

ORIGINAL ARTICLE

Chemotherapeutic drug sensitivity of primary cultures of epithelial ovarian cancer cells from patients in relation to tumour characteristics and therapeutic outcome

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Abstract

Background. A number of chemotherapeutic drugs are active in epithelial ovarian cancer (EOC) but so far choice of drugs for treatment is mostly empirically based. Testing of drug activity in tumour cells from patients might provide a rationale for a more individualised approach for drug selection. **Material and methods.** Sensitivity of EOC to chemotherapeutic drugs was analysed in 125 tumour samples from 112 patients using a short-term primary culture assay based on the concept of total cell kill. Sensitivity was related to tumour histology, treatment status and clinical tumour response. **Results.** For most EOC standard drugs serous high grade and clear cell EOC were the most sensitive subtypes and the mucinous tumours the most resistant subtype. Docetaxel, however, tended to show the opposite pattern. Samples from previously treated patients tended to be more resistant than those from treatment naïve patients. The activity of cisplatin correlated with that of other drugs with the exception of docetaxel. Tumour samples from two sites in the same patient at the same occasion showed similar cisplatin sensitivity in contrast to samples taken at different occasions. Samples from patients responding in the clinic to treatment were more sensitive to most drugs than samples from non-responding patients. At the individual patient level, drug sensitivity in vitro compared with clinical response showed sensitivities and specificities in the 83–100% and 55–83% ranges, respectively. **Conclusions.** Assessment of EOC tumour cell drug sensitivity in vitro provides clinically relevant and potentially useful information for the optimisation of drug treatment.

Epithelial ovarian cancer (EOC) is in general a chemosensitive disease. In advanced stages, surgical tumour reduction followed by systemic chemotherapy is the treatment of choice [1]. Combination of paclitaxel and carboplatin is the current gold standard treatment regardless of histological subtype or grade. However, although first line treatment is effective in most patients, the disease will relapse in a majority of patients. In this situation, chemotherapy

is generally less effective, but many patients respond to repeated platinum-based treatment. Besides platinum compounds, a number of other drugs have documented clinical activity [2].

Current chemotherapy in EOC follows principles based on empirical clinical knowledge and results of randomised clinical trials using standardised regimens in large patient cohorts. However, OC is a heterogeneous disease with regard to tumour biology

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and clinical behaviour. Although patient survival has improved, the 'one treatment fits all' approach may not be optimal. Obviously, it does not take into account variability of tumour- and patient-related characteristics of potential importance for the outcome. Therefore, attempts have been made to develop individualised cancer therapy, e.g. using in vitro chemosensitivity testing [3].

Chemosensitivity testing implies determination of tumour cell sensitivity to anticancer drugs in vitro [4]. Identifying which tumour diagnoses, subtypes and grades that might respond to an agent could prove useful. In addition, identifying inactive drugs could spare patients ineffective treatment that only produces side effects. Experimental treatments could be offered in disease refractory to established therapy.

The semi-automated fluorometric microculture cytotoxicity assay (FMCA) has been developed and used for individualised drug selection and drug development [5,6]. FMCA is a short-term in vitro drug sensitivity assay based on the concept of total cell kill. The FMCA is rapid and easy to perform, and reports clinically relevant cytotoxic drug sensitivity data [7–9]. A good correlation has been shown between the results obtained by the FMCA and by other methods for measuring drug-induced cytotoxicity, e.g. the DiSC assay [10].

We used the FMCA for analysis of samples from patients with EOC, ovarian borderline tumours (BOT) and primary peritoneal cancer. The aims were to assess drug sensitivity related to histological tumour subtypes and grades, patient treatment status and tumour sample type, as well as drug cross-resistance and drug activity in vitro in relation to treatment response in the clinic.

Material and methods

Patients and tumour sampling

Ovarian and primary peritoneal tumour samples from five hospitals were analysed for drug sensitivity at the Department of Clinical Pharmacology, Uppsala University Hospital, and were included in this study. Samples excluded were those which did not fulfil criteria for a successful chemosensitivity test (see below), leaving us with 130 samples from 116 patients. After histological review of slides for diagnosis by a specialist in gynaecologic pathology (SC), five samples from four patients were not considered to be consistent with epithelial ovarian or primary peritoneal malignancy, leaving for further analyses 125 samples from 112 patients. Two samples were obtained from each of 13 patients. Patient and sample characteristics are shown in Table I.

Tumour sampling by surgery, ultrasound guided biopsy or therapeutic removal of malignant ascites

Table I. Demographic features and sampling details of the patients and tumour samples included.

Variable	N
Samples	125
Patients	112
Mean age at diagnosis (range)	55 (23–84)
Tumour type at diagnosis	
Invasive ovarian cancer	99
Primary peritoneal cancer	2
Borderline tumour (BOT)	10
Borderline with microinvasion	1
Stage at diagnosis	
St I	13
St II	9
St III	70
St IV	19
St IVsuspected	1
Histological grade	
Grade 3 (high grade)	73
Grade 2 (medium grade)	16
Grade 1 (low grade)	4
Borderline (BOT)	11
Ungraded (Clear cell cancer)	8
Histological subtype	
Seropapillary (serous)	67
Endometrioid	25
Mucinous	6
Clear cell cancer	8
Mixed	6
Treatment status at sampling	
Untreated	48
Treated	77
Sample type	
Solid sample (surgery, biopsy)	80
Fluid sample (effusion)	45
Treatment lineage at sampling	
At diagnosis	46
At first relapse	28
At later relapse	51

or pleural fluid was most often part of the routine diagnostic or treatment-related procedures, but was done exclusively for the in vitro analysis in some cases. The samples were obtained either at diagnosis from chemotherapy naïve patients, or at relapse/progression, following various numbers of treatment lineages. Chemotherapy treatment decisions were made by the treating physician as clinically indicated and according to local treatment programmes and recommendations. The assay results were also available for the clinicians and may in some cases have influenced the choice of therapy. The study was approved by the research ethical committee of the Uppsala University Hospital and all patients provided informed consent.

Clinical data and assessment of clinical outcome

Relevant clinical data were extracted from patient records and entered into a database together with the results from the drug sensitivity test. Treatment

baseline evaluation and response evaluations were routinely performed before start of chemotherapy and after every third course of chemotherapy, i.e. approximately every second month. Tumour volume assessment was performed by gynaecologic examination under anaesthesia, at second look or interval surgery and/or by computerised tomography. Tumour response (complete response, CR; partial response, PR; stable disease, SD or progressive disease, PD) was evaluated based on WHO criteria. Serum levels of the tumour marker CA-125 were recorded and serological response was defined according to modified Rustin criteria [11]. For judgment of response to each treatment lineage, the combined final response evaluation at or within three weeks after the end of the last course of chemotherapy was used, by combining the clinician's judgment of the clinical, surgical and radiological response and the tumour marker results. Patients that had undergone radical surgery and/or had no measurable tumour at baseline were in some cases considered evaluable for tumour response based on the tumour marker CA-125 levels only [12].

Only patients treated with at least three courses of chemotherapy were considered as evaluable for tumour response, but one course was considered sufficient in case of unequivocal progression. Death from cancer was scored as PD.

Cell preparation

Tumour cells from solid tumour tissue were prepared by mincing the tissue to $<2 \text{ mm}^3$ pieces followed by digestion in culture medium containing collagenase whereas tumour cells from effusions were collected by centrifugation and purification as described previously [10].

The cells obtained were single cells or small cell clusters as judged by morphological examination of May-Grünwald-Giemsa-stained cytocentrifuge preparations. The fraction of viable cells was verified to be $>90\%$ by the trypan blue dye exclusion test. The cells were washed and re-suspended in complete medium, i.e. RPMI 1640 (HyClone) supplemented with 10% foetal calf serum (HyClone), 2 mM glutamine, 50 g/ml streptomycin, and 60 g/ml penicillin (HyClone), prior to seeding on culture plates.

Reagents, drugs and preparation of experimental plates

Fluorescein diacetate (FDA; Sigma) was dissolved in DMSO (Sigma) and was kept frozen as a stock solution (10 mg/ml) at -20°C protected from light. Seven chemotherapeutic drugs deemed as relevant in treatment of OC were chosen for the analyses. The drugs used were from commercially available

clinical preparations and were diluted according to instructions. The concentrations ($\mu\text{g/ml}$) tested in vitro were: cisplatin (Cis; Bristol-Myers Squibb, Stockholm, Sweden) 10, doxorubicin (Dox; Pfizer, Stockholm, Sweden) 2.5, docetaxel (Dtax; Aventis Pharma, Stockholm, Sweden) 5, the active metabolite of cyclophosphamide, 4-hydroperoxycyclophosphamide (4HC; ASTA Medica, Frankfurt, Germany) 10, topotecan (Topo; GlaxoSmithCline, Mölndal, Sweden) 2.5, mitomycin C (MitC; Medac, Varberg, Sweden) 2.5 and 5-fluorouracil (5-FU; Roche, Stockholm, Sweden) 50.

These drug concentrations have been derived empirically to produce a clinically relevant spectrum of drug activity in various tumour types [6] and correspond fairly well to the maximal plasma concentrations achievable in patients, whereas the total drug exposure is mostly higher in vitro than in vivo [13]. Due to shortage of tumour cells, all tumour samples were not investigated for every drug.

Experimental V-shaped 96-well microtitre plates (Nunc, Roskilde, Denmark) were prepared with a total of 20 μl /well of drug solution at 10 times the desired final concentration with a programmable pipetting robot (ProPette; Perkin Elmer, Norwalk, Connecticut). The plates were stored frozen at -70°C for up to two months until use. The experiments were performed with continuous drug exposure. Each drug was tested in triplicate wells.

Fluorometric microculture cytotoxicity assay

On day one, 180 μl /well of the tumour cell preparation (10 000 to 30 000 cells/well) were seeded in triplicates on microtitre plates prepared in advance with cytotoxic drugs as described above. Six blank wells received only culture medium and six wells with cells but without drugs served as controls. Under these culture conditions the tumour cells are in suspension as single cells or small cell clusters. The culture plates were then incubated at 37°C in humidified atmosphere containing 95% air and 5% CO_2 . After 72 hours the plates were centrifuged (200 g, 5 minutes), the medium was removed and the cells were washed once in phosphate buffered saline, followed by addition of 100 μl /well of a physiological buffer containing 10 $\mu\text{g/ml}$ FDA to all wells. After incubation for 30–45 minutes at 37°C , the fluorescence from each well was read in the fluorometer Fluoroscan 2 (Labsystems Oy, Helsinki, Finland).

Quality control and quantification of results

Quality criteria for a successful assay included $\geq 70\%$ tumour cells in the cell preparation prior to incubation and/or at the assay day, as checked by

microscopic inspection of stained cytocentrifuge slide preparations, a fluorescence signal in control cultures of $\geq 5 \times$ mean blank values and a coefficient of variation in control cultures of $\leq 30\%$. The overall success rate in the assay was approximately 75%, with a too low proportion of tumour cells in the cell preparation being the most common cause of assay failure. Other causes were shortage of cells and low fluorescence signal in controls. Only successfully analysed samples are reported in this study.

The results obtained by the indicator FDA are presented as survival index (SI) defined as the fluorescence of the test as a percentage of control cultures, with blank values subtracted. For the analysis of tumour cell drug sensitivity by different histological subtypes and grades, as well as for the analysis of drug cross-resistance, only the first sample taken was used for each patient with two samples. For the analysis of drug sensitivity versus treatment status, as well as drug sensitivity versus clinical response, the second samples taken at the same occasion were excluded.

For in vitro–in vivo comparisons at the individual patient level, the in vitro sensitivity was defined according to the sensitivity of the tumour cells to the best drug tested (i.e. the drug producing the relatively highest cell kill in vitro) that was actually given to the patient. If not all component drugs in the combination given to the patient were tested, the sample was excluded from this analysis. Post-test probabilities of response were calculated by using Bayes' rule [14].

Statistical analysis

For the comparison of SI values between different histological subtypes and grades, statistical inferences were calculated using the non-parametric Kruskal-Wallis test and, for pairwise comparisons between tumour types, the Mann-Whitney test, with Bonferroni correction for multiple testing. For analysis of correlations ('cross-resistance') between SI values for selected drugs the Spearman's rank correlation coefficient was calculated. The slope of the regression line was calculated with the least squares method. For comparisons between in vitro sensitivity and clinical response, 2×2 contingency tables were constructed and the statistical significance was calculated with Fisher's exact test. The level of significance was set to two-sided $p < 0.05$. The statistics software used was GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Demographic features and sampling properties

The most important demographic features of the material are shown in Table I. The samples taken at

diagnosis were almost all of solid tissue, whereas the relapse samples from previously treated patients were either solid tissue or fluid (effusion) samples. All samples of mucinous tumours were solid, but many of the samples of other histological types were fluid. Almost all mucinous samples were of grade 1 or of borderline histopathology, while all endometrioid samples were of grade 2 or 3, and the serous samples were of all grades. Clear cell tumours are considered as clinically aggressive but are generally not graded [15], and in this study they were classified as ungraded. The samples from early stage tumours (stages I and II) were most often of endometrioid or clear cell type.

Tumour cell drug sensitivity by histological subtype, grade, treatment status and drug cross-resistance

The distribution of SI values for the various cytotoxic drugs and for the histological subgroups of serous (ser), endometrioid (end), mucinous (muc) and clear cell tumours (ccc) is shown in Figure 1. Serous samples, constituting the largest group, are also shown according to grade. The mucinous samples were the most resistant and the clear cell and serous samples were the most sensitive sample types to the majority of drugs. For the following drugs, there was a significantly different in vitro sensitivity for any subtype compared to the serous type: Cis for ser versus muc ($p = 0.005$), Dox for ser versus end ($p = 0.024$) and ser versus muc ($p = 0.0099$), 5-FU (not shown) for ser versus muc ($p = 0.006$), and MitC for ser versus muc ($p = 0.021$). The exception to this pattern was Dtax, albeit non-significant, but with seemingly an almost inverse pattern of drug sensitivity for different tumour subtypes compared to that of the other drugs.

Of the serous samples, the high-grade samples were most sensitive and the low-grade/borderline samples most resistant to the majority of the drugs. However, a statistically significant difference in sensitivity as per grade was observed only for MitC ($p = 0.024$; not shown).

When comparing the sensitivities of solid samples of different grades, without considering the histological subtypes, there were some significant differences. Pairwise comparisons showed significantly higher SI values for low grade compared with high grade samples for Cis ($p = 0.038$), 4HC ($p = 0.026$) and Topo ($p = 0.047$), and for low grade compared with medium grade samples for MitC ($p = 0.025$) after correction for multiple testing (not shown).

The comparison of drug sensitivity in samples from treatment naïve and previously treated patients was partly compromised by patient selection and by the histological, clinical and sampling characteristics

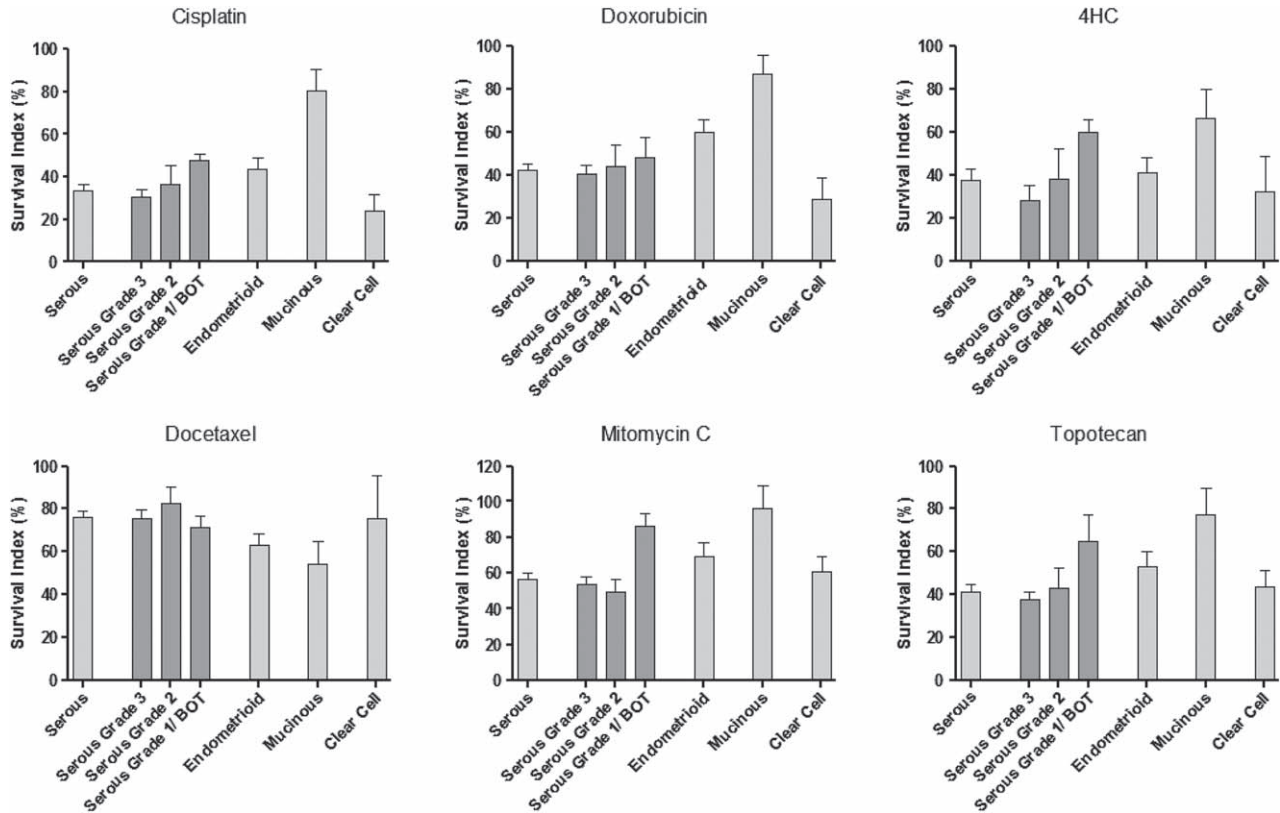


Figure 1. Survival index (mean + SEM) in different subtypes at empirically derived cut-off concentrations of the drugs indicated. For the serous type, data are also presented according to histological grade. 4HC, 4-hydroperoxycyclophosphamide.

being unevenly distributed between the two groups. Therefore, this analysis was restricted to the largest and most homogeneous group, i.e. the serous high grade tumour samples. The data for this subgroup are shown in Figure 2 divided into solid samples from untreated and treated patients and fluid samples

from treated patients. Solid samples from treated patients had higher median SI values compared to those from untreated patients for all drugs except for Dtax, but the difference was statistically significant only for MitC ($p = 0.008$) and Topo ($p = 0.011$). Median SI values for solid versus fluid samples from previously treated patients showed no significant difference, but there seems to be a larger spreading of the SI values for fluid samples for most drugs.

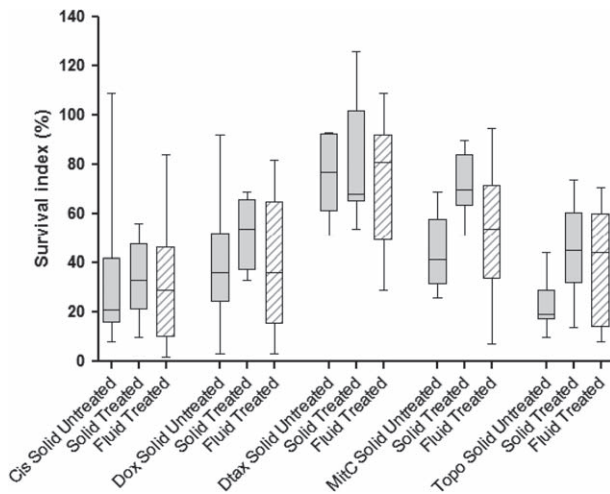


Figure 2. Survival index (median, quartiles and range) for solid samples of serous grade 3 tumours, divided into untreated and treated solid samples and treated fluid samples, at empirically derived cut-off concentrations of the drugs indicated. C; Topo, topotecan; Cis, cisplatin; Dox, doxorubicin; Dtax, docetaxel; MitC, mitomycin.

Concerning drug cross-resistance, significant positive correlations were observed between SI values for Cis versus Dox, 5-FU, 4HC, MitC and Topo with correlation coefficients of 0.79, 0.56, 0.74, 0.73 and 0.78, respectively (Supplementary Figure 1 available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2013.794956>). Interestingly, there was no significant correlation for drug activity between Cis and Dtax ($r = 0.11$).

Two samples from the same patient

There were 11 patients with two samples analysed for Cis sensitivity (Supplementary Figure 2 available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2013.794956>). In five patients, the samples were taken from two different sites on the same occasion, and in six patients, the samples

were taken on different occasions, with chemotherapy given in between. Cis sensitivity in samples taken from different locations on the same occasion was essentially equal. However, Cis sensitivity in samples taken on different occasions differed considerably, without a uniform pattern of change in the second compared to the first sample.

In vitro drug sensitivity versus clinical response

Drug sensitivity data analysed in relation to clinical response classification at group level for the largest reasonably homogeneous subgroup, the serous grade 2–3 solid samples, are detailed in Figure 3. The median SI values for clinical responders (CR or PR) were lower compared to those for non-responders (SD or PD) for several drugs, but with a great overlap between these groups. The difference was statistically significant for Cis ($p = 0.025$), Dox ($p = 0.041$) and 5-FU ($p = 0.049$). For in vitro–in vivo comparisons at the individual patient level, not all drugs received by the patients were tested due to a limited amount of tumour cells. For some drugs, SI values for related drugs were used as surrogate values. Thus, for patients who received carboplatin, epirubicin and paclitaxel that were not included for analyses within this study, the closely related drugs Cis, Dox and Dtax data were used as surrogate drugs, respectively, for defining sensitivity in vitro.

The in vitro drug sensitivity data were analysed in relation to the clinical response to the treatment that was given immediately after the in vitro test was performed. For this analysis, 47 individual in vitro–in vivo correlations were identified. The most common causes of drop-outs from this analysis were the following: the clinical response was not assessable, all drugs received by the patient were not tested, or the patient received no chemotherapy

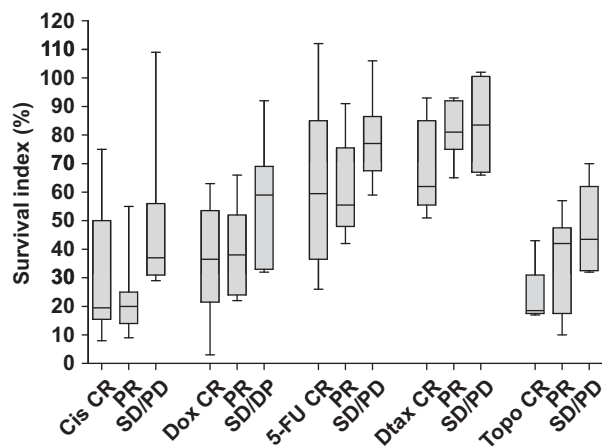


Figure 3. Survival index (median, quartiles and range) for solid samples of serous grade 2–3 tumours for the drugs indicated divided into categories according to clinical response.

immediately after the assay. The samples with in vitro–in vivo correlations were representative of the total material with respect to the features detailed in Table I (not shown).

In vitro–in vivo comparisons were performed using three different types of analyses, denoted ‘classic’, as used previously for this type of analyses [5], ‘delimited’ and ‘confined’ analysis, respectively. In all these analyses, the in vitro sensitivity was defined according to the sensitivity of the tumour cells to the most active drug tested that was actually given to the patient. In the ‘classic’ analysis, the median SI values for all tumour samples were used as a cut-off for in vitro sensitivity. In this heterogeneous, crude mix of solid and fluid samples from patients ($n = 47$) with various characteristics, sensitivity and specificity were low, i.e. 76% and 42%, respectively, and the distribution was not statistically significant (Supplementary Table IA available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2013.794956>). The ‘delimited’ analysis was limited to solid samples only ($n = 29$), with samples with an SI value \leq median for solid samples defined as sensitive, and resulted in improved sensitivity and specificity, 83% and 55%, respectively, and a statistically significant distribution (Supplementary Table IB available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2013.794956>).

The ‘confined’ analysis was restricted to the most homogeneous subgroup of patients in terms of histopathology, i.e. those with solid samples of serous grade 2–3 tumours, in order to compare ‘like with like’. The median SI values for serous grade 2–3 samples were used as a cut-off for sensitivity. This reduced the number of evaluable patients to 18 but provided a high sensitivity and specificity of 100% and 83%, respectively, and a highly statistically significant distribution (Supplementary Table IC available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2013.794956>).

Post-test probability of response

When using this kind of a test in the clinical setting, and when interpreting the results of the test, also pre-test probabilities of response should be taken into account. This approach is exemplified here for the results obtained, in relation to treatment lineage. The observed clinical response frequencies for chemotherapy in the different treatment lineages for all patients in our material were: primary treatment 72%, treatment at first relapse 32%, at second relapse 14%, at third relapse 4% and at fourth or later relapse 0%. These frequencies are fairly similar to those reported in the literature and were used as estimates of pre-test probabilities of response for the different

treatment lineages. Using the observed test sensitivity and specificity for solid samples, this gives the following post-test probabilities for response when the test showed drug sensitivity versus resistance: primary treatment 83% versus 44%, first relapse 47% versus 13%, second relapse 23% versus 5%, third relapse 7% versus 1%, and fourth or later relapse 0% irrespective of test result.

Discussion

The serous and clear cell tumours were the most platinum sensitive subgroups *in vitro* and the mucinous tumours the most platinum resistant. Among the serous tumours, platinum sensitivity seemed inversely related to the degree of differentiation. These observations are generally consistent with the clinical response behaviour of these tumours [16,17]. However, clear cell cancers are generally considered to be aggressive and to have a poor prognosis [18]. They are also considered to be platinum resistant with increased drug efflux and slow proliferation proposed as possible resistance mechanisms [19,20]. Thus, the relatively high cellular platinum sensitivity of these tumours in our material is not consistent with clinical experience. However, the number of clear cell tumour samples was small and mostly from effusions obtained at relapse. This highly selected group may not adequately reflect the drug sensitivity for this tumour subtype.

Interestingly, the *in vitro* sensitivity pattern among the EOC subtypes for Dtax was rather the opposite to that of Cis. This implicates that for some subtypes, notably the mucinous tumours, inclusion of a taxane drug might be more important than for other subtypes. However, to our knowledge there are no clinical data to support this notion.

When comparing SI values in solid samples of different grades, low grade/BOT samples were found to be more resistant *in vitro*. This finding is in line with an earlier *in vitro* report on chemosensitivity in low versus high grade serous carcinoma [21]. However, these tumours often tend to have an indolent course.

For most drugs, solid samples of serous high-grade tumours obtained from previously treated patients tended to be more resistant than those from untreated patients. These data should be interpreted with caution due to the problem of patient selection by treatment, but provide some support for the notion of acquired cellular drug resistance from chemotherapy [22]. The activities of all the drugs tested, except that of Dtax, showed a quite strong positive correlation with that of Cis, indicating a significant cross-resistance that should be considered when selecting drugs for combination treatment. However, the activity of Cis did not correlate with that of Dtax.

Thus, combining Cis and Dtax would provide a broader spectrum of anti-tumour activity than other combinations. This is in line with previous findings *in vitro* [23,24] and *in vivo* [25] and with the facts that platinum-taxane combinations have shown the best response rates in clinical trials [26] and that they constitute the standard first-line chemotherapy for EOC today [2].

Samples taken from different tumour sites in the same patient at the same occasion showed very similar Cis sensitivity. Although the number of observations is low, they indicate that concurrent tumour subclones with varying drug sensitivity are uncommon in OC. In samples taken at different occasions, however, the sensitivity for Cis had changed considerably and, somewhat unexpected, in either direction between the first and the second sample. This observation may indicate development of tumour subclones over time and/or by treatment.

Generally, among the solid samples from serous tumours of grade 2–3, clinical responders had lower median SI values compared with clinical non-responders, although with a considerable overlap between these response categories. This pattern indicates a correlation between drug activity *in vitro* with that in the patient, but also that the clinical response is influenced by other tumour and patient-related factors. For EOC, there are many well-known prognostic factors [27–31]. Many of these factors may not be reflected in an *in vitro* assay like the FMCA.

The heterogeneity of the patient population was a problem when comparing *in vitro* chemosensitivity to clinical response in individual patients. Not unexpectedly, the ‘classic’ analysis for comparisons did not perform very well in this mix of different sample and tumour types, since the optimal performance of an *in vitro* assay is based on a comparison of ‘like with like’. Due to this, reasonable alternative approaches were used in order to improve group homogeneity. In the ‘delimited’ analysis, fluid samples were excluded, which resulted in improved sensitivity and specificity.

In the ‘confined’ analysis, individual correlations were restricted to a reasonably homogeneous subgroup, i.e. solid samples of serous grade 2–3 tumours. Again, this analysis produced much better results than the ‘classic’ analysis with an impressive sensitivity and specificity of 100% and 83%, respectively. This gives support to the notion that different subtypes represent separate diseases, and should be analysed separately [32]. Considering the post-test probabilities of response for the different lineages, it appears that for primary chemotherapy, due to the high pre-test probability of response, there still is a relatively high chance of responding even when the test shows resistance. At the other end of the

spectrum, for late line therapies, the post-test probability of response is low, even if the test shows sensitivity. The situations in which an in vitro test would be most useful seem to be in patients receiving chemotherapy for the first or second relapse, when there still is a reasonable chance of obtaining a clinical response.

A number of previous studies have compared in vitro chemosensitivity and clinical outcomes in EOC [33–43]. In some studies [33,39,40,43], no significant association was found between assay results and clinical outcome, but in other studies, significant associations were found between assay results and various types of short- or long-term clinical endpoints [34–38,41,42]. In many of these studies, the study populations and sample types were markedly heterogeneous, which might well explain the lack of relationship between drug activity in vitro and clinical outcome in some of them. For example, survival analysis can be confounded by subtype differences [32]. It has been suggested that the different subtypes, in fact, may represent different diseases [32,44]. Consequently, in vitro assays should be developed, analysed and interpreted separately for each of these subtypes to improve their performance.

It should be acknowledged that the individual in vitro–in vivo comparisons presented here are based on limited data and should rather be regarded as explorative and in need of prospective validation taking into account the importance of disease heterogeneity, other biological and clinical prognostic variables and patient characteristics. If carefully interpreted, in vitro drug sensitivity testing could become a useful tool in efforts to optimise treatment of EOC.

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Supplementary materials available online

Supplementary Figures 1–2 and Table I.