Increased ERCC1 Protein Expression Is Associated with Suboptimal Debulking in Advanced Epithelial Ovarian Cancer

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Abstract. *Some biological features, such as amount of ascites and molecular tissue markers, have been found to correlate with debulking outcome in advanced epithelial ovarian cancer (EOC). This study investigated whether proteins involved in nucleotide excision repair of EOC affected the debulking outcome. Patients and Methods: The relationship between dichotomised clinical characteristics, ERCC1 and XPD protein expression levels in 78 patients were tested by univariate and multivariate analysis to determine the independent significance of factors for debulking outcome. Receiver operating characteristic (ROC) curves were constructed to determine their predictive value. Results: Pre- and postoperative CA125, ascites, menopause, and ERCC1 protein all significantly correlated with debulking outcome. However, only ERCC1 was the only independent factor, with the area under the ROC curve being 0.724. Conclusion: ERCC1 protein is an independent prognostic indicator for debulking outcome in advanced EOC.*

Epithelial ovarian cancer (EOC) is the leading cause of death from gynaecological malignancies and is most frequently diagnosed at an advanced stage (1). Currently, women with a diagnosis of EOC only have a 5-year survival rate of 29% (2). Staging laparotomy with cytoreduction followed by platinum-based chemotherapy is currently the standard treatment for women with previously untreated, advanced-stage EOC (1). Survival of patients with optimal tumour cytoreduction is significantly higher than that of patients with larger residual lesions. Furthermore, platinum-based regimens have produced higher overall response rates and an increase in median survival (1). However, despite improved methods of surgery and chemotherapy, the mortality rate in women with this type of cancer has remained largely unchanged for the last 5 decades (3). This result has led to the hypothesis that it is self-biological characteristics of ovarian cancer, rather than surgery methods and chemotherapy regimens, that have the most important effect on debulking outcome, response to chemotherapy and overall survival (4, 5).

Previously published studies (5-8) have found that serum CA125, amount of ascites and molecular tissue markers can predict surgical outcome. These parameters may be related to biological characteristics of ovarian cancer. Nucleotide excision repair (NER) is involved in the DNA repair process and is able to correct the majority of bulky lesions in DNA, including bulky chemical adducts (9). Of the proteins in NER pathway, xeroderma pigmentosum D (XPD) (10) and excision repair cross-complementation group 1 (ERCC1) (11) are involved in DNA damage helicase activities and incision of the DNA strand at sites flanking the DNA damage, respectively. A recent study has linked the regulation of ERCC1 and XPD protein expression to the observed differences in clinical behaviours of advanced EOC (12). The objective of this study was to explore the associations between ERCC1, XPD protein expression, clinical characteristics in patients with advanced EOC and residual disease categories after primary cytoreduction, and to assess the effect of tumour NER on the debulking outcome.

Patients and Methods

Patients. After obtaining Institutional Review Board approval, all patients who underwent primary cytoreductive surgery between January 1999 and October 2004, at the Women's Hospital, Zhejiang University School of Medicine, China were identified. The records of seventy-eight consecutive patients with FIGO stage III-IV were reviewed, including sixty-three cases with serous ovarian cancer and fifteen with clear cell cancer. All patients had undergone primary cytoreductive

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therapy with visible residual disease up to 6 cm, and the group consisted of 11 cases with no visible disease, 23 with ≤1 cm, 44 with >1 cm, at the Department of Gynaecological Oncology. Residual disease of more than 1 cm was defined as suboptimal debulking (44 cases), and residual disease less than 1 cm (34 cases) as optimal debulking. Exclusion criteria included prior surgical exploration for cytoreduction at another institution, histology consistent with carcinosarcoma, non-epithelial malignancies, or borderline tumours. Patients who had received neoadjuvant chemotherapy were also excluded. Informed consent was obtained from all patients prior to initiating treatment. Individual records were reviewed and the following preoperative information collected: gravidity, parity, smoking, contraception used, body mass index (BMI), age at surgery, date of surgery, and serum level of CA125. Intraoperative information recorded included presence of ascites, the largest diameters of tumours and their residuals. Information obtained from the final pathology report included stage, histology, and tumour grade. All patients were staged according to the FIGO system (1). Postoperative information collected included the available CA125 levels.

Immunohistochemistry. Ovarian cancer tissue samples from the primary tumour were obtained during surgery, collected prospectively by the pathologist. Hematoxylin- and eosin-stained sections of all cases were reviewed by a single pathologist who confirmed the presence and histological subtype of the tumour. In all cases, there was complete agreement between the reviewing pathologist's and the referring pathologist's diagnoses. The ovarian cancer specimens were made into formalin-fixed, paraffin-embedded tissue blocks and cut in approximately 6 μm sections, fixed onto glass slides, and shipped to the Authors' laboratory for further processing. An anti-ERCC1 monoclonal antibody was produced using BALB/C mice injected with full length recombinant human ERCC1 protein, which was obtained from DBS Biotechnology (Pleasanton, CA, USA). This antibody is supplied as a purified immunoglobulin fraction containing sodium azide as preservative. XPD is a rabbit polyclonal antibody raised against a recombinant protein corresponding to amino acids 611-760 mapping at the carboxy terminus of TFIIH p80 of human origin, was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Tissues determined previously to express ERCC1 and XPD were used as positive controls. The ERCC1 and XPD proteins were also expressed in the nucleus of the tumour cells. Tumour cells were defined as having negative expression when there was no detectable nuclear staining. Slides were immunostained and analysed using a blinded coding system. Staining procedures and microscopic assessments were performed without knowledge of the histopathological diagnosis and response to primary chemotherapy. Immunohistochemical investigations of ERCC1 and XPD protein expression were performed on paraffin-embedded tissue sections (13). Six-micrometer sections were dewaxed and rehydrated using xylene and alcohol. Endogenous peroxidase was blocked by dipping the sections in 3% aqueous H_2O_2 for 10 min and antigen retrieval was performed by boiling for 2 min at a temperature of 110˚C in 10 mM citrate buffer, pH 6.00. Following antigen retrieval, sections were incubated individually overnight at 4˚C with a mouse monoclonal antibody to the ERCC1 protein (1:50 dilution) or a rabbit polyclonal antibody to the XPD protein (1:50 dilution), and lightly counterstained with haematoxylin. Immunostaining was performed using the avidin-biotin peroxidase complex technique, using diaminobenzidine as a chromogen (14). The distribution of specific staining was evaluated according to an optical density scale using the values of all the positive nuclei of a given receptor. Any appreciable brown staining was considered positive and graded as follows: 0, negative; 1, barely detectable staining; 2, easily seen fine granules, present diffusely throughout the nucleus; or 3, staining so strong that nuclear nuclear detail was obscured. The quantitative H score (QH score) was adopted to calculate ERCC1 and XPD protein expression levels as follows: QH score=$\sum$ P (i+1), where i is the optical density graded as above and P is the percentage of stained cells for each given i (from 0% to 100%) (15).

Table I. *Baseline characteristics of the study population*.

Parameters	Residual tumour diameter		*p*-Value
	≤1 cm (34 cases)	>1 cm (44 cases)	
Age (years)	50.1±9.6	49.3±5.6	0.673
Gravidity	2.18±1.03	2.64±1.35	0.103
Parity	1.38±0.78	1.61±0.81	0.209
Smoking (%)	2(5.9)	4(9.1)	0.691
Contraception used			
None (%)	6(17.6)	4(9.1)	0.421
Condom (%)	9(26.5)	9(20.5)	
Oral pills (%)	4(11.8)	6(13.6)	
IUD (%)	10(29.4)	11(25.0)	
Other (%)	5(14.7)	14(31.8)	

IUD, Intrauterine device. Patients with residual tumours ≤1cm were, while patients with residual tumours >1 cm were considered to be suboptimally debulked.

Statistical analysis. The SPSS16.0 system (SPSS, Inc, Chicago, IL, USA) was used for all statistical analysis. Statistical analysis was carried out using the independent samples *t*-test for age, BMI, the largest diameter of tumour, ERCC1 and XPD proteins. The Mann-Whitney test was used to analyse gravidity, parity, serum CA125 levels and ascites. Their values are expressed as mean±standard deviation. The Pearson Chi-square or Fisher's exact test was used to analyse other parameters. Variables that were significant in univariate analysis at a level of $p<0.1$ were included in the multivariate model (logistic regression analysis). Variables were retained in the model if they remained significant at $p<0.05$. Receiver operating characteristic (ROC) curves were constructed to determine the optimal values of ERCC1, which plots the sensitivity on the y-axis and the false-positive rate (defined as 1-specificity) along the x-axis. A probability value of $p<0.05$ was considered to be significant.

Results

From the study database, 78 patients were identified with stage III-IV EOC who underwent primary cytoreductive surgery. Patient baseline characteristics compared between those with optimal and suboptimal debulking are given in Table I. There were no significant differences between the two groups.

Associated risk factors based on suboptimal debulking status are presented in Table II. Thirty-one (66%) menopausal patients underwent suboptimal debulking, while 13 (41.9%) of the non-

Table II. *Relationship of suboptimal debulking (residual >1 cm) of advanced EOC to associated risk factors.*

Parameters	Residual disease		Univariate		Multivariate†	
	≤1 cm (34 cases)	>1 cm (44 cases)	*p*-Value	OR	95% CI	*p*-Value
Preoperative CA125(kU/l)#	906±1416	2813±3244	0.000*	2.381	0.395-14.349	0.344
Postoperative CA125 (kU/l)#	329±612	1405±1510	0.000*	2.491	0.410-15.119	0.321
Ascites (l)	0.74±1.24	2.25±1.59	0.000*	1.581	0.948-2.638	0.079
BMI	22.1±3.3	23.8±4.1	0.053	1.144	0.950-1.379	0.157
Diameter of tumour (cm)	9.5±3.1	10.0±4.1	0.591			
ERCC1	2.03±0.57	2.48±0.46	0.000*	8.199	2.076-32.382	0.003*
XPD	2.22±0.64	2.43±0.61	0.136			
Histology						
Serous (%)	26 (41.3)	37 (58.7)	0.397			
Clear cell (%)	8 (53.3)	7 (46.7)				
Grade						
I-II(%)	19 (48.7)	20 (51.3)	0.361			
III (%)	15 (38.5)	24 (61.5)				
FIGO stage						
III (%)	34 (45.3)	41 (54.7)	0.253			
IV (%)	0 (0)	3 (100)				
Menopausal						
Yes (%)	16 (34.0)	31 (66.0)	0.036*	1.000		
No (%)	18 (58.1)	13 (41.9)		0.267	0.068-1.048	0.058

OR, odds ratio; CI, confidence interval. #logged values were used to analyse the distribution of CA125 levels in the logistic regression. †Logistic regression analysis was used for multivariate analysis; *denotes statistically significant difference.

menopausal patients did (p=0.036). Preoperative CA125 serum value ranged 11-13420 kU/l and postoperative CA125 was distributed from 10 to 5560 kU/l. Patients that were cytoreduced to small residual tumours (≤1cm) had statistically significant (p=0.000) lower preoperative CA125 serum values (906±1416 kU/l) than those with larger residual tumours (2813±3244 kU/l), as did the postoperative CA125 (329±612 *vs*. 1405±1510 kU/l, p=0.000). Ascites was present in 85.9% of the cases and ranged from 0-6 l. In the entire series of 78 patients, the optimal debulking rate for those with and without ascites was 37.3% and 81.8%, respectively (p<0.0001). The patients with optimal debulking had less ascites than those of suboptimal debulking (0.74±1.24 *vs*. 2.25±1.59l, p=0.000). ERCC1 (Figure 1A) and XPD protein expression levels in the patients who underwent optimal debulking were lower than those of suboptimal debulking (Figure 1B) in advanced EOC. In the analysis, patients who achieved an optimal debulking had a mean of 2.03 of ERCC1 protein expression and 2.22 of XPD protein expression, whereas those patients with suboptimal debulking had a mean of 2.48 of ERCC1 protein expression and 2.43 of XPD protein expression. A significant difference was found in ERCC1 expression (p=0.000) but not in XPD expression (p=0.136) between two groups. In addition, BMI in the suboptimal group was slightly higher than that of the optimal group (p=0.053). There were no significant differences between the two groups in terms of the largest diameter of tumour mass, histological type, histological grade and FIGO stage.

On multivariate analysis, comparing preoperative and postoperative CA125 (logged transformed values), ascites, menopause, BMI, and ERCC1 protein expression, only ERCC1 protein expression remained an independent variable affecting debulking outcome (p=0.003) (Table II).

The best predictor of cytoreductive outcome was the ERCC1 protein expression. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of performing suboptimal debulking at various ERCC1 threshold levels are shown in Table III. Figure 2 shows the ROC generated by the data; the area under the curve was 0.724 (95% confidence interval 0.611-0.838, p=0.001) representing a significant difference from the hypothetical 45˚ 'fifty-fifty' line. The point on the curve closest to the upper left corner corresponds to a threshold level of 2.30. This value gives an approximation of the proportion of the patients who underwent suboptimal debulking. In patients with a value less than or equal to 2.30, suboptimal debulking was resultant in 39.5% of the patients *vs*. 72.5% if the value was above 2.30 (p=0.006).

Discussion

For patients with advanced EOC, the current standard treatment consists of maximum cytoreductive surgery to reduce residual tumour to a minimum, followed by platinum-based chemotherapy. The size of residual disease

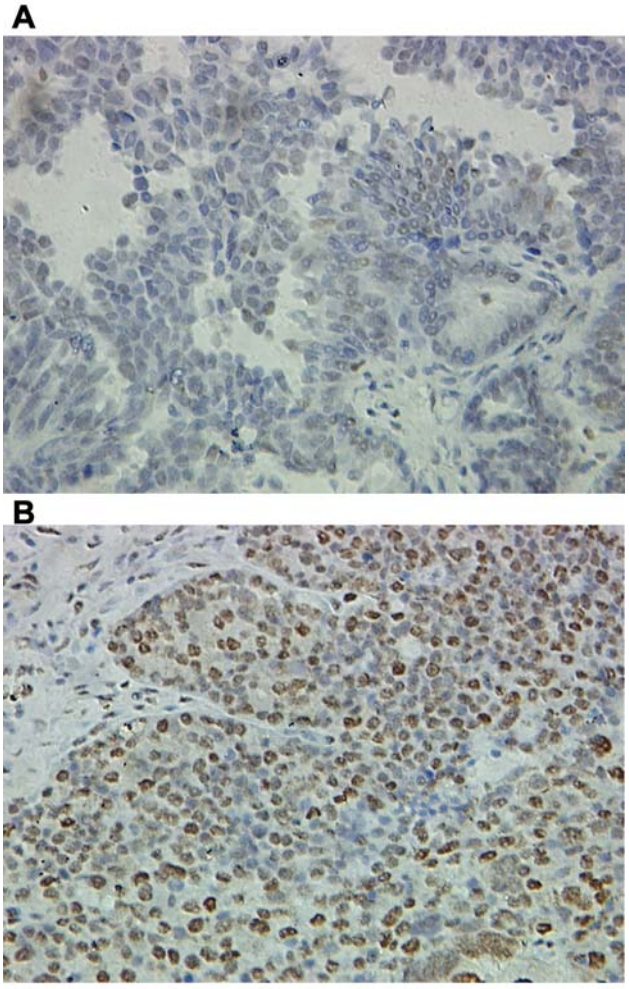

Figure 1. *The expression of ERCC1 protein in the patient with optimal debulking (A) versus suboptimal debulking (B) (original magnification ×400).*

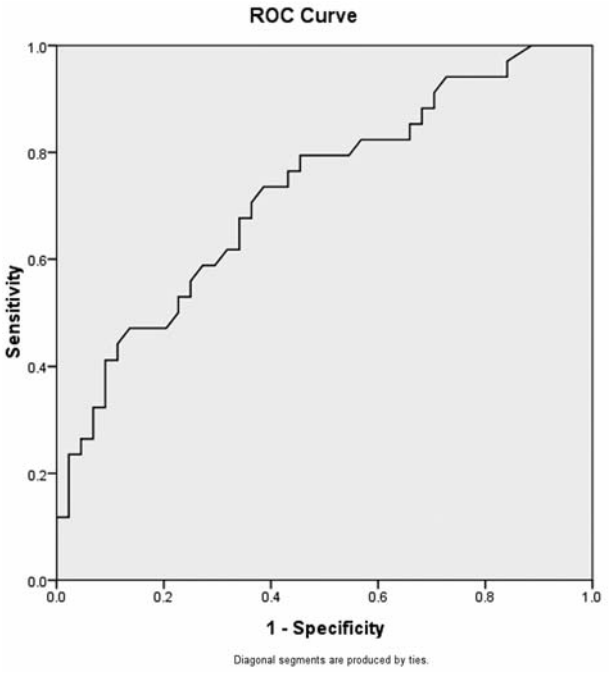

Figure 2. *Receiver operating characteristics (ROC) curve of correlation between sensitivity and false-positive (1–specificity) rate for suboptimal debulking using each ERCC1 level as a cut-off point.*

Table III. *Prediction of suboptimal debulking at various ERCC1 levels.*

ERCC1 cut-off level	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
1.49	98	21	61	87
1.74	93	27	62	75
1.82	91	32	64	73
1.96	89	41	66	74
2.02	84	47	67	70
2.08	77	50	67	63
2.17	73	59	70	63
2.30	66	68	73	61
2.40	57	74	74	57
2.49	55	79	77	57
2.63	43	82	76	53
2.78	32	88	78	50
2.86	25	94	85	49

after surgery is one of the most important prognostic factors for survival and the only prognostic factor can be influenced by the physician (16, 17). However, due to the initial extent of the disease, optimal debulking may often be difficult to achieve. It is a remarkable fact that suboptimal debulking in advanced EOC tends to be associated with adverse biological characteristics. On univariate analysis, data in this study showed that the patients with suboptimal debulking had a higher level of serum CA125, ERCC1 protein and larger volume of ascites than those with optimal debulking. Increased levels of these variables are often associated with widespread metastasis (18), enhanced DNA damage repair (19) and aggressiveness of the tumours (20, 21), respectively. In addition, from these data, menopause was associated with suboptimal debulking, indicating that it is a high risk factor for cytoreductive outcome. In agreement with previous studies (6, 22), these results suggest that women are optimally cytoreduced not only because of the surgery *per se,* but also because of the less aggressive nature of these tumours, which allows them to be more easily resected.

However, on multivariate analysis, only ERCC1 protein expression level remains an independent prognostic

indicator. Previous laboratory and clinical evidence suggests that the greater the tumour spread, the larger the bulk of the tumour is, and the greater the tumour aggressiveness is (6, 23–26). It is possible that the association between a high level of ERCC1 protein and suboptimal debulking status is due to these tumours having a greater ability to metastasize relative to those with reduced expression. The mechanism by which ERCC1 contributes to metastasis is not well understood, but as a rate-limiting factor in the NER pathway, it may play a central role in maintaining genomic integrity by counteracting insults from endogenous and exogenous damaging agents. Thus, a lower level of ERCC1, failing to correct molecular lesions and increasing early senescence and apoptosis of tumour cells, leads to a slower rate of development of metastases or the presence of a smaller number of lesions, which might well render such tumours more amenable to optimal debulking.

ERCC1 protein level, associated with debulking outcome, may therefore serve as a predictor of suboptimal debulking. The data in this study showed that there was a statistical difference in the suboptimal debulking rate between values of ERCC1 expression above and below 2.30. If this level were used to select patients for surgery, approximately half of the patients would not be candidates for a primary surgical attempt. However, the sensitivity and specificity of this threshold level in predicting suboptimal debulking in the study are only 66% and 68%, respectively. In this regard, besides biological features, surgical techniques have an important effect on debulking outcome (6, 27). Therefore, the ERCC1 protein level, as one of the biological features of the tumour, was not always in accordance with debulking outcome in advanced EOC. EOC is variable in its clinical behaviour, and gene expression underlies these differences (12). In previous studies with the highest optimal debulking rates, median survival of the optimal group has been noted to be much inferior to that seen in studies with lower rates of optimal debulking (28). This suggests that outcome is predetermined by the underlying biological characteristics more than by the extent of debulking.

In summary, this study suggests that self-biological features of tumour may decide the outcome in advanced EOC. It is conceivable that ERCC1 protein expression analysis of a preoperative biopsy samples could be used to predict the likelihood of achieving optimal debulking, which could facilitate a more rational selection of patients for debulking surgery. As a result of a more rational patient selection, other patients who are less likely to benefit from this approach might be able to undergo less extensive surgical debulking and be treated with *ERCC1* gene target therapy in order to alter their adverse outcome.

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