Prognostic evaluation of tumour type and other histopathological characteristics in advanced epithelial ovarian cancer, treated with surgery and paclitaxel/carboplatin chemotherapy: Cell type is the most useful prognostic factor

A. Bamias a,*, M. Sotiropoulou b, F. Zagouri a, P. Trachana a, K. Sakellariou a, E. Kostouros a, K. Kakoyianni a, A. Rodolakis c, G. Vlahos c, D. Haidopoulos c, N. Thomakos c, A. Antsaklis c, M.A. Dimopoulos a

a Dept. of Clinical Therapeutics, Medical School, University of Athens, Athens, Greece
b Histopathology Dept., Alexandra Hospital, Athens, Greece
c 1st Dept. of Obstetrics and Gynaecology, Medical School, University of Athens, Athens, Greece

ARTICLE INFO

Article history:
Available online 31 October 2011

Keywords:
Ovarian cancer
Grade
Tumour type
Cell type

ABSTRACT

Aim: Ovarian carcinomas have been classified into types I and II according to the hypothesised mode of carcinogenesis and molecular characteristics. The prognostic significance of this classification has not been studied.

Patients and methods: Five hundred and sixty-eight patients with histologically confirmed, ovarian, fallopian tube or peritoneal carcinomas, international federation of gynecology and obstetrics (FIGO) stages IIC–IV, treated with paclitaxel/platinum following cytoreductive surgery, were included in this analysis. Type I included low-grade serous, mucinous, endometrioid and clear-cell and type II high-grade serous, unspecified adenocarcinomas and undifferentiated carcinomas.

Results: Median overall survival (OS) was 49 months for type I versus 45 for type II (p = 0.576). In contrast to type II, there was considerable prognostic heterogeneity among the subtypes included in type I. Cox regression analysis showed that cell-type classification: low-grade serous, mucinous, endometrioid, clear-cell, type II (high-grade serous, unspecified adenocarcinomas, undifferentiated carcinoma) was an independent predictor of survival (respective median OS 121 versus 15 versus 64 versus 29 versus 45 months, p = 0.003). On the contrary, histopathological subtype or tumour type (I versus II) did not offer additional prognostic information.

Conclusion: The proposed model of ovarian tumourigenesis does not reflect tumour behaviour in advanced disease. Tumour-cell type is the most relevant histopathological prognostic factor in advanced ovarian cancer treated with platinum/paclitaxel.

© 2011 Elsevier Ltd. All rights reserved.

* Corresponding author: Address: Oncology Unit, Alexandra Hospital, 80 Vas. Sofias Ave., 115 28 Athens, Greece. Tel.: +30 210 3381546; fax: +30 210 3381511. E-mail address: abamias@med.uoa.gr (A. Bamias). 0959-8049/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2011.09.023
1. Introduction

Ovarian carcinomas are primarily classified according to the different epithelia of the reproductive female tract they arise from into serous, mucinous, endometrioid, clear-cell and transitional cell tumours. Clinical and pathological observations on the different histological types have suggested two pathways of carcinogenesis in the ovary: type I, where precursor lesions in the ovary have clearly been described and type II, where such lesions have not been described as yet. According to that model, type I tumours include low-grade serous, mucinous, endometrioid, clear-cell and transitional-cell tumours, while type II tumours comprise of high-grade serous, undifferentiated carcinomas and carcinosarcomas. Progress in molecular biology has enabled the clarification of certain aspects of carcinogenesis of these two types of ovarian cancer, and has suggested that there are common underlying molecular features characterising each tumour type. 

Advanced ovarian cancer (international federation of gynecology and obstetrics (FIGO) stages III and IV) is a prognostically heterogeneous group of tumours. Up to now the clinical relevance of tumour histology and grade remain uncertain. Histology is generally associated with prognosis, but from the specific subtypes only mucinous has consistently been accepted as an independent adverse prognostic factor. In addition, each histological subtype, apart from serous, accounts for less than 10% of the total in advanced disease. Finally, therapeutic approaches are similar for all histopathological subtypes, in spite of their well documented epidemiological, molecular and prognostic differences. Tumour grade has been associated with prognosis but opposite results have also been published. Again, such stratification is not very practical in advanced disease, since grade I tumours account for less than 10% of the total. Another drawback is the fact that all clear-cell carcinomas are classified as grade 3, while the prognostic significance of tumour grade may differ among different histological subtypes. More importantly, grade assignment lacks reproducibility with several systems (FIGO, World Health Organisation (WHO), gynecology oncology group (GOG), 3-tier, 2-tier) being used. The proposed classification into tumour types I and II combines the histological type and grade and might be advantageous, compared to each factor alone, since distribution of advanced tumours might be more balanced in this stratification. In general, type I tumours are considered slow-growing and they are often confined to the ovary at diagnosis. On the contrary, type II tumours evolve rapidly, metastasise early in their course and are highly aggressive. If the hypothesis of slow growth is true, it is expected that type I tumours would survive longer. Furthermore, it has been suggested that low-grade tumours may be less sensitive to chemotherapy than high-grade. For the above reasons, it could be argued that tumour type classification would be prognostically more useful when compared to histological classification or tumour grade. Still there may be considerable prognostic heterogeneity among the different histological subtypes included in the same tumour type. For example, mucinous and clear-cell carcinomas, which have been included within type I have been associated with inferior outcome, compared to the other subtypes of this group.

Information on the prognostic significance of the above tumour type classification is limited. We, therefore, retrospectively analysed the outcome of type I and type II advanced epithelial ovarian tumours.

2. Methods

2.1. Patients and assignment to histopathological characteristics

Patients were selected from our epithelial ovarian cancer (EOC) database according to the following criteria: histologically confirmed epithelial cancer of the ovary, fallopian tube or primary peritoneal carcinoma, FIGO stages IIC-IV and first-line chemotherapy with the combination of paclitaxel and a platinum compound. Patients receiving additional agents were also included. Patients with borderline or germ cell tumours were excluded from the analysis. All patients were operated, received chemotherapy and were followed up at our institution (Alexandra Hospital, Athens, Greece). Staging was performed according to FIGO guidelines: total abdominal hysterectomy and bilateral salpingo-oophorectomy, omentectomy and removal of all macroscopic diseases. Lymph node dissection has not been routinely performed in otherwise obvious stage IIIC disease, unless lymph node involvement was suspected by pre-surgical radiological assessment or clinically during surgery. Radical upper abdominal techniques were applied in carefully selected cases, within the scope of achieving complete tumour removal. Patients who received their first course of chemotherapy up to January 2010 were included, in order to ensure at least one year of follow-up, since our database was updated in January 2011.

Tumours were examined using sections. We studied the prognostic significance of the following three histopathological characteristics, defined as follows for the purposes of this analysis: (a) histological subtype (serous, endometrioid, mucinous, clear-cell, adenocarcinomas unspecified, undifferentiated carcinoma, transitional-cell); (b) tumour type (I: low-grade serous, endometrioid, mucinous, clear-cell, transitional-cell; II: high-grade serous, undifferentiated, adenocarcinomas unspecified); (c) cell type (low-grade serous, endometrioid, mucinous, clear-cell, transitional-cell, high-grade serous, undifferentiated, adenocarcinomas unspecified). Staging was performed according to FIGO and cell type was assigned using the WHO criteria. Mixed types were classified according to their highest-grade component or the predominant component if the constituent components were of similar grade. Grading of serous carcinomas was assigned according to FIGO until 2000, then the 3-tier system was used until 2008 and currently the 2-tier system is applied. According to the latter, serous carcinomas were stratified into low- and high-grades. For tumours diagnosed prior to 2008, grade 1 was considered low grade, while grades 2 and 3 as high-grades in keeping with the 2-tier system by Malpica. This classification has been used by other investigators and is in accordance with recent clinical findings and as well as the results of this study showing similar median overall survival (OS) between grades 2 and 3 serous carcinomas as Grade 2. All mucinous, clear-cell carcinomas and transitional-cell...
tumours were categorised in type I, while unspecified adeno-
carcinomas and poorly differentiated carcinomas were cate-
gorised in type II according to Kurman and Shih.1,21

The assignment of endometrioid carcinomas to type I or II
has not been unanimous. Low-grade endometrioid carcin-
mas have always been included in type I tumours, while
high-grade has been included in both types.20 In contrast to
serous carcinomas, grade 2 endometrioid tumours have been
classified as low-grade tumours,5,11 making the accuracy of
assignment of the grading particularly important for the
scope of this study. Since WHO17 and FIGO18 systems (which
have been used in our institution for the assessment of ova-
rian endometrioid carcinomas) can be considered impre-
cise,5,12 all endometrioid tumours were re-examined by a
gynaecologic oncology pathologist (M.S.). Separate analyses
were performed including high-grade endometrioid carcino-
mas in both types.

2.2. Statistical analyses

Analyses were performed using the SPSS statistical software
(SPSS for Windows, version 13, SPSS Inc., Chicago, IL, United
States of America (USA)). Chi-square test was used for the
comparisons of baseline characteristics between the different
tumour types. The variables analysed were: age (<70 years
versus >70 years), stage (IIC, IIIA, IIIB versus IIIC versus IV),
grade (I versus II + III), residual disease (<1 cm versus
>1 cm), performance status (PS) (0 versus 1–3) and histology
(serosal versus mucinous versus endometrioid versus clear-
cell versus unspecified adenocarcinoma versus undifferenti-
ated). Survival was calculated from the day of the initia-
tion of treatment until date of death or date of last follow-up,
using the Kaplan–Meier method and differences were studied
using the log-rank test. Cox proportional hazards model was
used for hazard ratio estimation, to study the interactions be-
tween tumour type and baseline characteristics and for mul-
tivariate analyses. Variables with a \( p \) value <0.1 in the
univariate analysis were entered in the multivariate Cox
regression model. Throughout analysis the level of 5% was
used to denote statistical significance.

Platinum-free interval was used to determine platinum-
resistance and was calculated from the day of last
chemotherapy until the date of last follow-up (for non-re-
lapsed patients), relapse or death from disease (for those with
non-documented progression). Diagnosis of relapse was
based on radiological or CA125 criteria (whichever came first).

3. Results

3.1. Patients and treatment

A total of 568 patients, treated from 13th February 1995 to
22nd January 2010, were included in the analysis. Their base-
line characteristics and the first-line treatment they received
are shown in Table 1. Median follow up was 102 months (13–
170 for alive patients). During follow-up 422 patients relapsed
(75%) and 385 died (67%). All but three patients (two deaths
due to cerebrovascular accident and one death due to sepsis,
probably related to treatment) died due to ovarian cancer.
Fifty-three patients (9%) received fewer than six cycles of
chemotherapy. Median OS was 45 months and median
progression-free survival (PFS) 19 months.

3.2. Significance of tumour grade within different
histopathological subtypes – classification to tumour types

Since serous and endometrioid carcinomas may be assigned
to different tumour types according to tumour grade, we
studied the significance of tumour grade across different histologies in order to explore the prognostic significance of this classification. There was a significant difference between low- and high-grade serous carcinomas: median OS 121 versus 45 months \( (p = 0.022) \) (Fig. 1a). Further division of high-grade tumours into grade 2 and grade 3, did not add to the prognostic significance of tumour grade: median OS for grade 2 39 versus 45 months for grade 3 \( (p = 0.452) \).

Review of endometrioid carcinomas resulted in the change of six of 48 cases to high-grade serous \( (n = 5) \) and clear-cell \( (n = 1) \) carcinoma. Tumour grade was changed from 2 to 3 in 15 cases. After review, three cases \( (7\%) \) were assigned as grade 1, 11 \( (26\%) \) as grade 2 and 28 \( (67\%) \) as grade 3. There was no OS difference according to tumour grade \( (p = 0.103) \) (Fig. 1b). More importantly, no difference between Grade 2 and 3 was noted \( (44 \text{ versus } 84 \text{ months, } p = 0.190) \).

### 3.3. Tumour type

Separate analyses including grade 3 endometrioid carcinomas to either tumour type were performed. Results were essentially similar. Furthermore, median OS of these tumours was almost twice that of the other type II carcinomas \( (84 \text{ versus } 45 \text{ months}) \). Although this difference was not significant, apparently due to the small number of endometrioid tumours in our series, these results taken together with the fact that grade 2 endometrioid carcinomas did not have a better outcome than grade 3 (as described in the previous section), indicate that it would be prognostically more intuitive to include all endometrioid tumours in the same group.

Type I was correlated with age \( \leq 70 \) \( (83\% \text{ versus } 72\%, \ p = 0.015) \) but not with optimal debulking \( (p = 0.076) \), stage \( (p = 0.119) \) or eastern cooperative oncology group (ECOG) PS \( (p = 0.834) \). Tumour type was not associated with OS \( \text{(type I: } 49 \text{ months versus } 45 \text{ months for type II, } p = 0.576) \) (Fig. 2). Subgroup analyses did not show any interaction of tumour type with age, stage, extent of residual disease or PS.

Stratification according to histological subtype showed essentially identical outcome for the subtypes included in type II, but significant differences within type I (Fig. 3, Table 2). Taking into consideration the differences in the survival within type I tumours, we stratified these tumours according to cell type (this term is used to distinguish this classification from histopathological subtype and tumour type), as described by Gilks et al.\(^5\). Transitional-cell tumours were not included in this analysis due to the very limited number of such cases \( (n = 2) \). This stratification produced five groups with both statistically and clinically significant differences in survival. The outcome according to cell-type is shown in Table 2 and Fig. 4.

### 3.4. Uni- and multi-variate analyses

These are shown in Table 3. Age, stage, grade, PS, residual disease, histology and cell type were associated with OS in univariate analysis. Since tumour grade and histology were the criteria for the definition of cell type, two separate multivariate analyses were performed: in the first, grade and histology but not cell type were included in the model, while in the second, cell type but not grade or histology was included in the cox regression model. Age, stage and histology were not independent predictors of survival, while grade, PS, residual disease and cell type retained their prognostic significance in multivariate analysis.

---

Fig. 1 – Overall survival of serous (a) and endometrioid (b) ovarian carcinomas according to tumour grade.

Fig. 2 – Overall survival of 568 women with advanced ovarian cancer according to tumour type I (—) or II (——).
3.5. Platinum-resistance versus type

Sensitivity to platinum-based chemotherapy was analysed only for 1st line chemotherapy, since no data for subsequent lines of treatment are included in the database. There were 150 (26%) platinum-resistant and 418 (74%) platinum-sensitive patients. Platinum resistance was not associated with histological subtype or tumour type (chi-square $p = 0.150$ and 0.495, respectively). On the contrary, there was a significant association with cell type: low-grade serous tumours showed the highest sensitivity (92%), mucinous showed the lowest (52%), while type II, endometrioid and clear cell showed intermediate sensitivity (74%, 69% and 69%, respectively) ($p = 0.044$).

4. Discussion

Recent advances in our knowledge of epithelial ovarian cancer tumourigenesis and molecular biology has led to the proposal that they should be classified into two tumour types, based on the existence of precursor lesions and common molecular characteristics. This classification could have prognostic and therapeutic implications, since type I tumours are supposed to be slow-growing and more resistant to chemotherapy. These hypotheses have not, however, been adequately addressed in clinical studies. We studied this hypothesis in a large series of 568 patients with advanced ovarian cancer. In the interpretation of our results it should be taken into consideration that retrospective analyses can introduce selection biases, while treatment at relapse was not homogenous. In spite of these limitations, we believe that our series is representative of contemporary practice in ovarian cancer. These women were homogenously diagnosed and treated in a tertiary referral centre and histopathological evaluation was performed by highly experienced gynaecological oncology pathologists. The median survival of 45 months is comparable with that of recent series in the taxane era, while stage, histology and grade distribution were the expected for otherwise unselected patients. Finally, the independent prognostic value of performance status and residual tumour volume are in concert with previous findings in similar populations.
We found no association of tumour type with OS. This is unlikely to be due to methodological limitations. Endometrioid tumours were reviewed by a gynaecological oncology pathologist in order to precisely assign grades 2 and 3, since the former are considered low-grade for the purposes of tumour type classification. In keeping with previous reports,\textsuperscript{11} seven of 48 cases required reclassification to serous or clear-cell carcinomas. Since high-grade endometrioid tumours have been assigned to both types,\textsuperscript{1,21} we performed both analyses, which yielded similar results. Further analyses showed strikingly similar or even better outcome for grade 3 compared to grade 2 tumours. In addition, grade 3 endometrioid carcinomas had a better outcome than other type II tumours. Both these results led us to group all endometrioid carcinomas together, instead of assigning grade 3 tumours to type II. We assigned all grade 2 serous carcinomas to high-grade without further review. This was within the concept of this analysis, which aimed not to assess the reproducibility of different grading systems, but to use information from routine practice to assess the prognosis of women with advanced ovarian cancer, but it was also felt that this was unlikely to substantially affect our results. As mentioned, grading was based on international criteria, initially on the 3-tier system\textsuperscript{11} and subsequently on the 2-tier system.\textsuperscript{19} Comparisons between the two systems have consistently shown that 95\% of grade I tumours will be classified as low-grade,\textsuperscript{27,28} while a similar percentage of grade II and III tumours were classified as high-grade.\textsuperscript{14} According to our results, the 2-tier system has prognostic relevance (in contrast to the 3-tier system) and is keeping more with the recent knowledge regarding the two pathways of oncogenesis in the ovary.\textsuperscript{1,3,21} It could be argued that type I tumours are underrepresented in this population, since they are more frequent in early stages of the disease. Indeed, we found a 44\% type I tumour in early (II-IIA) stage ovarian cancer in a recent study.\textsuperscript{29} Similarly to this report, we found that tumour type also had no prognostic value in the early stage disease. There was considerable prognostic heterogeneity among type I tumours. The proposed stratification to tumour types has, apart from the common pathway of oncogenesis, been based on common molecular characteristics, such as Kirsten rat sarcoma (KRAS), BRAF and p53. Nevertheless, important molecular differences among the different tumours included in type I have also been described:\textsuperscript{1} b-catenin and PTEN mutations have been described in endometrioid carcinomas but not serous or mucinous\textsuperscript{30}; similarly, a high frequency of mutations of the transforming growth factor II as well as the tumour-suppressor gene ARID1A have been found in clear cell compared to other ovarian carcinomas.\textsuperscript{31,32} Whether molecular differences could be responsible for the different outcomes have not been conclusively answered yet. Recent data from our group,\textsuperscript{29} as well as from others,\textsuperscript{33} have suggested a different prognostic role of histological subtypes in early stage disease, with serious histology being an adverse factor, while the other types have a rather similar outcome. This indicates that baseline molecular characteristics are unlikely to solely explain the differences in the behaviour of the different ovarian carcinomas. In contrast to type I, type II tumours had remarkably

<table>
<thead>
<tr>
<th>Table 3 – Multivariate analysis of prognostic factors in 568 patients with advanced ovarian carcinomas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables (n)</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Stage</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Grade</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Residual</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PS</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Histology</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cell type</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

HR: hazard ratio.
similar median OS. The reason for this finding cannot be explained from the existing data, regarding undifferentiated carcinomas, since the pathogenesis and the molecular characteristics of these tumours are poorly described. Unspecified adenocarcinomas are high-grade adenocarcinomas, for which distinction between serous and endometrioid is impossible. It seems that the behaviour of these tumours closely resembles that of high-grade serous tumours and not their endometrioid counterparts.

Our results support the previously proposed classification for optimally debulked and early-stage ovarian cancer, based on five cell types: low-grade serous, mucinous, endometrioid, clear-cell and high-grade serous. We propose to use this classification also for unselected advanced ovarian cancer treated with paclitaxel and carboplatin with the addition of undifferentiated carcinomas and unspecified adenocarcinomas to the last group. This has practical implications, since the distinction of these tumours based on pathological criteria can be problematic. Tumour grade was also an independent prognostic factor. Its significance, however, was restricted only to low- versus high-grade serous carcinomas, while prognosis of grade 2 and grade 3 tumours was similar. Tumour grade was of no prognostic significance in mucinous and endometrioid carcinomas and it is irrelevant in clear-cell carcinomas. Compared to tumour grade, cell type assignment is more reproducible and it may be further improved by the introduction of type-specific molecular markers, such as WT1 for serous and HNF1B for clear-cell. For these reasons, we believe that cell type is a more useful prognostic factor than tumour grade.

It has been suggested that type I tumours are chemoresistant. Although this is true for mucinous carcinomas, the other types included in type I were not. In fact, the most chemosensitive tumours were low-grade serous carcinomas. Previous reports indicating that this cell type is chemoresistant suffer from important limitations, such as low number of patients, use of objective responses as criterion for chemoresistance and considerable variability in the chemotherapy used. We believe that our findings are more reliable, since we used the current guidelines to define platinum-resistance. Our results also strengthen the notion that mucinous carcinomas are largely unaffected by current chemotherapy and quality of surgery is the most critical factor for outcome. No deaths occurred beyond the first two years from surgery and a plateau in the survival curve was evident.

In spite of the lack of prognostic significance, the classification into type I and II accurately reflects the carcinogenesis pathway. The different molecular characteristics of type I and II tumours may have therapeutic implications. The high incidence of KRAS and BRAF mutations in type I suggests that anti-epidermal growth factor receptor (EGFR) strategies are unlikely to be beneficial in these tumours. On the contrary, the high incidence of p53 mutations among type II tumours may indicate a high probability for platinum resistance, while ERBB2 overexpression in a significant percentage suggests that some patients might benefit from therapies targeting this receptor.

In conclusion, tumour cell type can be used to subclassify epithelial ovarian cancer into prognostically relevant groups, in contrast to the type of tumour origin and tumour grade. Cell types reflect differences in underlying molecular profile and sensitivity to current chemotherapy and should, therefore, be used to stratify patients included in clinical studies in advanced ovarian cancer.

Conflict of interest statement
None declared.

REFERENCES


