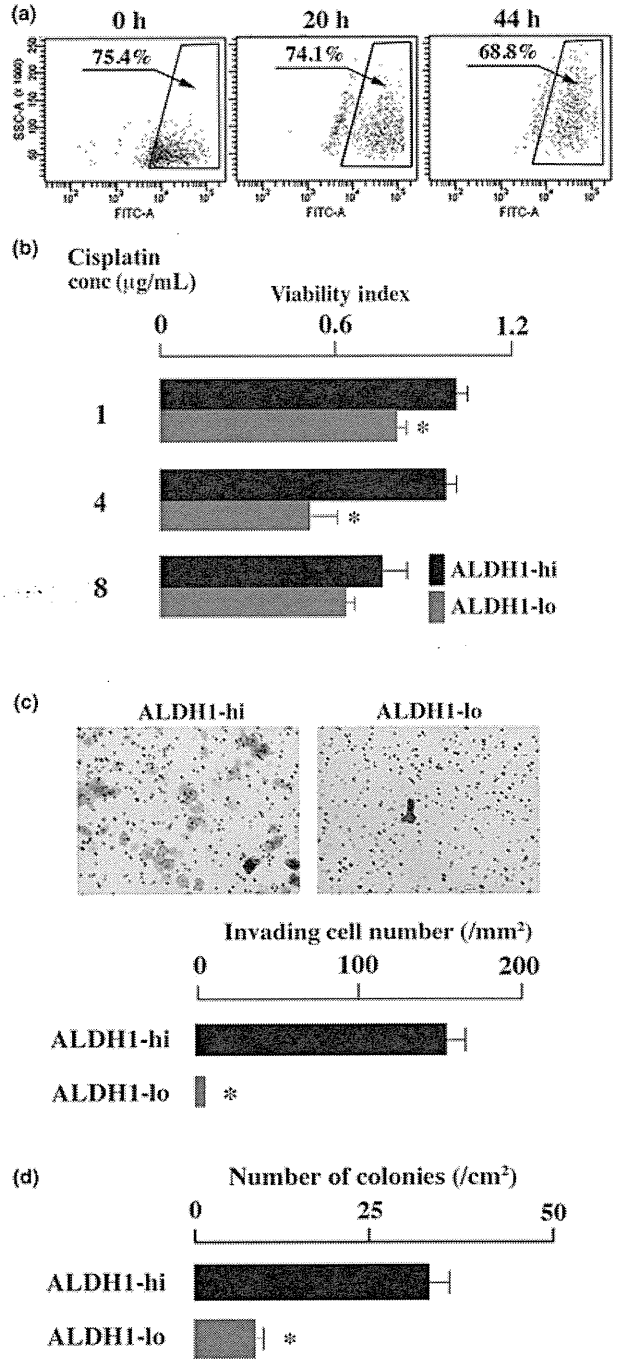

Fig. 4. Effects of ALDH1 on resistance to the anticancer drugs, cell invasion activity and *in vitro* colony formation activity. (a) Percentage of ALDH1-hi population in the sorted cells at 0, 20 and 44 h after sorting. (b) Viabilities of ALDH1-hi and ALDH1-lo HEC-1 cells compared in the presence of various amounts of cisplatin. (c) Matrigel invasion assay. HEC-1 cells invade through Matrigel (×40), and the number of invading cells per square millimeter are shown. (d) Comparison of colony number derived from the ALDH1-hi and ALDH1-lo HEC-1 cells per square millimeter. The values are the means ± SE of three experiments. *$P < 0.05$ (Student's *t*-test).

10% of cells positive for ALDH1 were regarded as ALDH1-hi: 40 (40.8%) of 98 cases were categorized as ALDH1-hi, and the remaining as ALDH1-lo.

Table 2. Correlation between ALDH1 expression in adenocarcinoma and clinicopathological parameters

	ALDH1 expression in cancer		*P*
	Low	High	
Tumor			
T1	43	27	
T2	5	3	
T3	10	10	0.047
Lymph node			
N0	50	23	
N1	8	17	0.002
Tumor histological grade			
Grade 1	28	10	
Grade 2	19	20	
Grade 3	11	10	0.065
Estrogen receptor status			
Positive	10	30	
Negative	11	47	0.474
Progesterone receptor status			
Positive	48	27	
Negative	10	13	0.080
Ki67 labeling index			
≥20%	47	35	
<20%	11	5	0.499
Response to chemotherapy			
Non-respond	4	13	
Respond	20	12	0.009
Recurrence			
Positive	7	13	
Negative	51	27	0.014
Prognosis			
Dead	5	10	
Alive	53	30	0.027

Expression of CD9, ER and PgR is one of the differentiation markers of endometrium. The expression level of CD9, ER and PgR was significantly lower in ALDH1-expressing cells than in non-expressing cells (Fig. 1c).

ALDH1 activity in endometrioid adenocarcinoma cell lines. ALDH1 activity was examined with Aldefluor assay in nine endometrioid adenocarcinoma cell lines: HEC-1, -1A, -108, -116, -6, -88nu and SNG-M contained ALDH-hi population, whereas HEC-251 and SNG-II did not (Fig. 2). In the subsequent experiments, HEC-1, which proliferates rapidly and is easy to handle, was used as a representative endometrioid adenocarcinoma cell line containing an ALDH-hi population.

Cancer-inducing cells are known to yield both CIC and non-CIC, whereas non-CIC does not yield any CIC. To examine whether ALDH1 could be used as a CIC marker for endometrioid adenocarcinoma, ALDH1-hi and ALDH1-lo HEC-1 were sorted separately. After culture for 5 days, cells derived from sorted ALDH1-hi HEC-1 yielded both ALDH-hi and ALDH-lo cells, whereas few ALDH1-hi cells were detected in cells derived from ALDH1-lo cells (Fig. 3).

Comparison of ALDH1-hi cells to ALDH1-lo cells in resistance against anti-tumor drug, abilities of invasion and *in vitro* colony formation. As cisplatin is commonly used for the treatment of endometrioid adenocarcinoma, the effect of ALDH1 on resistance of HEC-1 to cisplatin was examined. The ALDH-hi and ALDH-lo cells were cultured for 20 h, and cisplatin was added. Then, viability was examined at 44 h after sorting. Since 5-day culture of ALDH-hi cells yielded both ALDH-hi and ALDH-lo as described above, there is a possibility that the ALDH-hi cells

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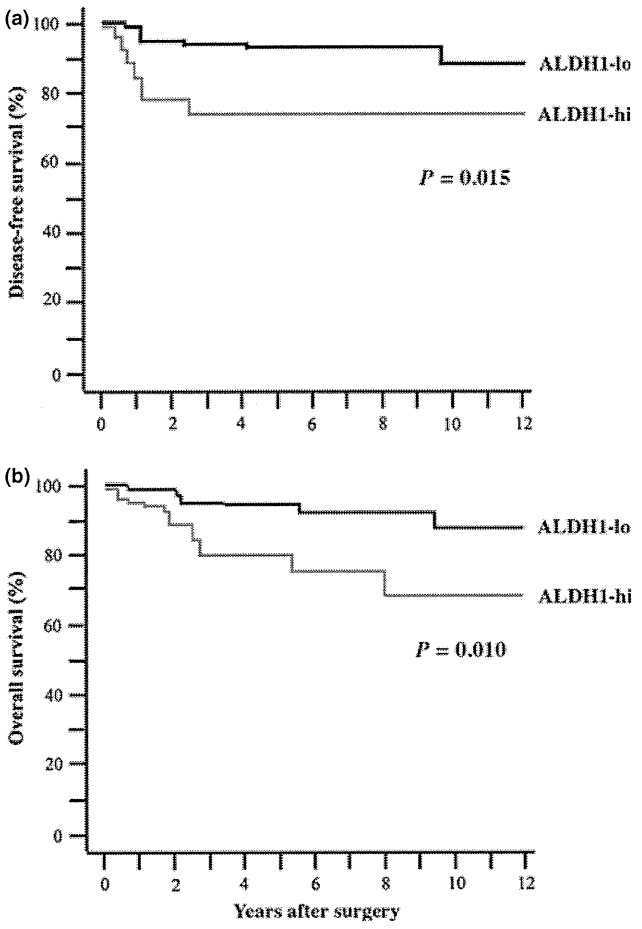

Fig. 5. Kaplan–Meier plots. Disease-free (a) and overall (b) survival curves are shown. The ALDH1-hi cases showed less favorable disease-free survival and overall survival.

yielded significant number of ALDH-lo cells at the time of cisplatin addition and viability check. However, this possibility was unlikely, because the percentage of sorted ALDH-hi cells remained at similar levels at 20 and 44 h to that just after sorting (Fig. 4a). The ALDH1-lo cells were more vulnerable to cisplatin than ALDH-hi cells (Fig. 4b). The invasion ability of ALDH-hi cells was compared to that of ALDH1-lo cells with Matrigel invasion assay: the number of invading cells was lower in ALDH1-lo cells than in ALDH1-hi cells, indicating that ALDH1-hi cells possessed stronger invasive capability than ALDH1-lo cells (Fig. 4c). Next, the ability for *in vitro* colony formation was evaluated. In comparison to ALDH1-hi cells, ALDH1-lo cells formed fewer colonies *in vitro* (Fig. 4d).

Correlation of ALDH1 expression with clinical variables. Correlation of ALDH1 expression with clinicopathological features was evaluated. Positive correlation was observed between ALDH1 expression and T factor ($P = 0.047$), lymph node metastasis ($P = 0.002$), resistance to chemotherapy $P = 0.009$), relapse rate ($P = 0.014$) and poor prognosis ($P = 0.027$). Other parameters including tumor histological grade, ER, PgR and Ki67 labeling index did not correlate with ALDH1 expression (Table 2). The 5-year DFS and OS were 86.7% and 90.6%, respectively. Tumors recurred in 22 patients. Of these, 14 patients died due to the tumors. There was a statistically significant difference in DFS ($P = 0.015$) and OS rates ($P = 0.010$) between patients with ALDH1-hi and ALDH1-lo tumors (Fig. 5).

Univariate analysis showed that T factor, lymph node metastasis, tumor histological grade and ALDH1 expression were significant factors for OS. For DFS, T factor, lymph node metastasis, tumor histological grade, PgR expression and ALDH1 expression were significant factors (Table 3). Multivariate analysis revealed that ALDH1 expression, lymph node metastasis and tumor histological grade were independent prognostic factors for OS, and ALDH1 expression was an independent prognostic factor for DFS (Table 3).

Discussion

Normal stem/progenitor cells of various lineages, such as hematopoietic, neural and mesenchymal stem cells, show high ALDH1 activity.[20–22] In addition, CIC have been reported to show high ALDH1 activity: the ALDH1-hi population is tumorigenic and resistant to chemotherapy in cancers of colon, breast, lung, pancreas, bladder, prostate and ovary.[23–27] To our knowledge, the role of ALDH1 in uterine cancer has never been studied. In the present study, we showed that ALDH1-hi endometrioid adenocarcinoma cells to be more tumorigenic, resistant to anti-cancer agents and invasive than ALDH-lo cells. Culture of the sorted ALDH-hi cells yielded both ALDH-hi and ALDH-lo cells, whereas culture of the ALDH-lo cells yielded ALDH-lo cells alone. These findings suggest that the ALDH-hi population possessed the character of CIC in endometrioid adenocarcinoma of uterus, like cancers of other organs.

In clinical specimens, ALDH1-expressing tumor cells were mostly negative for CD9, ER and PgR, indicating that ALDH1 expression was detected in tumor cells of a less mature state. Since CIC are in the immature state, this was consistent with the notion that ALDH1 was expressed in cells with CIC character.

The expression of ALDH1 was limited to a small portion of endometrioid adenocarcinoma cells, which were randomly

Table 3. Univariate and multivariate analyses of prognostic factors for overall and disease-free survivals

	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Tumor	3.32 (1.58–7.00)	0.002	1.59 (0.66–3.80)	0.302	3.29 (1.58–6.83)	0.002	1.49 (0.61–3.61)	0.383
Lymph node	2.64 (1.66–4.21)	<0.001	2.19 (1.21–3.97)	0.010	1.59 (1.26–2.00)	<0.001	1.29 (0.97–1.70)	0.077
Tumor histological grade	3.32 (1.58–7.00)	0.002	2.93 (1.11–7.74)	0.029	3.29 (1.58–6.83)	0.002	2.43 (0.97–6.12)	0.060
Estrogen receptor status	0.68 (0.23–2.00)	0.482			0.64 (0.22–1.89)	0.421		
Progesterone receptor status	0.40 (0.14–1.10)	0.076			0.36 (0.13–0.99)	0.048	0.44 (0.15–1.28)	0.132
Ki67 labeling index	2.56 (0.34–19.5)	0.364			2.85 (0.38–21.7)	0.311		
ALDH1 expression	3.78 (1.28–11.2)	0.016	4.89 (1.37–17.5)	0.014	3.51 (1.19–10.4)	0.023	3.65 (1.03–13.0)	0.045

CI, confidence interval; HR, hazard ratio.

located in the tumor tissues. Cells with CIC character in squamous cell carcinoma are reported to be located in the outer layer of cancer nests,[29,30] contrasting with the absence of any specific location of CIC in endometrioid adenocarcinoma.

Diffuse expression of ALDH1 was found in some clinical cases of endometrioid adenocarcinoma (Fig. 1d). This appeared to be incompatible with the concept that CIC comprise a small population of cancer cells with multiple differentiations and long-term repopulation capabilities. In several reports, CIC markers were expressed in most tumor cells in clinical samples, such as ALDH1 in breast cancers.[20,31] When most tumor cells possess CIC character, the tumor character might become aggressive. Alternatively, ALDH1 might be a marker of undifferentiated cancer cells but not a CIC marker.

The clinical implication of ALDH1 expression was evaluated in 98 cases of endometrioid adenocarcinoma. The characteristics of patients, such as age and stage, in the current study were similar to those in a previous report,[32] indicating that the results obtained from the current study are commonly applicable to endometrioid adenocarcinoma worldwide. The present study showed that a high level of ALDH1 expression was correlated with T category, lymphatic invasion, resistance to chemotherapy, recurrence, and prognosis of patients. Patients with higher ALDH1 expression showed poorer prognoses than those with lower expression ($P = 0.015$ for DFS and $P = 0.010$ for OS), and high ALDH1 expression was an independent poor prognostic factor. These findings were consistent with the previous observation that a high percentage of ALDH1-expressing cells in most types of epithelial tumors, such as breast, lung, pancreatic, bladder, ovarian and prostate, is associated with a poorer outcome of these patients.[23–27] Thus, ALDH1 might be a common marker for CIC among cancers of various organs.

Endometrioid adenocarcinoma is the most common invasive malignancy of the female genital system, and novel therapeutic strategies targeting CIC would be necessary to improve cure rate. Very recently, Yang *et al.*[33] reported that LIN28 positively and let-7 negatively regulates ALDH1 expression in breast and ovarian cancers, and suggested that targeting ALDH1 expression via a LIN28/let-7 axis by small chemical compounds could be a therapeutic modality. Then, ALDH1 would be an effective target for therapies to CIC not only in breast and ovarian cancers but also in endometrioid adenocarcinoma of the uterus. Further studies on ALDH1 regulation may open a new therapeutic modality for endometrioid adenocarcinoma.

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GYNECOLOGIC ONCOLOGY

Usefulness of computed tomography in predicting cytoreductive surgical outcomes for ovarian cancer

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Abstract
Purpose The objective of this study was to identify features of preoperative computed tomography (CT) scans that can best predict outcomes of primary cytoreductive surgery in ovarian cancer patients.
Methods Preoperative CT scans of 98 patients were evaluated retrospectively. Multiple logistic regression analysis was used to develop two models.
Results Although optimal surgical reduction was attempted in 98 patients, 12 had suboptimal results. Having tumor implants on the small or large bowel mesenteries (any size) or at other sites (cutoff index: ≥1 cm) was found to be significant ($p < 0.001$) for predicting a suboptimal cytoreduction outcome. Two predictive models were created using multiple logistic regression analysis; both consider diffuse peritoneal thickening (DPT), infrarenal para-aortic or pelvic lymph node involvement, a bowel encasement tumor (≥2 cm), and any tumor implants in the cul-de-sac as significant. Model 1 adds consideration to any tumors in the pelvic or retroperitoneum and has an accuracy of 90.8% for predicting a suboptimal surgery. Model 2 (accuracy of 93.9%) adds to the core of predictors the presence of tumor implants on the bowel mesenteries (≥2 cm), omental caking (≥2 cm), and ascites fluid.
Conclusion Using specific CT findings from patients with ovarian cancer, we have devised two predictive models that have an accuracy of greater than 90% for predicting whether cytoreductive surgery will completely remove all tumor tissue, which should greatly aid in the differential decision-making as to whether to attempt cytoreductive surgery first, or to advance directly to neoadjuvant chemotherapy.

Keywords Ovarian cancer · Cytoreductive surgery · Computed tomography · Prediction · Suboptimal

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Introduction

The number of deaths due to ovarian cancer is observably increasing. At the time of diagnosis over half of these patients already had advanced stage III or IV diseases (as defined by the International Federation of Gynecology and Obstetrics, FIGO) because they were largely asymptomatic at earlier stages. The standard therapy against these advanced ovarian cancers consists of a thorough primary surgical cytoreduction of the tumor masses followed by aggressive systemic taxane- and platinum-based chemotherapy. Although SEER (National Cancer Institute Surveillance, Epidemiology and End Results) has reported that the 5-year survival rate of stage III and IV ovarian cancer patients was significantly improved after treatment with cisplatin followed by paclitaxel, the long-term 10-year survival rate remains extremely poor, at less than 10% [1, 2].

Conducting a thorough primary cytoreductive surgery for patients with ovarian cancer is an essential factor for overcoming this disease. An extreme effort should be made to achieve maximal tumor cytoreduction, to the point of no macroscopically visible disease if possible (ideal), or to less than 1 cm of residual disease (optimal cytoreduction) in appropriate circumstances. In patients with optimal cytoreduction, a higher response rate to contemporary chemotherapy and improved survival continues to be observed [3].

Extreme surgical procedures that may be considered for optimal surgical cytoreduction include a radical pelvic dissection, bowel resection, diaphragm stripping, hepatic resection, and splenectomy. Patients who undergo a less successful suboptimal surgery do not have as much benefit, in spite of these attempted aggressive surgical procedures. Furthermore, the suboptimal result may actually worsen the patients' post-surgical performance status, delaying the start of the subsequent chemotherapy, consequently decreasing the survival rate of the patients.

There are reports of the effectiveness, for some advanced ovarian cancer patients, of first administering a neoadjuvant chemotherapy followed by an interval cytoreduction; reportedly, these patients showed a better prognosis compared with those who underwent the primary cytoreductive surgery first [4]. However, there are other reports, for example that of Vergote et al., showing that conducting the neoadjuvant chemotherapy first, followed afterwards by interval debulking surgery, was comparable in outcome to when the primary debulking surgery occurred first, followed by the chemotherapy. Achieving complete resection of all macroscopic disease remains the primary objective, regardless of when the cytoreductive surgery is to be performed [5].

Thus far, the perfect method for predicting beforehand the outcome of an attempted cytoreductive surgery in the patients with epithelial ovarian cancer has yet to be reported. We undertook our current study to develop a reliable model, comprised of CT radiographic features and clinical characteristics (such as serum marker levels of CA 125), for predicting the outcome of attempted primary cytoreductive surgery in patients with advanced ovarian cancer.

Patient and methods

At our Osaka University Hospital, from January 2002 to January 2009, 160 patients were diagnosed with epithelial ovarian cancer. Of these, 98 patients who underwent both a primary cytoreductive surgery (by experienced gynecologic oncologists) and a preoperative spiral CT scan of the abdomen and pelvis (within 4 weeks prior to surgery, and whose CT films were available for review) were enrolled in our retrospective study, which was approved by our Institutional Review Board and our Ethics Committee.

Table 1 Pathologic characteristics

	$n = 98$
Stage	
I	45
a	19
b	1
c	25
II	19
a	3
b	1
c	15
III	29
a	1
b	2
c	26
IV	5
Histology	
Serous	27
Mucinous	8
Endometrioid	25
Clear	35
Others	3

An analysis of their demographic and clinical data is shown in Table 1. Twenty-seven patients had serous adenocarcinomas, whereas 8 had mucinous adenocarcinomas, 25 had endometrioid adenocarcinomas, 35 had clear cell adenocarcinomas, and 3 had some other form of ovarian cancer. All patients had a World Health Organization (WHO) performance status of 0–2. Thirty percent of our patients had a FIGO (International Federation of Gynecology and Obstetrics) stage III disease, while 5% had stage IV disease.

A surgical procedure that left the patient with visible or imaging-detectable residual disease equal to or less than $\leq$1 cm in maximal diameter was considered to have received optimal cytoreduction. We classified as sub-optimal a surgery that left behind any residual disease larger than 1 cm.

All CT (computer tomography) images were obtained using a 5-mm collimation through the abdomen and pelvis with intra-venous contrast. Preoperative CT scans were independently, retrospectively, systematically re-reviewed by one radiologist and one gynecologist, who were blinded to the previous surgical outcomes. The results of the review by the radiologist and gynecologist were compared, and no significant difference in their interpretations was found (data not shown).

The CT scans were particularly analyzed for the presence or absence of certain preselected radiological features. These useful radiological features were chosen based on results from previous studies and these were supplemented

Table 2 Seventeen predictors initially selected for suboptimal cytoreduction of ovarian cancer

Advanced age	
Large volume ascites	
Diffuse peritoneal thickening	
Liver metastasis	
Lymph node swelling	Infrarenal para-aortic
	Pelvic
Unresectable tumors	Bowel encasement
	Others
Tumor implants	Peritoneum of diaphragm
	Porta hepatis or gallbladder fossae
	Omental caking
	Omental extension to spleen or stomach
	Small or large bowel mesentery
	Cul-de-sac
	Invasion of pelvic wall
	Pelvic retroperitonium
	Other places

with additional features from our own clinical experiences. These features included large volume ascites, diffuse peritoneal thickening (DPT), liver metastasis, omental caking, omental extension to the spleen or stomach, pelvic lymph nodes swelling, infrarenal para-aortic lymph nodes swelling, tumor implants on the small or large bowel mesenteries, peritoneum of the diaphragm, hepatic portal or gallbladder fossae, cul-de-sac, or other sites. We also looked for features of any tumors that were unlikely to be successfully surgically removed (unresectable), such as aggressive bowel encasement tumors and invasive pelvic sidewall tumors, or those which simultaneously involved multiple organs (i.e., bladder, colon, liver, etc.), and any tumors occurring in the pelvic and abdominal retroperitoneal spaces. All criteria were examined for the number and size of the tumors, except for the presence of ascites and DPT (Table 2).

In addition to radiological features, preoperative serum CA 125 levels were obtained within 4 weeks before surgery. Clinical data, laboratory values, intra surgical findings, and post surgical results were abstracted retrospectively from the patients' medical records.

Prediction sensitivity was defined as the number of correctly defined suboptimally debulked cases (true positives) divided by the total number of suboptimally cytoreduced patients. Specificity was defined as the number of correctly defined optimally debulked cases (true negatives) divided by the total number of optimally cytoreduced patients. NPV (negative predictive value) corresponded to the number of true negatives divided by the total number of negative results for each parameter, and PPV (positive predictive value) corresponded to the number of true positives divided by the total number of positive results for each parameter. Accuracy was calculated as the sum of the true positives and true negatives divided by the total number of patients in the study.

The Student *t* test and the Mann–Whitney tests were used to compare median age and median CA 125 levels between patients with optimal versus suboptimal cytoreduction. Cross-tabulation and univariate comparisons of the percentage of patients who underwent suboptimal cytoreduction were carried out using logistic regression for each of the potential radiologic features. Using multiple logistic regression analysis, we identified specific combinations of these radiologic features that predicted a suboptimal outcome for the subsequent surgery.

Results

Eighty-six (87.8%) of the 98 enrolled patients had optimal primary surgical results, i.e., they were cytoreduced to residual disease of $\leq$1 cm at the primary surgery. As might be expected, optimal cytoreduction was achieved in all 64 cases of patients with stage I and II diseases. In stage III patients, optimal surgery was achieved in 19 of 29 (65.5%) and in stage IV, in 3 of 5 cases (60%). Suboptimal surgery results were observed in 12 of the 98 patients (12.2%), 10 in stage III, and 2 in stage IV disease.

The sites of residual inoperable tumor (greater than 1 cm in diameter) were in the cul-de-sac, greater omentum, diaphragm, multiple sites on the rectum, colon, and vesicouterine pouch. The types of radical surgeries performed for patients to achieve an optimal cytoreduction result included large bowel resection (7 cases), liver resection (3 cases), appendectomy (7 cases), splenectomy (1 case), diaphragm resection (2 cases), and gastrectomy (1 case). In one patient with a suboptimal result, a palliative small bowel resection to avoid intestinal obstruction was performed.

The mean age of the study population was 54 years (range 33–79). There was no statistically significant difference in ages between the optimal and suboptimal cytoreductive groups ($p = 0.12$, 53.3 vs. 58.8) using the *t* test. The median preoperative CA 125 serum level was 176 units/mL (range 7–21,887 units/mL) for all patients; it was 126 units/mL (range 7–21,887 units/mL) in the optimal cytoreduction group and 431 units/mL (range 10–5,400 units/mL) in the suboptimal cytoreduction group. Having a CA 125 level greater than 250 or 500 was not predictive of surgical outcome. Using the Mann–Whitney test, no significant difference of CA 125 levels between the two groups ($p = 0.18$) was observed.

In age-adjusted models, univariate comparisons the CT features of all patients were carried out to determine their

Table 3 Univariate analysis of predictors for suboptimal cytoreduction

	Optimal ($n = 86$)	Suboptimal ($n = 12$)	p values	OR (95% CI)	Accuracy[a]	NPV	PPV
CA125 > 500	25	5	0.153	2.44 (0.72–8.29)	87.8	100	0
Ascites	21	6	0.013	4.94 (1.41–17.33)	87.8	100	0
DPT	2	2	0.006	28.33 (82.66–301.63)	89.8	98.8	25
Infrarenal para-aortic lymph nodes	21	6	0.087	3.76 (0.82–17.17)	87.8	100	0
Pelvic lymph nodes	11	4	0.007	14.00 (20.59–95.18)	88.8	97.7	25
Bowel encasement (≥2 cm)	6	4	0.002	9.52 (2.31–39.25)	87.8	100	0
Omental caking (≥2 cm)	7	4	0.122	3.25 (0.73–14.50)	87.8	100	0
Bowel mesentery							
(0–1 cm)	3	3	0	25.67 (5.86–112.51)	87.8	89.5	75
(1–2 cm)	0	1	0	29.25 (6.56–130.51)	88.8	90.7	75
(≥2 cm)	5	4	0	28.70 (6.25–131.82)	90.8	95.3	58.3
Tumor implants in cul-de-sac							
(0–2 cm)	1	0	0.005	6.96 (1.79–27.10)	87.8	100	0
(≥2 cm)	8	5	0.122	3.25 (0.74–14.50)	87.8	100	0
Tumor implants on the other places							
(0–1 cm)	2	3	0	22.8 (5.28–98.53)	87.8	100	0
(≥1 cm)	12	7	0	19.5 (84.79–79.36)	87.8	90.7	66.7

[a] Patients correctly predicted to have optimal and suboptimal/total

ability to predict a suboptimal cytoreduction outcome using logistic regression analysis for each of the 17 potential radiologic features listed in Table 2 and serum CA 125 levels >500 units/mL. Using cross-tabulation and the chi-square test, the features of advanced age, liver metastasis, tumor implants (peritoneum of diaphragm, porta hepatis or gallbladder fossae, omental extension to spleen or stomach, invasion to pelvic wall, and pelvic retroperitoneum) were excluded because they had too few positive numbers for suboptimal results. We set the cutoff index for each radiological criterion, using the size of the tumor as 0–1, 1–2, 2–4, and ≥4 cm.

Next, we studied the associations among all the features using a correlation matrix. The paired features of (tumor implants on the small or large bowel mesenteries—tumors implants on other places), (ascites—omentum caking), and (ascites—tumor implants on other places) have strong associations each other. All remaining features were subjected to univariate analysis to determine their ability to predict an outcome of suboptimal cytoreduction. Tumor implants on small or large bowel mesentery (cut off index: (1) 0–1 cm, (2) 1–2 cm, and (3) 2–4 cm) and 4.) and tumor implants at other places (cut off index: ≥1 cm) were found to be statistically significant ($p < 0.001$), as shown in Table 3. For these four features, each predictive probability for suboptimal surgery was 9/12 (75%), 9/12 (75%), 7/12 (58.3%), and 8/12 (66.7%), respectively.

To create predictive models using CT findings, we analyzed combinations of some of the most significant features of 17 predictors identified by our multivariate analysis. Finally, we created two useful models. Model 1, which uses a combination of CT-observable diffuse peritoneal thickening (DPT), swollen infrarenal (>0 cm) and pelvic (>0 cm) lymph nodes, bowel encasement tumor (≥2 cm), and other tumors in the pelvic- and retro-peritoneum (>0 cm), and cul-de-sac (>0 cm), would appear to be the most clinically useful model to predict suboptimal cytoreduction (Table 4). Model 1 provides an NPV: 96.5%, PPV: 50%, accuracy: 90.8%, AIC (Akaike's Information Criterion): 51.586, and Negelkerke R^2: 0.603. Model 2 uses a slightly different combination of CT features: ascites, diffuse peritoneal thickening (DPT), swollen infrarenal para-aortic lymph nodes (>0 cm), pelvic lymph nodes (>0 cm), bowel encasement tumor (≥2 cm), omental caking (≥2 cm), and tumors in the large bowel mesentery (≥2 cm), and cul-de-sac (≥2 cm) to predict whether optimal or suboptimal surgery will be achieved (Table 5). Model 2 represents NPV: 98.8%, PPV: 50%, accuracy: 93.9%, AIC: 56.371, and Negelkerke R^2: 0.593.

After stepwise down-selection, only the CT features of swollen pelvic lymph nodes (>0 cm), tumors in the cul-de-sac (>0 cm), and 'other tumors in the pelvic- and retro-peritoneum (>0 cm)' were left in Model 1 (Table 6). After stepwise down-selection, only DPT, tumor implants on the small or large bowel mesenteries (≥2 cm) and bowel encasement tumor (≥2 cm) were left in Model 2 (Table 6).

Table 4 Multivariate analysis of CT predictors for suboptimal cytoreduction in Model 1

	p value	OR (95% CI)
DPT	0.006	59.51 (1.89–1877.81)
Infrarenal para-aortic lymph nodes (>0 cm)	0.087	4.27 (0.18 –100.07)
Pelvic lymph nodes (>0 cm)	0.007	14.35 (0.26–784.93)
Bowel encasement (≥2 cm)	0.002	2.01 (0.16–24.93)
Tumor implants in cul-de-sac (>0 cm)	0.005	3.58 (0.50–25.49)
Tumor implants in other places (>0 cm)	0	17.88 (2.32–137.72)

Table 5 Multivariate analysis of CT predictors for suboptimal cytoreduction in Model 2

	p value	OR (95% CI)
Ascites	0.013	1.87 (0.18–20.07)
DPT	0.006	12.08 (0.27–533.89)
Infrarenal para-aortic lymph nodes (>0 cm)	0.087	1.72 (0.11–28.17)
Pelvic lymph nodes (>0 cm)	0.007	11.77 (0.26–537.48)
Bowel encasement (≥2 cm)	0.002	5.86 (0.43–79.25)
Omental caking (≥2 cm)	0.122	1.79 (0.12–27.71)
Tumor implants in bowel mesentery (≥2 cm)	0.000	20.51 (2.43–173.10)
Tumor implants in cul-de-sac (>0 cm)	0.005	1.06 (0.07–17.00)

Table 6 Two models to predict suboptimal cytoreduction

	Accuracy (%)[a]	NPV	PPV	AIC[b]	Negelkerke R^2
Model 1	90.8	96.5	50	51.586	0.603
After stepwise selection	89.8	98.8	25	50.495	0.479
Model 2	93.9	98.8	50	56.371	0.593
After stepwise selection	92.9	25	50	51.127	0.499

[a] Patients correctly predicted to have optimal or suboptimal cytoreduction/total

[b] Akaike's Information Criterion

Discussion

Although an optimal cytoreduction of the primary tumor and its observable metastases clearly contribute to a better prognosis in ovarian cancer patients, the rates of achievement of such optimal cytoreduction vary from institution to institution. Results are affected by the surgical team's experience, technique, effort, enthusiasm, and by their institutional policies concerning the need and use of radical aggressive surgery [3, 6]. When faced with advanced ovarian cancer patients, such as those patients with massive ascites and/or pleural effusion, or with a poor general health condition, gynecologists often ponder whether a primary aggressive radical cytoreduction surgery should be done first, or, alternatively, based on some recent reports, aggressive neoadjuvant chemotherapy should be done first.

To address this bedeviling dilemma, several researchers have reported on the benefits of neoadjuvant chemotherapy (NAC). Debulking after the receipt of NAC appears to be associated with less operative morbidity and improved rates of optimal cytoreduction achievement [4, 7]. Recent prospective randomized control study proved NAC followed by interval debulking surgery is not inferior to primary debulking surgery followed by chemotherapy as a treatment option for patients with advanced ovarian cancer, although the rate of a complete resection of the tumor was different in terms of the participating countries [5].

Other researchers have also reported on the prospect of predicting surgical outcomes by preoperative imaging, mainly (like us) using computed tomography. Nelson et al. retrospectively evaluated 34 patients with stage III/IV disease and reported that suboptimal cytoreduction was predictable using the following criteria: attachment of the omentum to the spleen, and tumors >2 cm in the mesentery, liver, diaphragm, gallbladder fossae, suprarenal para-aortic lymph nodes, pericardial lymph nodes, and the lungs; their PPV to predict suboptimal and optimal surgical outcome was 66.7% and their sensitivity 92.3%, so they concluded that CT scans are a valuable tool for predicting optimal cytoreduction [8].

Dowdy et al. found DPT, diaphragm or lung involvement, bowel encasement, large-volume ascites, and omental extension to the spleen or pancreas were statistically more common in patients that went on to have a suboptimal surgical result; however, only DPT was an independent feature in a multivariate model. They concluded that the combination of DPT and ascites on at least two-thirds of CT scans would appear to be the most clinically useful model to predict suboptimal cytoreduction, but they could not confirm this during cross-validation [9].

Diaphragm and large bowel mesentery implants were the only features that resulted in suboptimal cytoreduction on univariate and multivariate analysis by Axtell et al. [10]. Omentum disease was also more frequent in the suboptimal cytoreduction patients in the study of Everett et al. [11]. Bristow et al. proposed a scoring system using 13 CT features. Their Predictive Index score of ≥4 was highly accurate in predicting suboptimal cytoreductive surgery. They used a cutoff index in each feature size of 1 or 2 cm [12]. In addition, other recent studies discussing using CT prediction of surgical outcomes have been reported [13–15].

In this study, our Model 1 had a sensitivity of 50%, a specificity of 97%, an accuracy of 90.8%, a PPV of 50.0%, and NPV of 96.5% for suboptimal cytoreduction; Model 2 had a sensitivity of 58%, a specificity of 99%, an accuracy of 93.9%, a PPV of 50.0%, and NPV of 98.8% (Table 6). The significance of some features, such as diffuse peritoneal thickening, large volume ascites, tumor implants on the small or large bowel mesenteries, are compatible with the result of several former studies. Using our two new models, we were able to identify features that accurately predict those patients most likely to undergo optimal surgery. Among all predictors, having other tumors in the pelvic- and retro-peritoneum ($\geq$1 cm) and having tumor implants on the small or large bowel mesentery ($\geq$1 cm) together had the highest suboptimal cytoreduction predictive probability of 8/12 and 9/12, with a sensitivity of 67 and 75%, a specificity of 91 and 91%, an accuracy of 88 and 89%, a PPV of 50 and 12%, and a NPV of 95 and 96%, respectively.

DPT, infrarenal para-aortic lymph node and pelvic lymph node involvement, bowel encasements, and implants within the cul-de-sac were common factors in our two models. The detection of tumor implants on small or large bowel mesentery was highly associated with a suboptimal result, as previously reported by others [8]. These five factors are relatively easily detectable on pre-surgical CT scans and should be considered highly clinically relevant information prior to surgery.

In our study, the sites most likely to be under-diagnosed by imaging were for tumors in the cul-de-sac and elsewhere in the pelvic- and retro-peritoneum, whereas Nelson et al. reported liver and lymph node metastasis, omental attachment to the spleen, and tumors involving the gallbladder fossae were difficult to ascertain by their CT scan reviewers. It thus seems likely that the sites of metastasis likely to be under-called are highly dependent on the skills of the radiology team.

When considering the tumor marker CA 125 as a predictive factor for cytoreductive surgery, Chi et al. reported that 43 of 55 patients who underwent suboptimal cytoreduction were correctly identified using preoperative serum CA-125 levels above 500 U/mL as a cutoff value to predict suboptimal cytoreduction [16]. On the other hand, others have reported that the CA125 level has a poor predictive value of optimal resection [9, 11, 17]. It is not clear whether this discrepancy is due to differences in subtypes of ovarian tumors in the conflicting studies, ethnic population differences, or methods of determining CA 125 levels. We can only say that in our current study we did not find CA125 level to be a useful predictor of future suboptimal cytoreduction.

Salani et al. evaluated the potential for achieving optimal primary cytoreduction in patients with surgically documented advanced ovarian cancer at anatomic sites typically considered to be unresectable by conventional criteria (those having ascites fluid volume > 1000 mL, omental extension to spleen > 1 cm, parenchymal liver disease > 1 cm, porta hepatis involvement > 1 cm, diaphragmatic disease > 1 cm, carcinomatosis > 1 cm, or suprarenal adenopathy > 1 cm). They determined that an optimal primary surgical resection was still feasible in over 80% of their cases involved with metastatic disease in at least four conventionally unresectable anatomic regions, such as a large volume ascites, splenic involvement, and diaphragmatic disease. So they concluded, correctly in our opinion, that the likelihood of achieving an optimal primary resection might be more surgeon-dependent than disease-dependent [18]. We also have to consider that the patient's desires and financial/social situation can affect the consequences of treatment choices.

The standard treatment for advanced ovarian cancer, whose results we studied here, consists of complete surgical debulking and intravenous platinum and taxane-based chemotherapy. Although the toxicity was much higher in the i.p. arm when compared with standard intravenous treatment with the same compounds in the GOG 172 trial, platinum-based intraperitoneal chemotherapy (IPC) has been shown to give better results in term of overall and disease-free survival [19]. Hyperthermic intraperitoneal chemotherapy (HIPEC), with the synergy of hyperthermia, has now largely replaced IPC. Cytoreductive surgery is followed by immediate intraoperative HIPEC, which is followed by systemic chemotherapy. For a select group of patients with diffuse ovarian peritoneal carcinomatosis, with low tumor volume and no organ metastases, HIPEC has resulted in improved prognosis [20]. But even with this advance in ovarian cancer treatment, the key for success has been the cytoreductive step.

A perfect method for predicting the surgical resection outcome of patients with advanced ovarian cancer has yet to be developed. To date, several studies, including ours, have attempted to establish a predictive preoperative model to estimate successful tumor resectability (optimal cytoreduction) or to predict the likelihood of a suboptimal cytoreduction. To establish an international consensus, such an analysis will need large data sets from a number of studies, in multiple countries.

In conclusion, from our study we propose potential clinical applications for several CT scan features within two new predictive models for surgical cytoreductive outcomes in advanced ovarian cancers.

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Conflict of interest The authors declare no potential conflicts of interest.

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ORIGINAL ARTICLE

Salvage chemotherapy for recurrent or persistent clear cell carcinoma of the ovary: a single-institution experience for a series of 20 patients

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Abstract
Background Recurrent or persistent clear cell carcinoma (CCC) of the ovary is particularly chemotherapy resistant. The purpose of this study was to review our extensive institutional experiences with recurrent or persistent CCC with the aim of finding a more effective chemotherapy regimen.
Methods The medical records of 67 patients treated for CCC of the ovary were retrospectively reviewed to select patients subsequently treated for recurrence or persistence of the disease.
Results The review identified 20 patients treated for recurrent or persistent CCC. For these 20 patients, 9 chemotherapeutic regimens, with 125 cycles, were administered. Gemcitabine monotherapy showed the best response rate [1 partial response (20%) and 2 stable diseases out of 5 patients so treated]. A partial response was observed with a combination of docetaxel plus irinotecan in 1 of 11 patients (9%). Stable disease was observed in 1 of 9 cases on a paclitaxel/carboplatin doublet and in 1 case on a docetaxel/carboplatin doublet. The median overall survival time was 8 months (range, 2–52). One group of patients who received gemcitabine therapy showed significantly better survival ($n = 5$, median 18 months) compared with a group who did not ($n = 15$, median 7 months) ($P = 0.0108$, by univariate analysis). In addition, multivariate Cox proportional hazards analysis revealed that gemcitabine administration was a significant factor for survival (hazard ratio: 13.0, 95% CI: 1.4727–115.2255, $P = 0.02$).
Conclusion Although most chemotherapeutic regimens for recurrent or persistent CCC have little or no effect, gemcitabine showed modest activity and is the most effective agent we have tested to date.

Keywords Chemotherapy · Clear cell carcinoma · Gemcitabine · Ovarian cancer · Persistence · Recurrence

Introduction

Epithelial ovarian cancer (EOC) is the second most lethal of the gynecological malignancies (after cervical cancer), causing approximately 125,000 deaths annually worldwide [1]. Standard therapy for EOC includes maximal surgical debulking followed by chemotherapy with platinum and taxane drugs. Despite an initial response rate to this primary therapy of approximately 80%, most EOC patients suffer subsequent recurrence and mortality.

Clear cell carcinoma (CCC) is a subtype of EOC that is relatively uncommon in western countries, including the USA, where CCC comprises only 5–10% of ovarian tumors. In contrast, in Japan, CCC has a higher incidence rate, at 20–25% of all EOCs. The reason behind this significantly higher incidence is not yet fully understood [2].

CCC has distinct biological activities relative to other histological types of ovarian cancer. Sugiyama et al. [3] have reviewed the distinct chemo-resistance and poorer prognosis of CCC. Enomoto et al. [4] showed that this problem continues, even with our best current standard regimen of a paclitaxel/carboplatin doublet.

For recurrent EOC, the treatment strategy depends on the tumor's response to the primary chemotherapy. When

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recurrence occurs more than 12 months after the completion of the initial therapy, re-administration of the same chemotherapy can be effective in many cases, resulting in extended survival times. However, if the recurrence occurs before 6 months have passed, most chemotherapeutic agents are usually no longer effective [5, 6].

Recurrences of the CCC subtype of EOC tend to be highly chemoresistant to any previous chemotherapy regimen, no matter when they reoccur. Some medical groups have attempted to overcome this resistance by a number of different strategies. Irinotecan (CPT-11) combined with cisplatin (CPT-P) was introduced as an efficacious regimen for refractory or recurrent general EOC, and has been used specifically for CCC. A retrospective Japanese multi-center study reported that a CPT-P group showed significantly better progression-free survival than a group receiving standard TP (taxane plus platinum) [7]. In another strategy, postoperative whole-abdominal radiotherapy (WAR) was carried out. The 5-year overall and disease-free survival in the WAR group was significantly better than that for the standard platinum-based chemotherapy group. However, the adverse effects in the bowel were occasionally severe, causing some patients to require surgery [8].

Clinical trials using novel agents specifically for CCC are ongoing. For persistent or recurrent disease, sunitinib is being evaluated in a phase II study by GOG (NCT 00979992, http://www.clinicaltrials.gov). Another phase II study is evaluating temsirolimus in combination with a paclitaxel/carboplatin doublet followed by temsirolimus consolidation as a first-line therapy in the treatment of stage III–IV CCC (NCT 01196429, http://www.clinicaltrials.gov). The results of these studies should give us a clue as to how to overcome CCC.

In this review, we recount our past experiences with recurrent and persistent CCC, seeking clues for overcoming the scourge that is CCC of the ovary.

Patients and methods

During the period of 1998–2009, 67 cases of CCC of the ovary (all of Japanese descent) underwent cytoreductive surgery within the Department of Obstetrics and Gynecology at the Osaka University Hospital, Osaka, Japan. The medical records of the patients were reviewed, revealing that the FIGO (International Federation of Gynecology and Obstetrics) staging of these cases was distributed as follows: stage I in 46 cases (Ia; 16 cases, Ib; 1 case, and Ic; 29 cases), stage II in 5 cases (IIc for all), stage III in 14 cases (IIIb; 3 cases, and IIIc; 11 cases), and stage IV in 2 cases.

Study inclusion eligibility criteria for those patients who were treated for recurrent or persistent disease included the following: (1) pathological diagnosis of CCC of the ovary at the initial surgery, (2) subsequent measurable recurrent or persistent disease, (3) treatment for the recurrent or persistent disease with one or more systemic chemoregimens, and (4) availability of adequate clinical information. The following patient information was abstracted from their medical records: age; date of primary surgery; residual disease; stage of disease based on FIGO criteria; date of completion of the primary chemotherapy; date of first detected recurrence or progression; regimens of each systemic agent administered; date of start and completion of each treatment; number of cycles of each systemic agent; response to each systemic agent administered; status at the last patient contact; and the date of last contact or death. Responses to the systemic agents were recorded according to version 1.0 of the Response Evaluation Criteria in Solid Tumors (RECIST) criteria.

Statistical analyses were performed using MedCalc for Windows (version 11.3.3.0, MedCalc Software, Mariakerke, Belgium). Treatment-free interval (TFI) was defined as the time (months) from the completion of initial therapy to recurrence with a radiological confirmation. For recurrent disease, overall survival time (OS) was calculated from the date of first recurrence to either the date of death or date of last contact. For persistent disease, OS was calculated from the date of primary surgery to either the date of death or date of last contact. A multivariate Cox proportional hazards analysis with selected variables was used to determine the significantly important factors for survivals. The Kaplan–Meier statistical method was used to calculate the overall survival times. Statistical significance was analyzed by the log-rank test. We considered the results to be significant when the P value was less than 0.05.

Results

After reviewing the medical records of 67 patients with CCC of the ovary, 21 patients were identified as having subsequently had a recurrence or a persistent disease. Of these 21 patients, 1 patient refused to receive any systemic agents and was therefore excluded from this study. A total of 20 patients received systemic agents, thereby meeting the eligibility requirements for this study, and were subsequently analyzed.

The characteristics of these 20 patients are shown in Table 1. The median age was 53 years; ages ranged from 35 to 65. In stage I patients, recurrence occurred in 4 cases with stage Ic (recurrence rate 9% for stage I overall, 14% specifically for stage Ic). There was also a single case of stage IIc, which thus showed a recurrence of 20%. Thirteen of 14 cases (93%) with stage III showed recurrence or persistent disease. Both cases with stage IV had persistent

disease (100%). Ten of the patients were known to still have residual disease after the initial debulking surgery (50%); the remaining 10 patients were classified as having had recurrent disease (50%). Retroperitoneal (pelvic and para-aortic) lymphadenectomy was performed in 12 cases in their initial surgeries (60%).

In our hospital, until 2003, postoperative chemotherapy with paclitaxel/carboplatin doublet (TC) was administered as the standard regimen for all EOC, regardless of histological subtype. Five of our 20-patient pool underwent this regimen. Thereafter, starting in 2003, because of the low response rate of this TC regimen, a docetaxel plus irinotecan (DIr) regimen was used for postoperative chemotherapy for advanced stages of CCC, and of these, 14 study patients received this regimen [4]. Among the 10 patients who had no detectable residual disease after initial surgery, but thereafter showed recurrence, 6 had equal to or more than 6 months of TFI, and the remaining 4 had less than 6 months of TFI.

As shown in Table 2, 9 treatment regimens were administered. Paclitaxel/carboplatin doublet (TC) was administered to 9 patients, with a total of 28 cycles, where 1 cycle consisted of paclitaxel (175 mg/m^2) plus carboplatin (AUC = 5) every 3 weeks. A docetaxel/carboplatin doublet (DC) was administered for 1 patient, for a total of 3 cycles, where 1 cycle consisted of docetaxel (70 mg/m^2) plus carboplatin (AUC = 5) every 3 weeks. A weekly treatment of a paclitaxel/carboplatin doublet (wTC) was administered to 3 patients, for a total of 8 cycles, where 1 cycle consisted of paclitaxel (80 mg/m^2 on days 1, 8 and 15) plus carboplatin (AUC = 2 on days 1, 8, and 15) every 4 weeks. DIr was administered to 11 patients, for a total of 41 cycles, where 1 cycle consisted of docetaxel (30 mg/m^2 on days 1 and 8) plus irinotecan (60 mg/m^2 on days 1 and 8) every 3 weeks. The single-agent gemcitabine (GEM) was administered to 5 patients, for a total of 18 cycles, where 1 cycle consisted of gemcitabine (800 mg/m^2 on days 1, 8, and 15) every 4 weeks. The single-agent carboplatin was administered to 1 patient as a single cycle/single dose of AUC = 5. Oral etoposide was administered to 1 patient, for a total of 2 cycles, where 1 cycle consisted of oral etoposide (50 mg/day for 21 days) every 4 weeks. Pegylated liposomal doxorubicin (PLD) was administered to 1 patient for 2 cycles. One cycle consisted of PLD (40 mg/m^2 on day 1) once every 4 weeks. Wilms' tumor 1 vaccine (WT1) was administered to 2 patients, for a total of 22 cycles, where 1 cycle consisted of intradermal injections of an HLA-A*2402-restricted, modified 9-mer WT1

Table 1 Characteristics of patients with recurrent or persistent clear cell carcinoma of the ovary

Characteristics	$n = 20$	%
Age		
Median	53	
Range	35–65	
FIGO stage		
I	4	20
II	1	5
III	13	65
IV	2	10
Residual disease		
No	10	50
Yes	10	50
Postoperative chemotherapy		
None	1	5
Paclitaxel/Carboplatin	5	25
Docetaxel/Irinotecan	14	70
Disease status		
Recurrence	10	
TFI: <6 months	4	20
TFI: ≥6 months	6	30
Persistent disease	10	50

FIGO International Federation of Gynecology and Obstetrics, *TFI* treatment-free interval

Table 2 Regimens and maximum responses for recurrent or persistent clear cell carcinoma of the ovary

Regimens	No. of patients	Total cycles	Median cycles	No. of maximum responses, duration
Docetaxel + Irinotecan	11	41	3	1 PR, 6 m
Paclitaxel + Carboplatin	9	28	3	1 SD, 7 m
Gemcitabine	5	18	4	1 PR, 6 m; 2 SD, 4 and 5 m
Paclitaxel + Carboplatin (weekly)	3	8	3	PD
WT1 vaccine	2	22	6	PD
Docetaxel + Carboplatin	1	3	3	1 SD, 4 m
Carboplatin	1	1	1	PD
Pegylated liposomal doxorubicin	1	2	2	PD
Oral etoposide	1	2	2	PD

PR partial response, *SD* stable disease, *PD* progressive disease

Table 3 Details of responders who showed more than stable disease with recurrent or persistent clear cell carcinoma of the ovary

Case	Age	Stage	Residual tumor (sites)	First-line regimen, cycles	TFI (when recurrent) or response (when persistent)	Second-line regimen, cycles	Response, duration	Third-line regimen, cycles	Response, duration	Fourth-line regimen, cycles	Response, duration	Fifth-line regimen, cycles	Response, duration	Status
1	65	Ic(2)	No	TC ×6	TFI; 31 m	DC ×3	SD, 4 m							DOD
2	42	IIIc	Yes (om, pnm, msty)	DIr ×6	PD	wTC ×3	PD	GEM ×3	PR, 6 m					DOD
3	54	IIIb	Yes (om, pnm)	DIr ×6	PD	TC ×3	PD	GEM ×10	SD, 5 m					DOD
4	51	IIc(2)	No	DIr ×6	TFI; 7 m	TC ×6	SD, 7 m	WT1 ×6	PD	GEM ×4	SD, 4 m	PLD ×2	PD	AWD
5	56	IIIc	No	DIr ×6, T ×12	TFI; 5 m	DIr ×6	PR, 6 m							AWD

Ic(2) and IIc(2) positive cytology of ascites, *om* omentum, *pnm* peritoneum, *msty* mesentery, *TFI* treatment-free interval (months), *DC* docetaxel + carboplatin, *TC* paclitaxel + carboplatin, *T* paclitaxel (consolidation), *wTC* weekly paclitaxel + carboplatin, *DIr* docetaxel + irinotecan, *GEM* gemcitabine, *WT1* Wilms' tumor 1 vaccine, *PLD* pegylated liposomal doxorubicin, *PR* partial response, *SD* stable disease, *PD* progressive disease, *DOD* dead of disease, *AWD* alive with disease

peptide every week [9]. Dose reduction was performed in response to toxicity to the patient's hematological status.

The majority of these administered regimens did not show significant responsiveness. A few showed some modest clinical activity. For example, gemcitabine represented the best response rate, in 1 of 5 patients (20%) it gave a partial response as a third-line treatment, and in 2 stable diseases it gave a response as a third- or fourth-line treatment. A partial response was also observed with DIr in one of 11 patients (9%) when used as a second-line treatment. Stable disease was observed in 1 of 9 cases treated with TC and in 1 case treated with DC, both as second-line efforts. Details of the responders who showed equal to, or more than, stable disease are shown in Table 3.

The median overall survival time of the recurrent or persistent patients was 8 months (range, 2–52), as shown in Fig. 1. Using univariate analysis, a group of patients who received gemcitabine therapy ($n = 5$) showed significantly better survival (median 18 months) compared with a group who did not receive it ($n = 15$, median 7 months) ($P = 0.0108$). A multivariate Cox proportional hazards analysis with selected variables (age, stage, postoperative chemotherapy, TFI, chemotherapy for recurrence or persistent disease) was used to determine the significantly important factors in survival. The analysis revealed that use of DIr for postoperative chemotherapy ($P = 0.02$) and use of gemcitabine for recurrence or persistent disease ($P = 0.02$) were significant factors in survival, as shown in Table 4.

Discussion

Clear cell carcinoma (CCC) of the ovary is relatively rare in the USA and Europe; however, its incidence in Japan

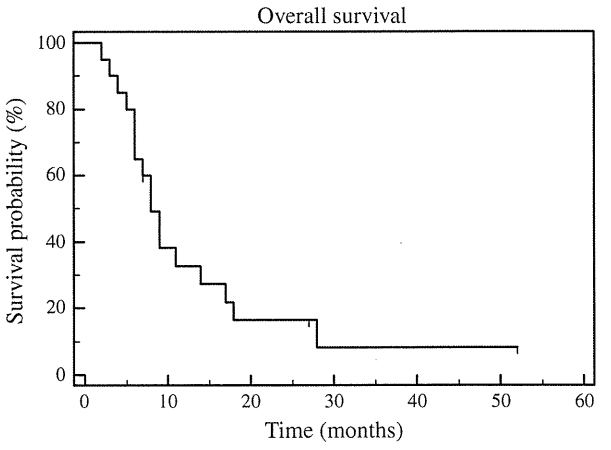

Fig. 1 Kaplan–Meier curve showing overall survival time of 20 patients with recurrent or persistent CCC. The median survival time was 8 months

Table 4 Multivariate Cox proportional hazards analysis for recurrent or persistent clear cell carcinoma of the ovary

Variables	Hazard ratio	95% CI	*P* value
Age			
<53 ($n = 9$)	1		0.18
≥53 ($n = 11$)	0.37	0.0898–1.6041	
Stage			
I/II ($n = 5$)	1		0.18
III/IV ($n = 15$)	6.16	0.4298–88.5383	
Postoperative chemotherapy			
TC ($n = 5$)	1		0.02
DIr ($n = 14$)	0.05	0.004–0.5792	
TFI			
<6 months ($n = 14$)	5.39	0.8834–32.9883	0.06
≥6 months ($n = 6$)	1		
Chemotherapy for recurrent or persistent disease			
DIr administration ($n = 11$)	1		0.23
Without ($n = 9$)	2.54	0.5407–11.9450	
TC administration ($n = 9$)	1		0.99
Without ($n = 11$)	0.99	0.2205–4.5342	
Gemcitabine administration ($n = 5$)	1		0.02
Without ($n = 15$)	13.0	1.4727–115.2255	

TFI treatment-free interval, *DIr* docetaxel/irinotecan, *TC* paclitaxel/carboplatin

accounts for roughly 20% of all EOC. We treated a total of 67 primary cases of CCC, along with 20 examples of recurrence or persistent CCC disease, during the 10-year study period from 1998 to 2009.

As previous reports have described, we also found that recurrent and persistent CCC was extremely chemoresistant. We noted that among the 9 different chemotherapy regimens we attempted, gemcitabine monotherapy showed the better response rate. Our patients who received gemcitabine therapy showed significantly better survival compared with a group who did not receive it. Furthermore, multivariate Cox proportional hazards analysis revealed that gemcitabine administration was a significant factor for survival (hazard ratio: 13.0, 95% CI: 1.4727–115.2255, $P = 0.02$). Therefore, we propose that gemcitabine may be an active chemotherapeutic agent for recurrent or persistent CCC.

Gemcitabine (2′,2′-difluorodeoxycytidine), a synthetic nucleoside analog of cytidine, has already been demonstrated to be an active agent for various other solid tumors, such as non-small-cell lung, pancreatic, genitourinary, and breast cancers [10]. As described in pioneering work from the Plunkett laboratory, gemcitabine is a prodrug that is metabolized to gemcitabine diphosphate and triphosphate, whose incorporation into DNA results in chain termination by inhibiting DNA polymerase activity [11]. Consequently, tumor cells are blocked in the G1 phase of the cell cycle. Gemcitabine triphosphate metabolite can be also incorporated into RNA, thus inhibiting RNA production [12].

Gemcitabine was studied for the first time as a single-agent treatment for recurrent EOC at a dose of 800 mg/m^2 on days 1, 8, and 15 every 28 days, thereafter, in a population of platinum-resistant ovarian cancers that included all histological subtypes [13]. In a review by Lorusso et al. [14], the results from a total of 411 patients treated by the single-agent gemcitabine were combined from 12 reports. The combined and re-analyzed data showed a mean gemcitabine response rate of 19%.

Recently, several large randomized control studies have been performed using gemcitabine in ovarian cancer patients. Mutch et al. have shown the safety and efficacy of gemcitabine monotherapy compared with PLD in their phase III trial in patients with platinum-resistant (Pt-R) recurrent ovarian cancer. In their report, gemcitabine and PLD seem to have comparable therapeutic indices, indicating that single-agent gemcitabine may be an acceptable alternative to PLD for patients with Pt-R recurrent disease [15]. For platinum-sensitive (Pt-S) recurrent disease, Pfisterer et al. reported that the addition of gemcitabine to carboplatin significantly improved progression-free survival and response rate compared with carboplatin alone without worsening quality of life in their phase III study [16]. Thus, gemcitabine is recognized as an active agent for both Pt-R and Pt-S recurrent ovarian cancer.

In most reports, gemcitabine's adverse effects and toxicity were easily manageable, transitory, noncumulative, and rarely represented a cause for dose reduction or treatment interruption. Gemcitabine has a well-proven activity in platinum and/or paclitaxel-resistant ovarian cancer patients, and seems to cause no cross-resistance with platinum compounds. However, it should be noted that

most of these studies represented treatments for mainly serous adenocarcinomas, with CCCs accounting for less than 5% of the cases. Therefore, the efficacy of gemcitabine for CCC is still largely unknown.

There are reports which suggest that gemcitabine may have a beneficial clinically active effect for CCC. Crotzer et al. [17] analyzed 51 patients treated for recurrent CCC. Their series received a total of 105 regimens with 344 cycles. In the platinum-sensitive setting, a partial response was observed in only 9% of cases, much lower than the response rates of 50–90% reported for platinum-sensitive disease in all cell types of EOC combined [18]. Among patients with platinum-resistant disease, only 1 patient had a partial response to gemcitabine and 1 patient had stable disease in response to 2 different regimens, paclitaxel and gemcitabine. Generally, second-line chemotherapy for platinum-resistant disease gives response rates of 15–20% when using an active agent.

Komiyama et al. [19] reported successful control with gemcitabine of a single case of peritonitis carcinomatosa presenting with massive ascites in a patient with a heavily pretreated recurrent CCC. Ferrandina et al. described a case of multi-drug-resistant CCC of the ovary showing a selective susceptibility to gemcitabine at first administration and again at re-challenge. Moreover, they showed that the tumor expressed a certain molecular profile that likely made it highly sensitive to gemcitabine [20]. Their finding points out that, although most reports of chemotherapy for CCC are highly disappointing, case-by-case molecular targeting therapy may be the key to combating this difficult to treat disease.

In conclusion, gemcitabine may be a key chemotherapeutic agent for the treatment of aggressive CCCs of the ovary. Additional adjunct molecular targeting therapy should also be considered.

Conflict of interest The authors declare that there are no potential conflicts of interest.

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GYNECOLOGIC ONCOLOGY

Investigating the relative efficacies of combination chemotherapy of paclitaxel/carboplatin, with or without anthracycline, for endometrial carcinoma

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Abstract
Purpose Recently a combination of paclitaxel and carboplatin (TC) (without an anthracycline) has begun to be used as an adjuvant or remission induction therapy, without any critical supportive evidence of its efficacy relative to a combination chemotherapy of taxane, platinum and anthracycline such as TEC (paclitaxel, epirubicin and carboplatin). The aim of our present study was to conduct the required clinical evaluations of the relative effectiveness of TC compared to TEC.
Methods A retrospective comparison between the efficacy of TEC and TC regimens used for endometrial carcinoma at the Osaka University Hospital and the Osaka Medical Center for Cancer and Cardiovascular Diseases in Osaka, Japan, respectively, from 1999 to 2009 was performed. The clinical characteristics of the patients who received either TEC or TC were not significantly different, and TEC and TC therapies were initiated based on similar indications for chemotherapy. TEC regimen was paclitaxel (150 mg/m^2), epirubicin (50 mg/m^2) and carboplatin (AUC 4). TC regimen consisted of paclitaxel (175 mg/m^2) and carboplatin (AUC 5).
Results TEC was demonstrated to provide significantly better survival than TC as an adjuvant therapy for resected Stage III/IV diseases ($p = 0.017$ for progression-free survival and $p = 0.014$ for overall survival, by the log-rank test). However, in recurrent or more advanced cases, TC and TEC demonstrated similar effects on survival ($p = 0.55$ for progression-free survival and $p = 0.63$ for overall survival).
Conclusions TEC should be offered as an adjuvant therapy to Stage III/IV patients. TC may be considered for recurrent or unresectable cases as a remission induction therapy.

Keywords Endometrial carcinoma · Platinum · Taxane · Anthracycline · Survival

Abbreviations

TAP or AP	Doxorubicin and cisplatin (with or without paclitaxel)
TC or TEC	Paclitaxel and carboplatin (without or with epirubicin)
TEP	Paclitaxel, epirubicin and cisplatin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the plasma drug concentration versus time curve
CR	Complete response
G-CSF	Granulocyte-colony stimulating factor
GOG	Gynecologic Oncology Group
5-HT$_3$	5-Hydroxytriptamine-3
JGOG	Japanese Gynecologic Oncology Group
OS	Overall survival

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PD Progressive disease
PFS Progression-free survival
PR Partial response
SD Stable disease

Introduction

Endometrial cancer is the most common gynecological cancer in the United States, and its incidence has increased significantly during the last three decades. Current surgical endometrial cancer therapy consists of a hysterectomy, bilateral salpingo-oophorectomy and retroperitoneal lymph node dissection [1, 2]. A randomized study by the Gynecologic Oncology Group (GOG) revealed that a combination chemotherapy of AP (doxorubicin and cisplatin) was superior to the traditional whole abdominal irradiation as an adjuvant therapy (GOG #122). Unfortunately, significant hematological and cardiac toxicity and treatment-related death was soon associated with AP treatments [3].

Platinum and anthracycline have long been used as the gold standard drugs for advanced or recurrent endometrial carcinomas [4, 5]. Recently, taxane has been added to this group [6, 7]. A recent study showed better survival following a TAP therapy (paclitaxel, doxorubicin and cisplatin) than for AP (GOG #177) [8]; however, neurological toxicity was even greater for patients receiving TAP, with 39% of the patients suffering Grade 2–3 peripheral neuropathy.

Lissoni et al. [9] reported that TEP (paclitaxel, epirubicin and cisplatin) exhibited superior anti-tumor activity against advanced endometrial carcinoma. Recently, TEC (paclitaxel, epirubicin and carboplatin) was shown to have improved activity against metastatic and recurrent endometrial carcinomas, and was found to be relatively tolerable when given with G-CSF support [10]. In our own recent phase I/II prospective studies of TEC, we analyzed the optimal dose for TEC therapy of our Japanese population, which we subsequently determined to be 150 mg/m^2 paclitaxel, 50 mg/m^2 epirubicin, and AUC 4 carboplatin Takata et al. [18]. Based on these findings, TEC has become our new standard for endometrial carcinoma treatment. Recently, however, TC (paclitaxel and carboplatin) has been begun to be widely applied for treatment of endometrial carcinoma, based on its initial reported effectiveness and high tolerability [11–13]. However, its equivalency or superiority to TEC has never been rigorously demonstrated, thus TC therapy is not currently an established regimen for endometrial carcinoma.

Gynecologic Oncology Group (GOG) has an ongoing study (#209) to compare TC with TAP, and the Japanese Gynecologic Oncology Group (JGOG) is performing a similar prospective study (JGOG #2043) to compare three combination chemotherapies: TC, DP (docetaxel and cisplatin) and AP. It has been of great interest to gynecologists whether TC alone is a sufficient chemotherapy for endometrial carcinoma or whether anthracycline is additionally required.

In our present study, we performed a retrospective comparison of TEC versus TC against endometrial carcinoma. We compared the patients' data for TEC therapy, which was exclusively performed at the Osaka University Hospital as part of our phase II study of TEC therapy (submitted), with those of TC, which was administered at our sister Osaka Medical Center for Cancer and Cardiovascular Diseases.

Materials and methods

A retrospective comparison was conducted between the relative efficacies of the TEC and TC regimens, which were performed for endometrial carcinoma cases at the Osaka University Hospital and the Osaka Medical Center from 1999 to 2009. During this period, TEC therapy was exclusively used at the Osaka University Hospital for all its endometrial carcinoma cases with indications for chemotherapy. A TEC dosage of 150 mg/m^2 for paclitaxel, 50 mg/m^2 for epirubicin and AUC 4 for carboplatin was used, based on our phase I results with a Japanese population (submitted). On the other hand, at the Osaka Medical Center, a TC regimen of paclitaxel 175 mg/m^2 and carboplatin AUC 5 (based on the results of our phase I study for ovarian carcinoma in a Japanese population, preliminarily reported by Ueno et al. [14]) was used instead of TEC for all their endometrial carcinoma cases with indications for chemotherapy. The gynecologic surgeons who performed the surgical treatments were all trained at the Osaka University Hospital, and the surgical procedures, and the indications for pelvic and para-aortic lymph node dissection, were identical in the two hospitals. Moreover, adjuvant chemotherapy was performed using the similar indicators.

Eligibility for TEC and TC chemotherapies required that the patient have adequate findings in the following: hematology (WBC $\geq$3,000/μl, platelets $\geq$100,000/μl, granulocytes $\geq$1,500/μl and hemoglobin $\geq$10 g/dl), renal (creatinine $\geq$2 mg/dl) and hepatic [bilirubin $\geq$3 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\geq$2 times the international normal value]. A relative performance status of 0–2 was needed. The tumors needed to be histopathologically diagnosed as being either a primary or recurrent endometrial carcinoma. In the current study, the clinicopathological features of the cases in which TEC or TC chemotherapy were performed, including the age of the patient, the histology and the stage of the disease, and the adverse effects of each chemotherapy regimen were retrospectively reviewed utilizing their clinical records,

including physical examination notes, radiological reports, operative records, and histopathology reports. The histological diagnoses were made by authorized pathologists from the Departments of Pathology of the Osaka University and the Osaka Medical Center, who were all trained at the Osaka University Hospital.

In order to evaluate the efficacy of TEC and TC chemotherapies against endometrial carcinoma, progression-free survival (PFS) and overall survival (OS) were calculated. PFS was measured from the administration of chemotherapy to the date of the radiologic or pathologic diagnosis of relapse, or to the date of the last follow-up. OS was defined as the period from the start of chemotherapy to the patient's disease-specific death or to the date of the last follow-up. In order to evaluate the anti-tumor effect of TEC and TC chemotherapies against the advanced diseases which were unresectable by surgery and the recurrent diseases, previously described standard criteria from the World Health Organization [15] and Pectasides et al. [16] were used. The tumors were assessed by CT scan and/or MRI at baseline and every three treatment courses thereafter. A complete response (CR) was defined as the disappearance of all known disease, determined by two observations not less than 4 weeks apart. We used RECIST (Response Evaluation Criteria in Solid Tumors, version 1.0) for evaluating the therapy response. A CR required regression of all tumors. A partial response (PR) required >30% reduction in the largest diameter of the largest lesion. A progressive disease (PD) was defined as one in which new lesions appeared, or the largest diameter of the largest lesion enlarged more than 20%. All others were considered to be stable disease (SD).

Adverse treatment effects were also analyzed. They were graded based on the National Cancer Institute's Common Toxicity Criteria (version 2.0). Granulocyte-colony stimulating factor (G-CSF) was administered to improve immune function whenever the total WBC/neutrophil count decreased to under 1,000/500 per μl, or when febrile neutropenia was observed. A histamine H1 5-HT_3 antagonist was administrated orally before the paclitaxel to prevent both emesis and an allergic reaction. Other antiemetic drugs were administered as needed.

A regimen of either TEC or TC was administered every 3–4 weeks for 3 weeks against resected Stage I/II diseases with risk factors, which included a myometrium invasion depth of >1/2 and or an atypical histology (such as endometrioid adenocarcinoma Grade 3, clear cell carcinoma or serous papillary carcinoma). TEC or TC was given as an adjuvant therapy for 6 weeks against resected Stage III/IV diseases and unresectable or recurrent diseases. The cases which were classified as Stage IIIa due to positive peritoneal cytology alone, without any other risk factors, were excluded from as having an indication for adjuvant chemotherapy. In order to compare the efficacy of TEC and TC regimes accurately, all the cases in which these chemotherapies were attempted were included in this analysis, including the cases in which these chemotherapies were canceled underway due to severe toxicities or to the patient's intermittent desire to stop chemotherapy.

The Osaka University Hospital protocol for TEC administration was to be given to only patients who were 70 years of age or less; the Osaka Medical Center protocol for TC allowed few patients who were over 70 years of age. The present comparative analysis was conducted only for those patients who were 70 years of age or less. In all cases, chemotherapy was performed only in those patients who were expected to have an estimated remaining survival of greater than 3 months.

Statistical analysis

MedCalc (MedCalc Software, Mariakerke, Belgium) was used for the statistical analyses. The distribution of patients' age, tumor histology and stage were analyzed by the Mann–Whitney *U*-test, the Chi-square test, or Fisher's exact test. PFS and OS curves were constructed using the Kaplan–Meier method and were evaluated for statistical significance by the log-rank test. The frequency of adverse effects in the two groups and the response of each chemotherapy were compared by Fisher's exact test. Results were considered to be significant when the p value was less than 0.05.

Results

Clinical characteristics of the patients with unresectable or recurrent disease who received TEC or TC chemotherapy as a remission induction therapy

TEC therapy was intended for 28 patients with unresectable or recurrent disease at the Osaka University Hospital, and TC therapy was attempted in 23 patients with similar diseases at the Osaka Medical Center. Distributions of age, histology and disease status did not exhibit any significant differences between the TEC and the TC groups (Table 1).

In the recurrent cases, all the patients underwent surgical treatment as a first treatment. The initial tumor stage in the TEC group was Stage I in three cases, II in one case and III in six cases; in the TC group it was I in five cases, II in two cases, III in three cases and IV in one ($p = 0.42$ by the Chi-square test). Adjuvant chemotherapy or radiation had been performed postoperatively in 8 (80%) of 10 cases in TEC group and 8 (73%) of 11 cases in TC group. TEC or TC was administered as the first treatment in all the recurrent disease cases. In advanced cases with unresectable disease, 7 cases were at Stage III and 11 cases were in Stage IV in

Table 1 Clinical characteristics of the advanced and recurrent cases in which TEC or TC chemotherapy was performed as a remission induction therapy

Characteristic	TEC ($n = 28$)	TC ($n = 23$)	p value
Age	57 (34–69)	56 (32–70)	0.95
Histology			0.54
Endometrioid	10 (36%)	7 (30%)	
Non-endometrioid	18 (64%)	16 (70%)	
Disease status			0.41
Advanced	18 (64%)	12 (52%)	
Recurrent	10 (36%)	11 (48%)	

Clinical characteristics of the primary endometrial carcinoma cases with unresectable diseases and the recurrent cases are shown. Distributions of age, histology and disease status did not exhibit any significant differences between the TEC and TC groups

the TEC group, and 5 cases were in Stage III and 7 cases were in Stage IV in the TC group, demonstrating no significant difference ($p = 0.88$ by Fisher's exact test).

Anti-tumor effect of TEC and TC therapies in the patients with unresectable or recurrent disease

Complete response (CR) or PR was achieved in 14 of 18 (78%) and 6 of 12 (50%) advanced cases by TEC and TC, respectively ($p = 0.11$ by Fisher's exact test), and 5 of 10 (50%) and 6 of 11 (55%) recurrent cases by TEC and TC, respectively ($p = 0.83$ by Fisher's exact test) (Table 2). In total, the response rate of TEC therapy against recurrent or advanced diseases was 68% (19 of 28 cases), and that of TC was 52% (12 of 23 cases). This 16% better difference in response rates between TEC and TC therapies was not statistically significant ($p = 0.25$ by Fisher's exact test).

Survival effect of TEC and TC therapies in the patients with unresectable or recurrent disease

Both OS and PFS did not demonstrate any significant difference between the TEC and TC groups ($p = 0.63$ for OS and $p = 0.55$ for PFS, by the log-rank test) (Fig. 1). By analyzing the effect of TEC and TC on survival in subgroups of recurrent cases and advanced (unresectable) cases, TEC therapy was demonstrated to be relatively more effective than TC in unresectable cases ($p = 0.17$ and $p = 0.75$ for PFS and OS, respectively). TC therapy, on the other hand, provided a better survival effect than TEC in recurrent cases ($p = 0.32$ and $p = 0.22$ for PFS and OS, respectively), however these differences were not statistically significant.

Table 2 Anti-tumor effect (response rate) of TEC and TC chemotherapies

Response to chemotherapy	TEC ($n = 28$)	TC ($n = 23$)
CR/PR	19 (68%)	12 (52%)
SD/PD	9 (32%)	11 (48%)

The anti-tumor effect of TEC and TC was evaluated in advanced or recurrent cases. Response rates were 68% in the TEC group and 52% in the TC group. This difference was not statistically significant

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

$p = 0.25$ (Fisher's exact test)

Clinical characteristics of the completely resected patients in Stage III/IV who received TEC or TC chemotherapy as an adjuvant therapy

Because in our previous study (submitted for publication), we could not demonstrate a significant survival improvement in Stage I/II cases using TEC compared to radiation as the adjuvant therapy, and because the effects of TEC and TC therapy on survival were not different significantly in our present study, a comparison analysis of the survival effects of TEC and TC was focused completely on resected Stage III and IV cases.

TEC was administered to 47 patients at the Osaka University Hospital, and TC to 30 patients at the Osaka Medical Center. Their clinical features are shown in Table 3. Distributions of age, histology and stage did not exhibit any significant difference between the TEC and the TC groups. The cases which were classified as being in Stage IIIa due to positive peritoneal cytology alone (without any other risk factors) were excluded from adjuvant chemotherapy, and thus this study.

Survival effect of adjuvant TEC and TC therapies in the completely resected patients in Stage III/IV

The OS and PFS curves of the TEC and TC groups were shown in Fig. 2. The median follow-up period was 38 months (2–105 months). PFS exhibited a statistically significant difference between the TEC and TC groups ($p = 0.017$ by the log-rank test, Hazard Ratio: 0.3838; 95% CI: 0.1709–0.8623). Moreover, OS also exhibited a statistically significant difference between TEC and TC groups ($p = 0.014$ by the log-rank test, Hazard Ratio: 0.3108; 95% CI: 0.1048–0.9214). Thus, TEC therapy was demonstrated to provide a significant improvement of survival as an adjuvant therapy for resected Stage III/IV diseases.

Adverse effects of TEC and TC therapies

Adverse effects of the TEC and TC chemotherapies were evaluated in 187 patients whose accurate data were available (Table 4).Hematological toxicity tended to be more