Resistance to first line platinum paclitaxel chemotherapy in serous epithelial ovarian cancer: The prediction value of ERCC1 and Tau expression

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Abstract. In oncology, a rational approach to identify patients who are likely to benefit from therapy, already before initiation of treatment, is urgently required. Excision repair cross-complementation group 1 enzyme (ERCC1) has been proposed as a molecular predictor of clinical resistance to platinum-based chemotherapy. Other data suggest Tau protein expression as a predictor of clinical outcome in cancer patients treated with paclitaxel-based chemotherapy as low tau expression may render microtubules more vulnerable to paclitaxel. Therefore, the combination of ERCC1 and Tau may be a valuable predictor of sensitivity to platinum/paclitaxel treatment. The primary aim of the study was to investigate whether ERCC1 and Tau protein expression correlates with patient outcome in newly diagnosed epithelial ovarian cancer (EOC) patients. Formalin-fixed, paraffin-embedded tissue sections from 227 newly diagnosed EOC patients were used for immunohistochemical staining for ERCC1 and Tau proteins. All patients received standard first-line combination platinum and paclitaxel chemotherapy. The patients were divided in a training set of 84 patients and an independent validation cohort of 143 patients. Neither ERCC1 nor Tau expression was associated with clinical response or platinum resistance in both the training and validation sets. Patients with ERCC1-positive tumors had significantly shortened progression-free and overall survival compared to patients with ERCC1-negative tumors, $p<0.00001$ and $p=0.0006$. In multivariate analysis ERCC1 also proved as an independent predictor of PFS and OS with HR of 3.86 and 1.98, respectively but the data could not be confirmed in the validation set. Tau expression was not associated with PFS or OS in this study. ERCC1 and Tau might serve as biomarkers of DNA repair and for paclitaxel sensitivity but the present study could not validate ERCC1 or Tau protein expression in tumors as pre-treatment tools to predict sensitivity to first-line platinum/paclitaxel chemotherapy.

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Introduction

Resistance to cytostatic drugs remains a major problem in clinical oncology. Most malignant tumors are sensitive from the start of treatment but develop drug resistance during the course of chemotherapy but a minor part are resistant already from the onset of treatment. The mechanisms behind chemotherapy resistance are multiple and complicated. The current knowledge in the field is far from sufficient to explain the biology. From a clinical point of view there is an urgent need of markers able to predict the effect of a certain regimen. The issue has been a central topic in clinical cancer research the last two decades but so far without much success. This is also true in ovarian cancer. Internationally, the combination of platinum and a taxane is still the cornerstone in the first-line treatment.

Despite an often initially good response to platinum-based chemotherapy the vast majority of tumors in patients with advanced disease will recur (1). In cases of platinum-resistant disease, the response to further platinum chemotherapy is negligible and usually only associated with side effects causing discomfort to the patient. Contemporary second line therapies are therefore palliative. A major challenge in oncology is therefore an urge to personalize the treatment more effectively and conveniently for each patient based on the patients unique tumor biology, expectation of treatment responsiveness, prolongation of good quality of life and consideration of potential side effects. It would therefore be a rational approach to identify patients unlikely to benefit from chemotherapy already before initiation of treatment. Ovarian cancer is characterized by a number of distinct diseases with heterogeneous biology not only among tumors but also intratumoral. Causes of platinum resistance are multifactorial, but one of the more compelling reasons of drug resistance is the ability of cancer

cells to repair DNA damage (2-4) caused by carboplatin. As alkylating agents, platinum compounds induce DNA damage by causing intrastrand/interstrand structural cross links, and formation of DNA adducts which are often lethal to the cell due to inhibition of DNA synthesis. If the cancer cells feature excessive DNA repair enzymes, they are better able to repair the chemotherapy-induced DNA damage and hence survive chemotherapy. One of these key DNA repair systems is excision repair cross-complementation group 1 (ERCC1), which is one of the essential proteins in the nucleotide excision repair (NER) pathway of several proteins that interact in a coordinated way to recognize DNA damage and repair of DNA (5-7). Excision of the formed DNA adducts is carried out by nucleotide excision repair proteins (8) that recognize the DNA damage and excise the cytotoxic platinum-DNA adducts from the injured DNA strand. ERCC1 gene and protein expression have been found to be inversely correlated with response to platinum-based chemotherapy in ovarian cancer patients (9-11). In particular, several lung cancer studies (12-14) have shown that overexpression of ERCC1 is associated with poorer prognosis and chemotherapy resistance.

Resistance to the first line chemotherapeutic combination platinum/paclitaxel may also be associated with resistance to paclitaxel. Microtubule associated protein (MAP) Tau binds to β-tubulin in the same place as paclitaxel and may consequently compete with this drug (15). Some studies focused on breast and gastric cancer revealed that patients with low expression of Tau protein benefit from paclitaxel therapy (16-20). Nevertheless, other studies, based on larger groups of breast cancer patients did not confirm these results (21,22). At the same time high Tau expression was indicated as a good prognostic factor in breast cancer (21), especially while co-expressed with estrogen receptor (22). There is very sparse data concerning the role of Tau protein in ovarian cancer (23).

To date, no validated tool exists that allows physicians to identify patients unlikely to respond to platinum and taxane agents to prevent patients suffering from unnecessary toxicities.

The overall purpose of this study was to explore whether ERCC1 and Tau expression correlates with patient outcome in newly diagnosed epithelial ovarian cancer patients.

Multiple molecular biology studies have shown that the various histological subtypes do not share the same mutation pattern and do not have the same treatment response (24-26). The majority of epithelial ovarian cancer histology is the serous subtype. To make our study as uniform and unambiguous as possible, we therefore focused on examining patients with serous histology only.

Furthermore, the purpose was also to attempt to validate results obtained and therefore the present study was divided into a predefined training set and the findings from this dataset was sought for validation in a larger and independent validation set.

Materials and methods

Study population. Formalin-fixed, paraffin-embedded tumor tissue and clinical data were collected from chemotherapy-naive patients with histological confirmed epithelial ovarian, primary peritoneal or fallopian tube cancer.

Patients were treated at four different institutions in Denmark (Departments of Clinical Oncology at Aalborg, Herning, Odense and Vejle Hospitals) and in one institution in Poland (Department of Oncology, Military Institute of Health Services, Warsaw, Poland). The data from the patients included in the training set have previously been published (11,23). Unlike the previous report, the present study only included patients with serous histology and the follow-up time is much longer with mature overall survival data.

The study was carried out in compliance with the Helsinki II Declaration. The Regional Scientific Ethics Committee for Southern Denmark and the Danish Data Protection Agency approved the study according to Danish law. The Local Research Ethics Committee approved the study according to Polish law.

All specimens were collected prior to chemotherapy, either as a biopsy before neoadjuvant chemotherapy or as tumor tissue removed at up-front debulking surgery. Patients were treated with combination chemotherapy with carboplatin (AUC5/AUC6) and paclitaxel (175 mg/m^2) or cisplatin (75 mg/m^2) and paclitaxel (135 mg/m^2) for some of the Polish patients treated in the early 2000s. Treatment was administered every 3 weeks for six cycles. From each patient one representative formalin-fixed, paraffin-embedded tissue block was selected and sections at 3.5 μm were cut.

ERCC1 immunohistochemistry. The sections were initially deparaffinized, rehydrated and blocked for endogenous peroxidase with 3% H_2O_2 followed by heat induced epitope retrieval in Tris-EGTA buffer pH 9.0 in microwave oven.

The immunostaining was performed on the Dako autostainer link 48 with the monoclonal mouse antibody against human full-length ERCC1 protein (clone 8F1, Thermo Scientific, Cheshire, UK, code no. MS-671-P, 1:1,000 dilution). Detection of the immunoreaction was obtained using the Super Sensitiv™ Polymer-HRP IHC kit (BioGenex, Fremont, CA, USA) as described previously (10). Please refer to Table I for further details.

The lot numbers of the antibody and of the detection kit used for the validation set were not the same as used for the training set. Therefore, adjustments were made in order to obtain similar staining intensity as for the training set. We performed the optimization carefully on different tissues and patient samples and finally, we restained a small group of 11 samples from the training set, to ensure that the originally staining could be reproduced. Three different lots of the primary antibody and two lots of the detection kit were tested and we found that there was a minor lot to lot variation for the ERCC1 antibody, but there were significant lot to lot variations for the detection kits. As a consequence, we had to dilute the primary antibody 3-fold. We stained all samples in the validation set with the same lot of ERCC1 and detection kit. Under these conditions we could reproduce the staining from the training set.

The slides were scored as described by Olaussen *et al* (14) and similar to our previous studies (10,11). Scoring of the immunohistochemistry results was generated by multiplication of the values for the percentage of immunopositive epithelial tumor cells and for staining intensity. In brief, staining intensity was graded 0-3, with 3 as the highest intensity. The

Table I. Antibodies used for ERCC1 and Tau staining.

Antibody	ERCC1 clone 8F1 (training)	ERCC1 clone 8F1 (validation)	Tau
Producer	Neomarkers	Thermo Scientific (Neomarkers)	Dako Cytomation
Code nr/Cat nr	MS-671-P	MS-671-P	A 0024
Antibody dilution	1:300	1:1,000	1:100
Deparaffinazation	Tissue-Clear®	Tissue-Clear®	Xylene 2x10 min; ethanol: absolute, 96%, 70%, 50%, water - each 2 min
Epitope retrieval	MWO/EGTA/pH9 15 min boiling/ 15 cooling RT	MWO/EGTA/pH9 15 min boiling/ 15 cooling RT	None (according to the manufacturer's product specification)
Time	30 min room temperature	30 min room temperature	30 min room temperature
Amplification	Super enhancer (from the detection system)	Super enhancer (from the detection system)	None
Detection system*	Super Sensitiv™ Polymer-HRP IHC kit (BioGenex, no QD430-XAKE)	Super Sensitiv™ Polymer-HRP IHC kit (BioGenex, no QD430-XAKE)	K 4002; Dako Envision™+ System

percentage of positive tumor cells in each slide were scored as 0 if 0% of the tumor cells were positive, 0.1 if 1-9%, 0.5 if 10-49% and 1.0 if ≥50% were positive. A semi-quantitative H-score for cutoff point was calculated by multiplying the staining intensity with the percentage score and the tumor was scored as positive when the H-score was >1.0 as also described in previous reports (10,11,14) for separating ERCC1-positive tumors from ERCC1-negative tumors. The interpretation of ERCC1 expression was done by a senior gynecological pathologist (M. Waldstrøm). At the time of interpretation, the authors had no knowledge of the clinical data.

Tau immunohistochemistry. Immunostaining was performed with anti-Tau polyclonal rabbit antibody that recognizes all isoforms of human Tau irrespectively of its phosphorylation status (1:100 dilution; code A 0024; Dako Cytomation, Glostrup, Denmark). Formalin-fixed, paraffin-embedded 3.5-μm sections of ovarian cancer were incubated with anti-Tau antibody for 30 min in room temperature. Anti-rabbit horseradish peroxidase-labeled secondary antibody was used to generate the signal (code K 4002; Dako Envision™+ System). Normal ovarian epithelium serving as control tissue was derived from a benign ovarian cyst in a 51-year-old patient. Omission of primary antibody served as a negative control. Specimens were assessed by means of light microscope with x20 magnification lens. Tau staining of tumor cells was scored according to a previous report (4) with our modification as follows: IHC score 0, no staining; 1+, poor focal staining or very poor diffuse staining; 2+, average diffuse staining or strong staining in <25% cells; 3+, strong staining in ≥25% of tumors cells. Tau expression was recorded as negative (0 and 1+) or positive (2+ and 3+). This dichotomization of staining results was determined by using staining intensity of normal epithelial cells as a reference. Slides were scored without knowledge of the clinical outcome.

Statistical analyses. The χ^2 test was used for testing a possible association between ERCC1/Tau immunohistochemistry and clinicopathological parameters and response to chemotherapy.

Progression-free survival was defined as the time from date of diagnosis (date of primary surgery or diagnostic biopsy) until disease recurrence or death from any cause, whichever came first. Overall survival was calculated as the interval from the time of diagnosis until death from any cause. Univariate overall survival analysis was performed using the Kaplan-Meier method and log-rank statistics for comparison of survival plots. Multivariate overall survival analysis was determined by the Cox regression model. Graphical checks of proportional hazards assumption were made for all variables before entering the variables into the Cox regression model. The parameters entered in the Cox analysis were ERCC1 status (negative and positive), Tau status (negative and positive), FIGO stage, grade and residual disease status as categorical variables and age at diagnosis as a continuous variable. FIGO stage was divided into stage I/II vs. stage III/IV, grade into grade 1 vs. grade 2/3/not-graded to avoid too many parameters entered into the Cox model according to the number of events if not merged into fewer variables. A value of $p<0.05$ was considered statistically significant. Statistical analyses were performed with the NCSS software (version 2007, Kaysville, Utah, http://www.ncss.com).

Results

Patient characteristics. Paraffin-embedded tumor specimens were obtained from all 227 patients enrolled into the two

Table II. Patient characteristics.

Clinicopathological parameters	Training set N=84	Validation set N=143	p-value
Age median	63.5	60.6	0.13
Age range	32-79	31-89	
FIGO stage			0.20
I	4 (4.8%)	11 (7.7%)	
II	10 (11.9%)	11 (7.7%)	
III	56 (66.7%)	83 (58.0%)	
IV	14 (16.7%)	38 (26.6%)	
Grade			0.14
1	3 (3.7%)	8 (7.4%)	
2	29 (35.4%)	49 (45.4%)	
3	50 (61.0%)	51 (47.2%)	
	(Not graded - e.g. clear cell, or biopsy only: 2)	(Not graded - e.g. clear cell, or biopsy only: 35)	
Residual tumor			0.0001
0 cm	21 (25.6%)	74 (54.8%)	
<1 cm	17 (20.7%)	16 (11.9%)	
≥1 cm	44 (53.7%)	45 (33.3%)	
	(Data not available: 2)	(Data not available: 8)	
ERCC1 staining			0.06
Negative	73 (86.9%)	106 (76.8%)	
Positive	11 (13.1%)	32 (23.2%)	
		(Staining not done: 5)	
Tau staining			0.24
Negative	24 (29.3%)	44 (37.3%)	
Positive	58 (70.7%)	74 (62.7%)	
	(Staining not done: 2)	(Staining not done: 25)	

studies (N=84 for the training study and N=143 for the validation study).

Patients included in the training set were all consecutively included at the Danish hospitals during the period 2005-2007. The validation cohort consisted of all patients from the Polish center (included in the period 2001-2009) and of patients from the Danish centers consecutively enrolled during 2007-2010. The patient characteristics are outlined in Table II.

Six cycles were administered to 81.5% of the patients and 98% received at least three cycles. Nineteen patients received more than six cycles due to an expectation of a further tumor response with prolonged treatment up to a maximum of nine cycles.

The predictive value of ERCC1 and Tau protein expression. Staining of ERCC1 and Tau was not significantly associated with each other (p>0.05, Fisher's exact test). Furthermore, neither ERCC1 nor Tau was associated with age, histological grade, FIGO stage, or residual tumor size (data not shown).

Thirty-six percent of patients with positive ERCC1 expression in the training set progressed during first line chemotherapy compared to progression in only eight percent of patients with negative ERCC1 expression (p=0.05, Table IIIA) although the numbers of patients progressing during first line chemotherapy were rather small.

However, patients with early relapse and platinum resistance had highly significantly increased levels of ERCC1 expression (p=0.0004, Table IIIB). Platinum resistance was defined as recurrence within 6 months after completion of first line platinum-based chemotherapy or progression during the first line platinum-based chemotherapy.

Positive ERCC1 staining was not significantly associated with treatment outcome in the validation set. Tau expression was not associated with clinical response or with platinum resistance in the training or validation set.

The prognostic value of ERCC1 and Tau protein expression. At the time of the analysis, 63 patients were deceased in the training set material. For the 21 patients still alive, the median

Table III. Association between ERCC1 expression and chemotherapy response (A)/platinum resistance (B).

A, Association between ERCC1 immunohistochemistry and clinical response (evaluated by RECIST version 1.0 and/or GCIG CA125 criteria)

	ERCC1 status					
	Training set P=0.05			Validation set P=0.72		
Response	Negative ERCC1 IHC	Positive ERCC1 IHC	All	Negative ERCC1 IHC	Positive ERCC1 IHC	All
Complete response	43 (66%)	5 (46%)	48	38 (48%)	10 (46%)	48
Partial response	14 (22%)	2 (18%)	16	28 (35%)	8 (36%)	36
No change	3 (5%)	0 (0%)	3	8 (10%)	1 (5%)	9
Progression	5 (8%)	4 (36%)	9	6 (8%)	3 (14%)	9
Non evaluable for response			8			41
All	65	11	84	80	32	143

B, Association between ERCC1 immunohistochemistry and platinum resistance (platinum resistance defined as recurrence within 6 months after completion of first line platinum-based therapy)

	ERCC1 status					
	Training set P=0.0004			Validation set P=0.58		
Platinum resistance	Negative ERCC1 IHC	Positive ERCC1 IHC	All	Negative ERCC1 IHC	Positive ERCC1 IHC	All
Platinum-sensitive	48 (66%)	1 (9%)	49	75 (71%)	21 (66%)	96
Platinum-resistant	25 (34%)	10 (91%)	35	31 (29%)	11 (34%)	42
All	73	11	84	106	32	138

follow-up time was 70 months (range, 53-87 months). In the validation set, 63 patients were deceased at censoring time and the median follow-up for the 80 patients still alive was 36 months (range, 16-110 months). There were no statistical differences in PFS or OS between the training and the validation sets (p=0.10 and 0.16, respectively).

Training set. Patients with ERCC1-positive tumors had significantly shortened progression-free survival compared to patients with ERCC1-negative tumors, p<0.00001 (Fig. 1). The Kaplan-Meier estimate of median PFS for patients with ERCC1-positive tumors was 8.1 months (6.1-8.9 months) 95% CI while the median PFS of patients with ERCC1-negative tumors was 13.4 months (11.9-21.1 months) 95% CI.

The difference in PFS also translated into a significant advantage in overall survival for patients with ERCC1-negative tumors, p=0.0006 and with >2 years difference in median OS (16.8 vs. 42.9 months, Fig. 1). Tau expression had no significantly association with PFS or OS, p=0.87 and 0.59, respectively.

Validation set. For ERCC1, the findings from the training set could not be confirmed in the validation set as there were no significantly differences in PFS or OS according to ERCC1 tumor expression (p=0.55 and 0.90, respectively, Fig. 1). For Tau expression the findings were the same as in the training set, with no influence of Tau expression on PFS or OS.

Multivariate analysis. The Cox proportional hazard model was used in order to study the independent effect of ERCC1 and Tau. FIGO stage, suboptimal debulking (residual tumor <1 and ≥1 cm) and positive ERCC1 (HR 5.71, 95% CI; 2.65-12.3) were found to be independently associated with worse PFS in the training set (p=0.021, p=0.003/0.002 and $p<10^{-3}$, respectively).

However, in the validation set, only FIGO stage and residual tumor were independent predictors of PFS (Table IVA). FIGO stage (p=0.028), residual tumor <1 and ≥1 cm (p=0.031 and 0.002, respectively) and ERCC1 (p=0.008) were also associated with OS in the training set. Again, ERCC1 and Tau were not associated with OS in the validation set. The results of the multivariate OS analysis are summarized in Table IVB.

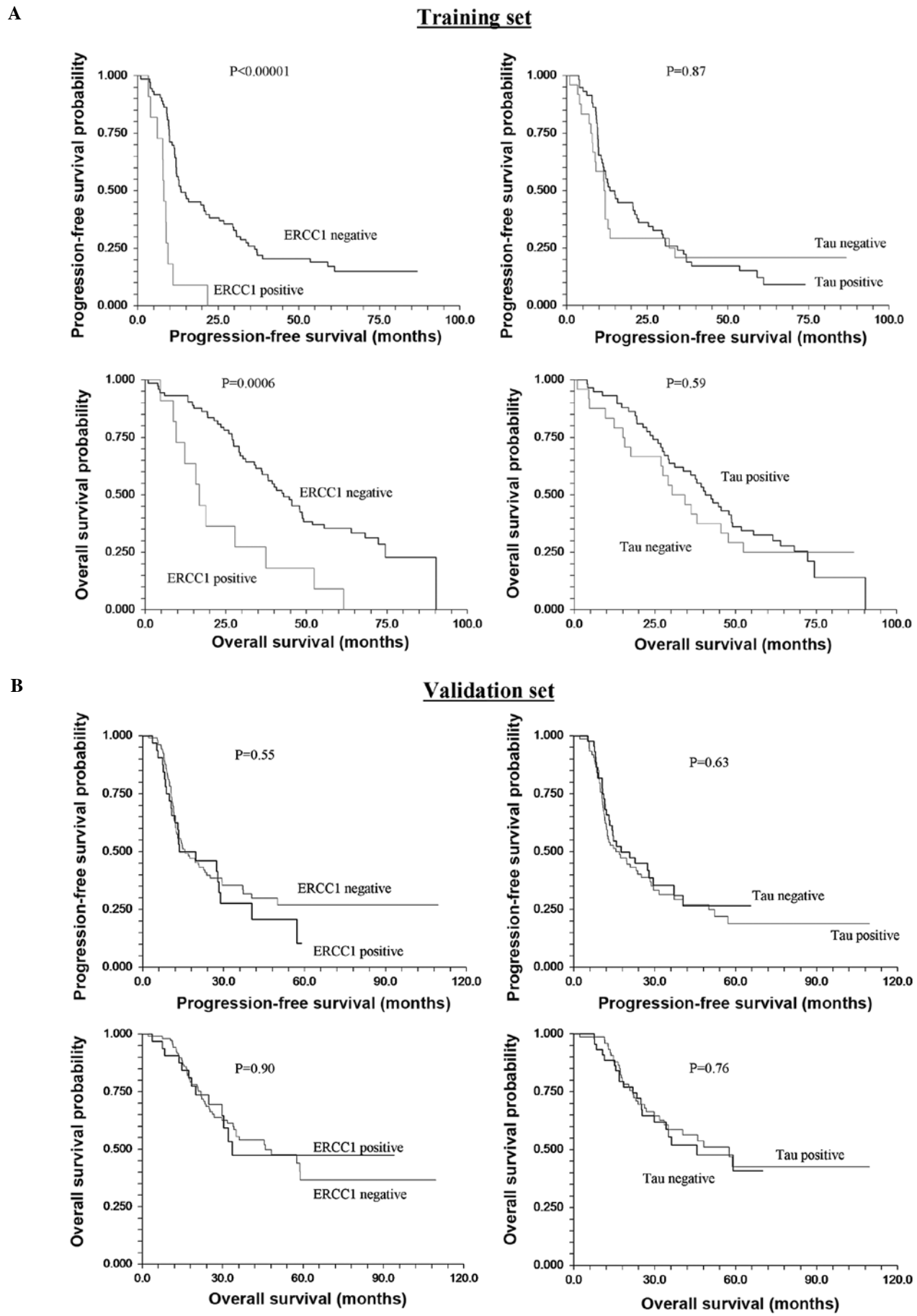

Figure I. Progression-free and overall survival according to Tau and ERCC1 status. (A) Univariate progression-free and overall survival analysis according to ERCC1 and Tau expression for the training set. (B) Univariate progression-free and overall survival analysis according to ERCC1 and Tau expression for the validation set.

Table IV. Multivariate progression-free survival (A) and overall survival (B) analysis for both the training and validation set.

A, Overall survival

	Training set			Validation set		
PFS	HR	95 % CI	p-value	HR	95 % CI	p-value
Age	0.99	0.96-1.02	0.622	1.00	0.98-1.02	0.892
FIGO stage						
I/II	1.00			1.00		
III/IV	2.71	1.16-6.34	0.021	4.43	1.54-12.8	0.006
Tumor grade						
1	1.00			1.00		
2/3/not graded	2.82	0.37-21.3	0.315	1.66	0.58-4.70	0.342
Residual tumor						
0 cm	1.00			1.00		
<1 cm	3.73	1.56-8.93	0.003	1.73	0.85-3.50	0.129
≥1 cm	3.14	1.54-6.42	0.002	2.10	1.21-3.64	0.009
ERCC1 staining						
Negative	1.00			1.00		
Positive	5.71	2.65-12.3	$<10^{-3}$	0.85	0.45-1.59	0.611
Tau staining						
Negative	1.00			1.00		
Positive	0.63	0.35-1.14	0.124	1.30	0.80-2.10	0.290

B, Multivariate OS analysis

	Training set			Validation set		
OS	HR	95 % CI	p-value	HR	95 % CI	p-value
Age	1.01	0.98-1.04	0.540	1.02	0.99-1.04	0.152
FIGO stage						
I/II	1.00			1.00		
III/IV	3.20	1.13-9.01	0.028	9.26	1.23-69.9	0.031
Tumor grade						
1	1.00			1.00		
2/3/not graded	1.42	0.18-10.9	0.735	2.73	0.66-11.5	0.172
Residual tumor						
0 cm	1.00			1.00		
<1 cm	2.92	1.10-7.77	0.031	1.82	0.81-4.11	0.146
≥1 cm	3.75	1.61-8.74	0.002	1.73	0.91-3.28	0.096
ERCC1 staining						
Negative	1.00			1.00		
Positive	2.69	1.30-5.56	0.008	0.59	0.25-1.43	0.245
Tau staining						
Negative	1.00			1.00		
Positive	0.76	0.40-1.44	0.392	1.19	0.66-2.14	0.569

Discussion

Management of patients with relapsed ovarian cancer constitutes a therapeutic challenge. Predicting and overcoming chemotherapy resistance and platinum-induced resistance in particular might be one of the key issues for improving the efficacy of ovarian cancer therapies, both in the first-line setting and in the treatment of relapses.

Previously published reports from our group have focused on ERCC1 as one of these potential tools for in advance to pinpoint patients who are likely not to benefit from platinum-based chemotherapy if they have high expression of ERCC1 (10,11,27). Specifically, it is striking how large the survival difference is between patients with ERCC1-positive and ERCC1-negative tumors concerning both PFS and OS in the training set, which is identical with the patient material included in a previous report (11). Although the previous report included all epithelial histological subtypes. In the present report, this patient cohort is now updated with much longer follow-up which further confirms the data as described in the previous report in which PFS but not OS (p=0.099 in univariate analysis) on the publication time were significantly different in patients with ERCC1-positive tumors compared to ERCC1-negative tumors. Now, the OS has as well become significantly disadvantageous (p=0.0006 and 0.008 in univariate and multivariate OS analysis, respectively) for patients with ERCC1 overexpression presumably because of longer follow-up and more mature data. It is therefore surprising that the data can not be validated. There are several considerations on possible causes for this. The most obvious reason is, of course, that ERCC1 is not an unambiguous and reliable marker of platinum resistance. Data have so far been inconsistent and recently, a report from another group, in agreement with our present report, also described that ERCC1 expression was unable to predict platinum resistance (28). This GOG study consisted of a heterogeneous group of 408 patients treated with seven different platinum-containing regimens and moreover, they used a different ERCC1 antibody (FL297 polyclonal antibody) for immunohistochemical detection. Thirdly, the immunohistochemical scoring method was somewhat different as the scoring of ERCC1 was performed in a binary fashion where any nuclei staining in a specimen was defined as positive and lack of any nuclei staining as negative, making a straight comparison of studies difficult. Cancer biology is not simple, and ERCC1 is only one of the proteins involved in a complex pathway of the NER system with contributions from many other players also likely to influence DNA repair.

Another potential criticism of the present study is an ongoing scientific discussion concerning the quality of the used ERCC1 antibody, which is also stated by the authors of the GOG report (28) and their viewpoint on why they can not demonstrate a clinical importance of ERCC1 staining in the GOG study. A report by Bhagwat *et al* (29) argued that the most commonly used antibody, 8F1, which was also the one used in our study and in many others was not suitable for immunodetection in tissue and recommended the use of the polyclonal FL297 antibody. This conclusion was drawn on the basis of an observed additional band with extremely close molecular weight in western blots. As a response to this Olaussen and Soria (30) reinvestigated the signal from the 8F1 antibody and did not observe a higher band very close to ERCC1 suggesting that it is rarely present in carcinoma cells. The 8F1 signal also correlated with ERCC1 mRNA expression in the cells suggesting antibody specificity. Consequently, Olaussen and Soria argued that the 8F1 antibody is an acceptable tool to determine nuclear ERCC1 protein expression in human formalin-fixed paraffin-embedded solid tumors of epithelial origin, whereas FL297 lead to a puzzling cytoplasmic staining.

None of the above explanations holds true in the present study as they do not explain the difference between the training and the validation set.

Another possibility may have its origin in diversity of the two sets of material. In the training set there were far less optimal debulked patients (26%) compared with the validation set which was a more recent and more extensive surgically operated cohort with nearly twice as many patients operated with optimal debulking (55%). One can speculate whether ERCC1 and Tau have greater significance in patients with residual tumor as it may be assumed that the effect related to chemotherapy depends on the size of the residue after surgery. The issue is of general importance as the value of predictive markers may vary considerably with tumor stage. The predictive value in early stage tumors of a disease allocated for adjuvant treatment may well be different from that of advanced disease with major residual tumor. This may at least partly explain why the results from the training set were not confirmed.

ERCC1 and Tau might serve as biomarkers of DNA repair in tumors and for paclitaxel sensitivity, respectively, but the present study could not confirm ERCC1 or Tau protein expression in tumors as pre-treatment tools to predict sensitivity to first line platinum/paclitaxel chemotherapy.

The results presented here illustrate a major problem in the search for biomarkers and their validation. There is an enormous amount of literature identifying predictive and/or prognostic markers but most studies are based on insufficient patient material or never validated. Consequently, very few have been accepted for clinical use. The situation calls for markers to be incorporated in prospective and preferably randomized clinical trials as a prerequisite for clinical routine applications.

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