that although some variables have correlation with histologic subtype or tumor grade, morphometric variables do not have any value in predicting prognosis.

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