# Multivariate analysis by Cox proportional hazard model on prognosis of patient with epithelial ovarian cancer

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**Summary**

*Purpose of investigation:* To evaluate the influence of various clinicopathological and biochemical factors on the survival of patients with epithelial ovarian cancer (EOC) after radical resection. *Methods:* A retrospective analysis was made for 183 cases of epithelial ovarian cancer treated from January 1997 to January 2001. Six clinicopathological factors, including menopause, histological type, histological grade, lymph node metastasis, FIGO stage and chemotherapy that could possibly influence survival were selected. The expression of COX-2 and VEGF protein as two biochemical factors were detected in EOC tissues using immunohistochemical staining. Independent variables were first analyzed by univariate methods. A multivariate analysis of these variables was performed using the Cox proportional hazard regression model. *Results:* The ovarian cumulative survival rate was 48.71% for three years and 30.71% for five years. Univariate analysis of overall survival involving all the patients indentified five factors that were associated with a significant outcome: menopause, histological grade, FIGO stage, COX-2 or VEGF expression level ($p < 0.05$). The expression of COX-2 was positive in 140 (76.5%) of these 183 cases, but was not associated with menopause, histological type, histological grade, lymph node metastasis or FIGO stage. Median survival time was 24.56 months for the patients with COX-2 positive expression, and 47.52 months for those with COX-2 negative expression ($p < 0.05$). VEGF protein overexpression was examined in 117 (63.93%) of all 183 cases, and was associated with lymph node metastasis ($p < 0.05$), but not associated with menopause, histological grade, histological type or FIGO stage. The median survival time was 23.36 months for the patients with VEGF detected expression, and 42.09 months for those with no VEGF detected expression ($p < 0.05$). When the interactive effects of these factors were taken into account, COX-2 expression, FIGO stage, VEGF expression and histological grade were the four most important prognostic factors by multivariate analysis using the Cox proportional hazards model. Risk of death for the patients with COX-2 positive expression was 2.8 times than that with COX-2 negative expression, and for FIGO stage, VEGF expression and histological grade, risk of death was 2.2, 2.1, and 1.84 times, respectively. *Conclusion:* COX-2 expression, FIGO stage, VEGF expression and histological grade are the most important prognostic factors for EOC after curative resection.

*Key words:* Ovarian cancer; Clinical pathological factors; COX-2; VEGF; Prognosis; Cox's proportional hazard regression model.

## Introduction

Ovarian cancer is one of three gynecologic malignancies. Although current treatment of ovarian cancer entails a combination of surgery and chemotherapy, the prognosis has not changed. The 5-year survival rate is still 20%-30%. Thus it is necessary to research and analyze the operative prognosis factor and to entail suitable methods to improve prognosis. Recently early diagnostic surveillance of disease and evaluation of prognosis has become an important subject and has achieved some advancement [1].

The prognosis of ovarian cancer is difficult and complex. Clinicopathological and biochemical factors are more strictly related to prognosis [2]. Many studies have noted that poor prognosis is associated with highly malignant biochemical characteristics and behavior [3-5]. In addition clinicopathological and biochemical parameters can reflect and express the biologic behavior of ovarian cancer systematically, especially biochemical parameters which have more clinical value [5].

Today there are more reports on clinicopathological parameters and less for biochemical parameters, many of which are analyzed by univariate analysis. Thus it is necessary to evaluate the function of these factors by multivariate analysis. Analysis of the clinicopathological and biochemical parameters by Cox's proportional hazard regression model has not been reported before.

Cyclooxygenases (COXs) are involved in the control of inflammatory reactions and catalyze the rate-limiting step in the biosynthesis of prostaglandins the conversion of arachidonic acid to prostaglandin H2. There are two COX isoenzymes encoded by different genes: COX-1 and COX-2. Studies show: COX-2 is expressed in many carcinomas [6].Vascular endothelial growth factor (VEGF) regulates vascular permeability, is an important mediator of vasculogenesis and angiogenesis. And the main COX-2 product, PGE2 induces VEGF and basic fibroblast growth factor [7]. Many studies shows COX-2 and VEGF might be the parameters for evaluating the carcinoma prognosis [8].

We analyzed and evaluated the relation of normal clinicopathological and biochemical factors to ovarian cancer by Cox's proportional hazard regression model to select the most significant factor which could help in treatment of epithelial ovarian cancer (EOC) systematically.

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## Materials and Methods

*Clinical materials*

A selected 183 patients with EOC underwent surgery at the Chinese Medical University Affiliation Shengjing Hospital between January 1997 and January 2001. All patients were diagnosed by the pathologist. Median age was 43.15 (range 20-74). All patients were not treated by chemotherapy or radiotherapy before surgery, and 147 of 183 cases were followed-up completely. All tissues were fixed in 10% formalin and paraffin-embedded according to standard procedures.

*Biological factors*

Immumohistochemial examination was performed for determination of expression of COX-2 and VEGF on ovarian epithelial cancer tissue samples.

*Main regent*

Rabbit anti-human COX-2 monoclonal antibody, rabbit anti-human VEGF monoclone and S-ABC were used according to the manufacturer's instructions (Boster Co.).

*Inmmunohistochemical studies*

Inmmunohistochemical staining with antibodies to COX-2 and VEGF was performed using a standard protocol according to laboratory manual instructions. Staining steps were carried out strictly according to standand procedures. The COX-2 and VEGF were heated in a microwave oven to retrieve masked antigens. Colon carcinoma sections showing immunoreaction were scored as positive. PBS replaced the first biotin as negative.

For the assessment of COX-2 and VEGF expression levels, the staining intensity and the percentage of stained cells were analyzed. Staining intensity was scored as 0 (negative), 1 (weak), 2 (medium), or 3 (strong), and percentage of stained cells was scored as 0 (0), 1 (< 30%), 2 (30%-60%), 3 (> 60%); both combined, 0-1 was negative, and 2 or more was positive.

*Clinicopathological factors*

1. Menopause: 94 cases were in premenopause and 89 in postmenopause.
2. FIGO stage: I + II ,72; III + IV, 111.
3. Histological grade: high = 54 cases; median and low = 129 cases.
4. Histological type: There were 120 serous cystadenocarcinomas (SCAC), 29 mucinous cystadenocarcinomas (MAC), 34 others (including 16 endometrioid carcinomas, 12 clear cell carcinomas, 4 of Wolffian duct origin cystadenocarcinomas and 2 undifferentiated carcinomas).
5. Lymph node transmission: 88 cases had involvement and 95 did not.
6. Chemotherapy: 151 cases underwent chemotherapy and 32 did not.

Quantified clinicopathological and biochemical factors are shown in Table 1.

*Follow-up*

In the 183 EOC patients, 147 were followed-up completely, and 36 were lost. The follow-up cases had the same survival time.

*Statistical analysis*

The $\chi^2$ test was used to analyze the distribution of COX-2 positive and VEGF positive cases according to the clinicopathological features. Median and life tables were computed using the product-limit estimate by the Kaplan-Meier method. Comparison of survival time of both (or more groups) was analyzed by the Wilcoxon method or Kruskal-Wallis test. Cox's proportional hazard regression model was used to analyze the role of the clinicopathological and biochemical parameters (COX-2 and VEGF).

Table 1. — *Quantified clinicopathological and biochemical factors*.

| Variables | Parameters |
|---|---|
| Menopause | Premenopause; postmenopause |
| FIGO stage | I + II; III + IV |
| Histological grade | high; median and low |
| Lymph node transmission | Negative; Positive |
| Histological type | SCAC; MAC; Others* |
| Chemotherapy | Yes; No |
| COX-2 | Positive; Negative |
| VEGF | Positive; Negative |

SCAC: serous cystic adenocarcinoma; MAC: mucinous adenocarcinoma; *Others: endometrioid carcinoma, clear-cell carcinoma, Wolffian duct origin cystadenocarcinoma, undifferentiated carcinoma.

Table 2. — *Relationship between COX-2, VEGF, and clinicopathological factors*.

| Characteristics | n | No. of COX-2 positive cases (%) | $X^2$ | *p* | No. of VEGF positive cases (%) | $X^2$ | *p* |
|---|---|---|---|---|---|---|---|
| *Menopause* | | | | | | | |
| Premenopause | 94 | 74 (78.72%) | 0.03 | n.s. | 61 (64.89%) | 0.014 | n.s. |
| Postmenopause | 89 | 66 (74.16%) | | | 56 (66.67%) | | |
| *FIGO stage* | | | | | | | |
| I + II | 72 | 53 (73.61%) | 3.65 | n.s. | 48 (66.67%) | 0.387 | n.s. |
| III + IV | 111 | 87 (78.38%) | | | 69 (62.16%) | | |
| *Histological grade* | | | | | | | |
| High | 54 | 43 (79.63%) | 1.51 | n.s. | 38 (70.37%) | 3.416 | n.s. |
| Median and low | 129 | 97 (75.19%) | | | 79 (63.24%) | | |
| *Lymph node transmission* | | | | | | | |
| Positive | 88 | 61 (69.32%) | 3.69 | n.s. | 60 (68.18%) | 3.86 | n.s. |
| Negative | 95 | 79 (83.16%) | | | 57 (60.00%) | | |
| *Histological type* | | | | | | | |
| SCAC | 120 | 95 (79.17%) | | | 79 (65.83%) | | |
| MAC | 29 | 20 (68.97%) | 3.08 | n.s. | 16 (55.17%) | 2.92 | n.s. |
| *Others | 34 | 25 (73.53%) | | | 22 (64.71%) | | |

SCAC: serous cystic adenocarcinoma; MAC: mucinous adenocarcinoma; *Others: endometrioid carcinoma, clear-cell carcinoma, Wolffian duct origin cystadenocarcinoma, undifferentiated carcinoma; n.s.: not significant.

## Results

*COX-2 and VEGF expression in EOC*

COX-2 and VEGF immunostaining was observed mainly in the cytoplasm of tumor cells. One hundred and forty cases (76.5%) were scored as COX-2 positive and 117 (63.93%) were scored as VEGF positive (Figures 1 and 2).

*Correlation with clinicopathological parameters*

Table 2 shows the distribution of positive COX-2 and VEGF according to clinicopathological characteristics. COX-2 positive was not distributed differently according to menopause, FIGO stage, histological grade, lymph node transmission, or histological type. The expression rate of VEGF was 68.18% and 60.0% for lymph node transmission and no lymph node transmission, respectively. The difference was significant. However, VEGF posi-

Table 3. — *Univariate analysis for clinicopathological and biochemical parameters*.

| Characteristics | No. | U value or H value | *p* value |
|---|---|---|---|
| *Menopause* | | | |
| Premenopause | 77 | 4496.00 | < 0.05 |
| Postmenopause | 70 | | |
| *FIGO stage* | | | |
| I + II | 55 | 5192.52 | < 0.05 |
| III + IV | 92 | | |
| *Histological grade* | | | |
| High | 47 | 6137.31 | < 0.05 |
| Median and low | 100 | | |
| *Lymph node transmission* | | | |
| Positive | 76 | 1640.00 | n.s. |
| Negative | 71 | | |
| *Histological type* | | | |
| SCAC | 101 | 0.869 | n.s. |
| MAC | 20 | | |
| Others* | 26 | | |
| *Chemotherapy* | | | |
| Received | 121 | 1602.50 | n.s. |
| Did not receive | 26 | | |
| *COX-2 status* | | | |
| Positive | 109 | 6929.50 | < 0.05 |
| Negative | 38 | | |
| *VEGF status* | | | |
| Positive | 91 | 5571.00 | < 0.05 |
| Negative | 56 | | |

SCAC: serous cystic adenocarcinoma; MAC: mucinous adenocarcinoma.

Table 4. — *Multivariate analysis*.

| | β | SE | Exp (β) | 95% CI for Exp (β) Lower | Upper | *p* |
|---|---|---|---|---|---|---|
| COX-2 positive | 1.038 | 0.321 | 2.825 | 1.506 | 5.299 | 0.001 |
| FIGO stage | 0.830 | 0.283 | 2.292 | 1.316 | 3.992 | 0.003 |
| VEGF positive | 0.718 | 0.242 | 2.051 | 1.277 | 3.295 | 0.003 |
| Histological grade | 0.628 | 0.288 | 1.873 | 1.066 | 3.291 | 0.029 |

SE: standard error.

tivity was not distributed differently according to menopause, FIGO stage, histological grade, or histological type.

Of 183 EOC cases both COX-2 and VEGF were expressed in 95 cases (51.9%). COX-2 positive and VEGF negative occurred in 49 cases (26.78%). COX-2 negative and VEGF positive occurred in 21 cases (11.48%). COX-2 and VEGF were not expressed in 18 cases (9.84%).

*Survival analysis*

Follow-up data were available for 147 patients, 64 of whom were alive for more than three years and 43 of whom were alive for more than five years. The 3-year and 5-year survival rate was 45.71% and 30.71%, respectively.

In 147 follow-up cases, patients with tumors negative for COX-2 had an increased median survival time (47.52 months, n = 38) compared to patients with tumors positive for COX-2 (24.56 months, n = 109). The comparison of survival was significantly differently. Figures 3 and 4 show the 3-year and 5-year survival curves according to COX-2 status in EOC cases. The 3-year cumulative survival rate was 37.31% for patients with tumors positive for COX-2 and 77.32% for negative tumors. The 5-year cumulative survival rate was 19.11% for patients with tumors positive for COX-2 and 69.11% for negative tumors. The 3-year and 5-year survival rate for patients with negative tumors was higher than for patients with positive tumors.

In 147 follow-up cases, patients with tumors negative for VEGF had an increased median survival time (42.09 months, n = 56) compared to patients with tumors positive for VEGF (23.36 months, n = 91). The comparison survival was significantly different. Figures 5 and 6 show the 3-year and 5-year survival curves according to VEGF status in EOC cases. The 3-year cumulative survival rate was 31.35% for patients with tumors positive for VEGF and 68.92% for negative tumors. The 5-year cumulative survival rate was 18.75% for patients with tumors positive for VEGF and 59.75% for negative tumors. The 3-year and 5-year survival rate for patients with negative tumors was higher than for patients with positive tumors.

*Univariate analysis*

We compared the survival among all patients with EOC by univariate analysis according to the six clinicopathological parameters (menopause, FIGO stage, histological grade, lymph transmission, histological type, and chemotherapy) and two biochemical parameters (COX-2, VEGF). Significant prognostic markers in univariate analysis were menopause, FIGO stage, histological grade, histological grade, COX-2 positive and VEGF positive. Lymph node transmission, histological type, and chemotherapy were not significant (Table 3).

Three clinicopathological factors were selected: menopause, FIGO stage, and histological grade. Figure 7 shows the 5-year Kaplan-Meier curves for eachfactor.

Figure 7A shows the different survival curves for patients in premenopause (median survival, 34.9 months) and postmenopause (median survival, 25.7 months). Postmenopausal patients had a 5-year survival rate of 67.61%, whereas premenopausal patients had a 5-year survival rate of 27.61%.

Figure 7B shows the different survival curves for patients with earlier FIGO stage (median survival, 47.18 months) and later stage (median survival, 20.52 months). Early-stage patients had a 5-year survival rate of 67.71%, whereas premenopausal patients had a 5-year survival rate of 18.61%.

Figure 7C shows the different survival curves for patients with high diffusion (median survival, 46.36 months) and median and low cases (median survival, 23.04 months). High diffusion patients had a 5-year survival rate of 67.21%, whereas the median and low diffusion patients had a 5-year survival rate of 38.18%.

*Multivariate analysis*

We used a multivariate regression analysis based on Cox's proportional hazard regression model to test the independent value of each parameter selected by univariate analysis. The variables used in Cox's model are shown in Table 4. Expression of COX-2 was an independent

Fig. 1

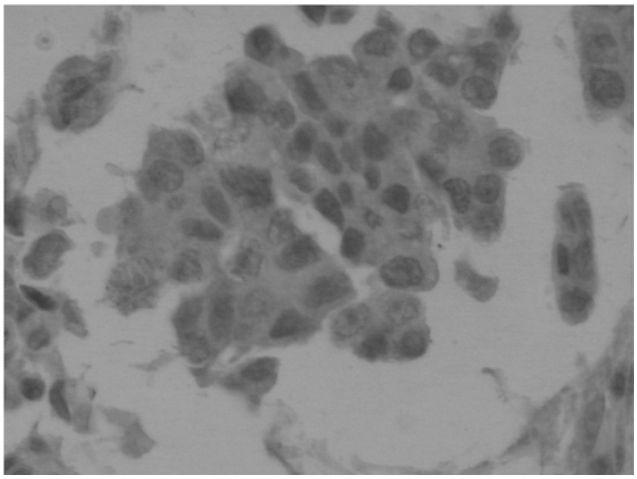

Fig. 2

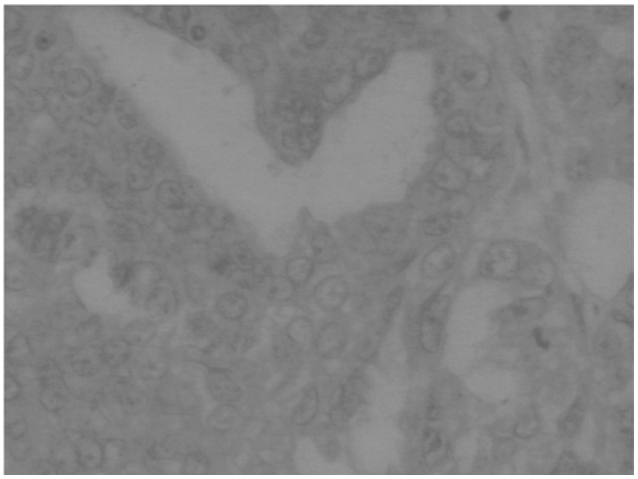

Figure 1. — Expression of COX-2 in ovarian epithelial cancer investigated by immunohistochemistry (SABC x 400).
Figure 2. — Expression of VEGF in ovarian epithelial cancer investigated by immunohistochemistry (SABC x 400).

Fig. 3

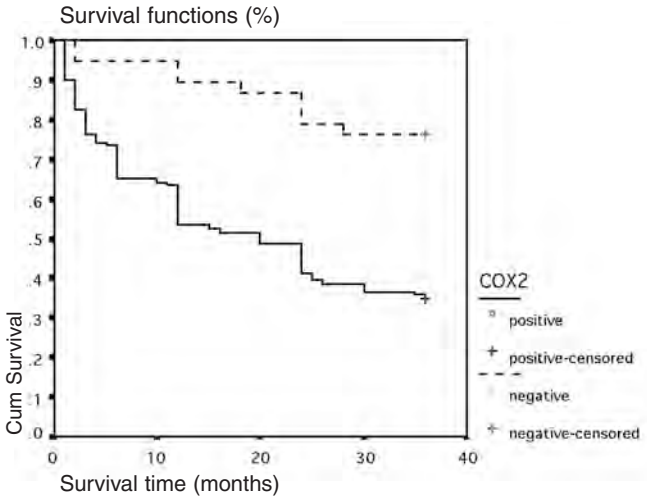


Fig. 4

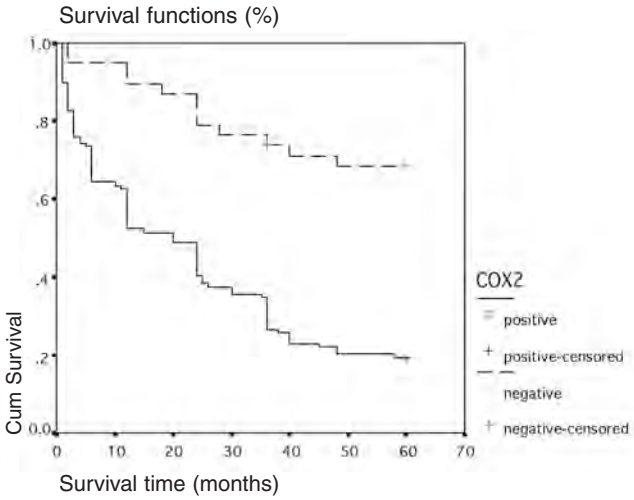


Fig. 5

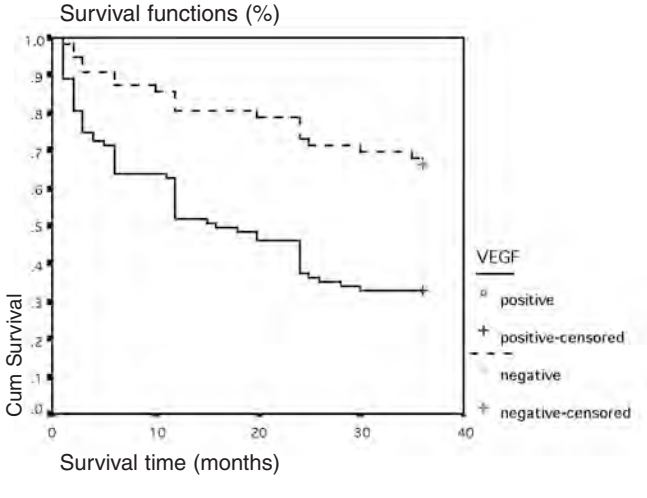


Fig. 6

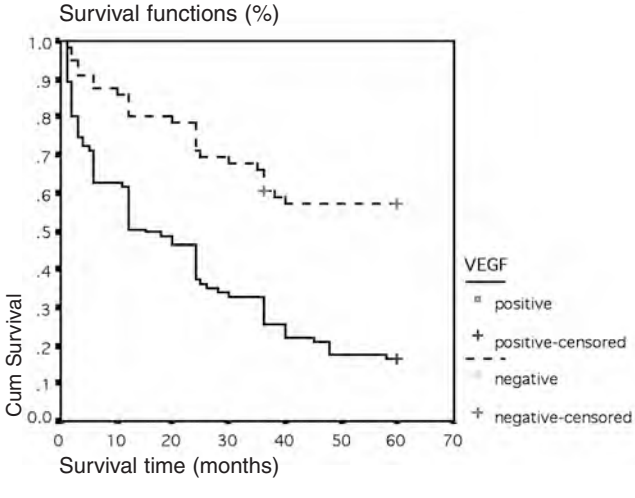


Figure 3. — Three-year Kaplan-Meier curves to COX-2 status in ovarian epithelial cancer.
Figure 4. — Five-year Kaplan-Meier curves to COX-2 status in ovarian epithelial cancer.
Figure 5. — Three-year Kaplan-Meier curves to VEGF status in ovarian epithelial cancer.
Figure 6. — Five-year Kaplan-Meier curves to VEGF status in ovarian epithelial cancer.

Fig. 7A

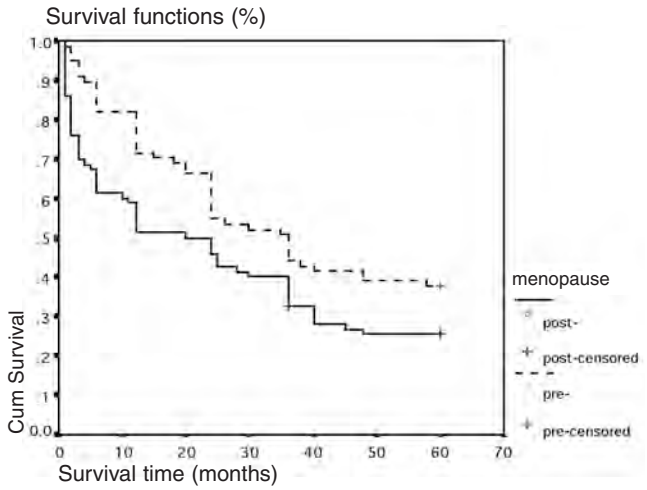


Fig. 7B

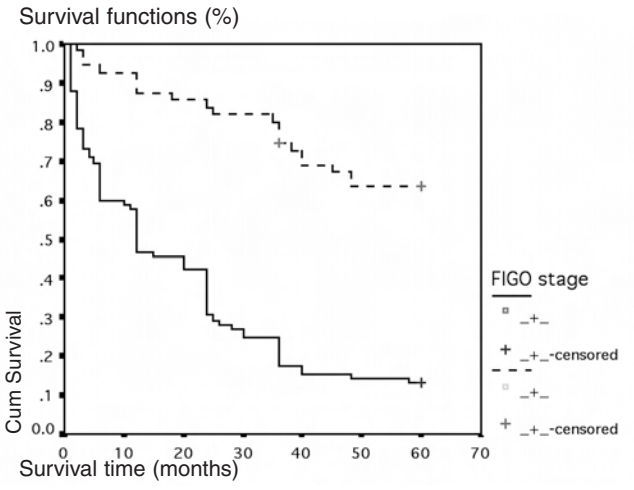


Fig. 7C

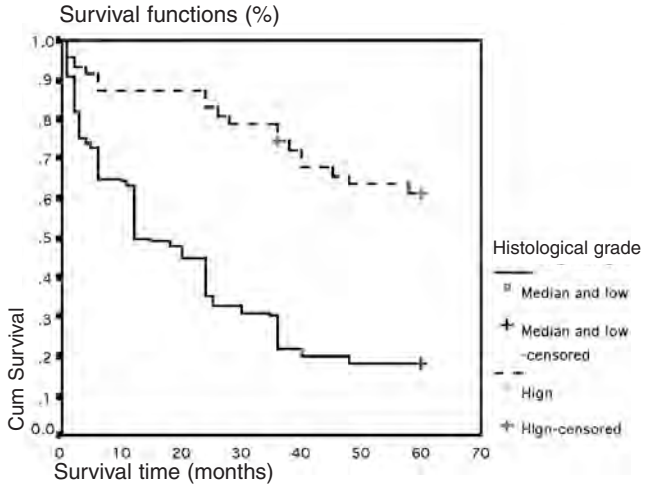


Figure 7. — Menopause (A), FIGO stage (B), histological grade (C) 5-year survival.

prognostic factor for poor survival (relative risk (RR) 2.825: 95% CI 1.506 to 5.299). Other independent prognostic factors associated with poor prognosis were FIGO stage (RR, 2.292), expression of VEGF (RR, 2.051), histological grade (RR, 1.873).

## Discussion

*COX-2/VEGF expression in EOC*

COX-2 is the rate-limiting enzyme in prostanoid biosynthesis and is involved in tumor progression. Several functions of inducible COX-2 have been described in the biology of various carcinomas: increased cell proliferation [9], inhibition of apopotosis, stimulation of angiogenesis, as well as inhibition of immunosurveilance [10]. VEGF can stimulate normal epithialial cell increases as well as promote some tumor cell growth.

Numerous studies show COX-2 is rapidly inducible when cells are stimulated and plays a role in pathology, physiology, and procession, including inflammatory processes as carcinogenesis [11]. COX-2 and VEGF overexpression have been described in various malignancies. Trifan and Hla showed COX-2 plays a role in carcinogenesis [12]. Gupta *et al*. showed COX-2 overexpression in prostate adenocarcinoma [13].

In our study COX-2 and VEGF both overexpressed in EOC, 76.5% and 63.93, respectively. We failed to demonstrate an association between COX-2 status and any of the clinicopathological characteristics (menopause, FIGO stage, histological grade, lymph node transmission, and histological type). VEGF status did have an association with lymph node transmission, but not with menopause, FIGO stage, histological grade, and histological type. Lee *et al*. had results similar to ours [14]. VEGF status is associated with lymph nodes and can be a helpful marker in determining lymph node transmission.

Recent experimental evidence indicates that carcinogenesis is a multi-factoral and multi-stepped procedure [15, 16]. More than two proteins were involved and different proteins play different roles in different stages, In our studies COX-2 and VEGF both expressed cases is 95 (51.90%) and neither is 18 (9.84%). There are some studies that show COX-2 and its product, prostaglandin $E_2$ ($PGE_2$), promote carcinogenesis together. COX-2 was found to be up-regulated VEGF expression to promote the vessel [17].

*Association between COX-2 and VEGF protein expression in EOC and prognosis*

In our study, patients with tumors negative for COX-2 had an increased median survival time compared to patients with tumors positive for COX-2 and increased cumulative survival time compared to patients with tumors positive for COX-2. The cumulative survival rate for COX-2 negative expression was higher than COX-2 positive expression showing that the expression of COX-2 protein is associated with prognosis in EOC patients. It could be a good parameter to determine the prognosis of ovarian cancer [18]. As is now known, COX-2 and $PGE_2$ can promote increased cells to inhibit apoptosis, enhance tumor transformation, and enhance tumor invasion, all of which can affect carcinoma prognosis.

VEGF can regulate vascular permeability and is an important mediator of vasculogenesis and angiogenesis. Our study shows the median survival time for VEGF negative-expression was longer than VEGF positive expression. The accumulation of negative VEGF was higher than that of positive VEGF showing VEGF can be a parameter for ovarian carcinoma [19].

*Factors affecting surgical prognosis of EOC and future application*

The research shows that the rate of three-year survival was 45.71% and the rate of five-year survival was 30.71% in the 147 cases with precise follow-up records. According to the latest reports about EOC we found that the rate of three-year survival is from 35.74% to 49.06% and the rate of five-year survival is from 25% to 40% [20, 21]. From the data we can see that although both basic research and clinical diagnosis have been improved in recent years, the prognosis shows little change, and the survival rate remains the same.

Cox's proportional hazard regression model is a mathematical model in survival analysis which was put forward by British biological statistician D.R. COX [22]. This model is a perfect solution to the three main problems that once existed in survival analysis, and the analysis has had breakthrough progress to be a more comprehensive system. Now the COX proportional hazard regression model has become one of the most important mathematical models in survival analysis and has been applied worldwide as a multivariate analysis method [23].

Univariate analysis showed that the surgical prognosis of epithelial ovarian cancer is influenced by many factors, including menopause, FIGO stage, histological grade, COX-2 protein positive expression, and VEGF protein positive expression ($p < 0.05$). In order to remove the mixed or overlapping factors in this study, Cox's proportional hazard regression model was used to give further multivariate analyses to the factors above. The results indicated that COX-2 protein positive expression, FIGO stage, VEGF protein positive expression, and histological grade are the four most significant factors which affect the surgical prognosis of EOC. The menopause factor in multivariate analysis was removed because its role does not appear significant when many factors have mutual influence on the surgical prognosis of EOC.

COX-2 protein positive expression is the most important factor. According to the data we can see that the COX-2 protein positive expression death risk is 2.83 times as COX-2 protein negative expression death risk. A study by Fujimoto *et al*. [18] found that patients with epithelial ovarian cancer who had positive COX-2 protein expression have poor prognosis, in line with our report. The reason may be that the COX-2 protein in tumor tissues has high proliferative activity and poor biological action.

FIGO stage has always been considered an important factor affecting the surgical prognosis of ovarian cancer. Indeed, Cox's model analysis proved pathologic staging to be the second most important factor. Survival time between early stage (phase I + phase II) and advanced stage (phase III + IV) revealed significant differences ($p < 0.05$). The latter death risk is 2.29 times higher as for early stage. Research shows that the sooner EOC is pathologically staged, the better the prognosis. Ovarian cancer patients in phase I have a 5-year survival rate of about 87%, while phase III-IV patients have a 5-year survival rate of only 5-10% [24], showing that early diagnosis and treatment can improve the prognosis.

VEGF protein positive expression has been proved to be the third most important factor affecting the surgical prognosis of EOC. VEGF positive protein expression and negative expression have significant differences in the median survival period ($p < 0.05$). The former has a risk which is 2.051 times higher than for negative expression. It could be that the poor prognosis of VEGF protein positive expression is because VEGF stimulates peripheral blood vessels and growth of lymphatic endothelial cells, which plays an important role in cancer growth and metastasis. The investigation on 83 cases of Phase III ovarian cancer patients launched by Raspollini *et al*. [25] found that microvessel density and VEGF are directly related to survival rate, also confirmed in this study.

According to Cox's model analysis, the importance of the histological grading factor is in fourth place. Highly differentiated EOC shows great differences in the median survival period of the medium and low differentiated period with the latter risk of death 1.87 times higher than the former. EOC histological grading has always been considered to be related to prognosis. Scorilas *et al.'s* [26] study confirmed that the five-year survival rate of a highly differentiated patient group was higher than the rate of the medium and low differentiated group. The results of this study are also consistent with our results in that the prognosis of medium and low differentiated EOC cases is poor.

The results mentioned above are instructive to clinical practice. The four factors (COX-2 protein positive expression, FIGO stage, VEGF protein positive expression, and histological grade), which have been confirmed to be the most influential on the surgical prognosis of EOC by COX multivariate analysis, are all related – with inherent biological characteristics and action of the tumor itself. Thus, in order to improve the surgical prognosis of EOC, it is necessary to adopt comprehensive treatment measures which use surgery as the main stay method to deal

with EOC. In accordance with the research, we suggest that COX-2 protein positive expression is the most important factor to prognosis. Our study used immunohistochemical methods which can detect COX-2 protein expression. This method is very easy to adopt and will hopefully be applied in routine pathologic examination. Other researches have also confirmed that COX-2 selective inhibitors, e.g. NS398, have a proliferative inhibition function on human ovarian cancer cells and can induce apoptosis in ovarian cancer cells; this suggests COX-2 is likely to be an effective chemical control target in ovarian cancer and NS398 is expected to become an effective chemoprophylactic drug in ovarian cancer though further development in detailed treatment methods and measures are necessary. Furthermore, although there are many clinical pathologic factors which affect the surgical prognosis of EOC, pathologic staging is the most obvious and important. Thus, emphasis on the importance of early diagnosis and early treatment of EOC can improve the prognosis. Finally, what needs to be pointed out is that there are many factors which influence the surgical prognosis of EOC besides the ones which have been analyzed in this report, and further researches on their function and significance is needed.

## References


[1] Dannenberg A.J., Altorki N.K., Boyle J.O., Dang C., Howe L.R., Weksler B.B., Subbaramaiah K.: "Cyclo-oxygase 2: a pharmacological target for the prevention of cancer". *Lancet Oncol*., 2001, *2*, 544.

[2] Saklesa E.: "Prognostic markers in epithelial ovarian cancer". *Int. J. Gynecol. Pathol*., 1993, *12,* 156.

[3] Bauknecht T., Runge M., Schwall M., Pfleiderer A.: "Occurrence of epidermal growth factor receptors in human adnexal tumors and their prognostic value in advanced ovarian carcinomas". *Gynecol. Oncol*., 1988, *2,* 147.

[4] Rajkumar T., Gooden C.S.R., Lemoine N.R., Gullick W.J., Goden C.S.: "Expression of the c-erbB-3 protein in gastrointestinal tract tumours determined by monoclonal antibody RT". *J. Pathol*., 1993, *170,* 271.

[5] Zheng H.: "The biochemical prognosis factors of epithelial ovarian cancer". *Obstet. Gynecol*., 2004 *31*, 37.

[6] Soslow R.A., Dannenberg A.J., Rush D. *et al*.: "COX-2 is expressed in human pulmonary, colonic, and mammary tumors". *Cancer,* 2000, *89,* 2637.

[7] Davies G., Salter J., Hills M., Martin L.A., Sacks N., Dowsett M.: "Correlation between cyclooxygenase-2 expression and angiogenes is in human breast cancer". *Clin. Cancer Res*., 2003, *9,* 2651.

[8] Zhi Y.H., Liu R.S., Song M.M., Tian Y., Long J., Tu W., Guo R.X.: "Cyclo-oxygenase-2 promotes angiogenesis by increasing vascular endothelial growth factor and predicts prognosis in gallbladder carcinoma". *World J. Gastroenterol*., 2005, *11*, 3724.

[9] Sheng H., Shao J., Morrow J.D., Beauchamp R.D., DuBios R.N.: "Modulation of apoptosis and bcl-2 expression by prostaglandin E2 in human colon cancer cells". *Cancer Res*., 1998*, 58,* 362.

[10] Huang M., Stolina M., Sharma S., Mao J.T., Zhu L., Miller P.W. *et al*.: "Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: upregulation of interleukin 10 and down-regulation of interleukin 12 production". *Cancer Res*., 1998, *58,* 1208.

[11] Inoue H., Yokoyaya C., Hara S., Tone Y., Tanabe T.: "Transcriptional regulation of human prostaglandin-endoperoxide synthase-2 gene by lipopoly-saccharide and phordol ester in vascular enlothelial cells". *J. Biol. Chem*., 1995*, 270*, 24965.

[12] Trifan O.C., Hla T.: "Cyclo-oxygenase-2 modulates cellular growth and promotes tumorigenesis". *J. Cell. Mol. Med*., 2003, *7,* 207.

[13] Gupta S., Srivastava M., Ahmad N., Bostwick D.G., Mukhtar H.: "Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma". *Prostate,* 2000, *42,* 73.

[14] Lee J.S., Choi Y.D., Lee J.H., Nam J.H., Choi C., Lee M.C. *et al*.: "Expression of cyclooxygenase-2 in epithelial ovarian tumors and its relation to vascular endothelial growth factor and p53 expression". *Int. J. Gynecol. Cancer,* 2006, *16* (suppl. 1), 247.

[15] Land H., Parada L.F., Weinberg R.A.: "Tumorigenic conversion of primary embryo fibroblasts reguires at least two cooperating oncogenes". *Nature,* 1983, *304,* 596.

[16] Slamon D.J., Dekernion J.B., Verma I.M., Cline M.J.: "Expression of cellular oncogenes in human malignancies". *Science*, 1984, *224,* 256.

[17] Siironen P., Ristimaki A., Narko K., Nordling S., Louhimo J., Andersson S. *et al*.: "VEGF-C and COX-2 expression in papillary thyroid cancer". *Endocr. Relat. Cancer,* 2006, *13*, 465.

[18] Fujimoto J., Toyoki H., Sakaguchi H., Jahan I., Alam S.M., Tamaya T.: "Clinical implications of expression of cyclo-oxygenase-2 related to angiogenesis in ovarian cancer". *Oncol. Rep*., 2006, *15*, 21.

[19] Nishida N., Yano H., Komai K., Nishida T., Kamura T., Kojiro M.: "Vascular endothelial growth factor C and vascular endothelial growth factor receptor 2 are related closely to the prognosis of patients with ovarian carcinoma". *Cancer,* 2004, *101,* 1364.

[20] Tang J., Cai S.M., Zhang R.Y., Wang H.Y.: "The significance of abdominal residue after primary cytoreductive surgery for Stage III ovarian cancer". *China Oncol*., 2004, *14*, 31.

[21] Zhang X.Y.: "Practical Obstetrics & Gynecology". Edition 2, Beijing, People's Medical Publishing House, 2004,·686.

[22] Cox D.R.: "Regression models and life table". *JR Statist. Soc. Ser B*., 1972, *34,* 187.

[23] Luo F.T., Hu M.X.: "The role of Cox proportional in the analysis of cancer prognosis factors". *Cancer,* 1991, *10,* 84.

[24] Gao X.Z., Xu S.X.: "Current cancer diagnosis and treatment". Beijing Scientific and Technological Literature Publishing House, 1997, 323.

[25] Raspollini M.R., Amunni G., Villanucci A., Baroni G., Boddi V., Taddei G.L.: "Prognostic significance of microvessel density and vascular endothelial growth factor expression in advanced ovarian serous carcinoma". *Int. J. Gynecol. Cancer,* 2004, *14,* 815.

[26] Scorilas A., Borgono C.A., Harbeck N., Dom J., Schmaifedt B., Schmitt M., Diamandis E.P.: "Human kallikrein 13 protein in ovarian cancer cytosols: a newfavorable prognostic marker". *J. Clin. Oncol*., 2004, *22*, 678.



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