ID (HLA-A type)	Peptides for vaccination	Antigen expression in tumor tissue	IgG response (FIU)			CTL response (IFN-γ producing cells/10^5 cells)			Numbers of IgG positive peptides			Best clinical response	Overall survival (months)	Prognosis
			Pre	6th	12th	Pre	6th	12th	pre	6th	12th			
	WHSC2-103		74	192	582	0	0	0						
FOV-036 (A24/A31)	SART2-93	na	184	491	354	0	0	0	20	22	20	PD	13.8	AWD
	SART3-109		30	3314	204	0	0	0						
	PAP-213		99	19701	20055	114	377	244						
	Lck-486		240	713	1672	0	0	147						
	Lck-488		618	1571	20091	0	340	0						
	PSMA-624		26	21092	15900									
FOV-037 (A24/A31)	SART2-93	na	27	27	20	0	0	na	10	7	na	PD	4.6	DOD
	Lck-486		25	25	17	0	0	na						
	Lck-488		31	31	21	193	0	na						
	PTHrP-102		11	11	0	150	0	na						
FOV-038 (A2/A24)	SART2-93	na	139	85	na	0	0	na	19	18	na	PD	7.9	DOD
	SART2-161		0	39	na	0	0	na						
	Lck-486		217	113	na	0	0	na						
	Lck-488		338	189	na	0	0	na						
	PSMA-624		52	29	na	0	0	na						
FOV-039 (A24/A26)	SART2-93	na	130	125	15304	984	760	599	8	6	8	PD	10.3	AWD
	SART3-109		54	42	54	800	392	0						
	PAP-213		65	52	15235	840	241	0						
	Lck-488		119	69	8511	784	0	573						
FOV-040 (A11/A26)	SART3-511	na	6092	2011	na	0	0	na	8	8	na	PD	2.2	DOD
	SART3-734		3012	1991	na	0	0	na						
	Lck-90		52	46	na	0	0	na						
	PAP-248		72	33	na	0	0	na						
	WHSC2-103		104	59	na	0	0	na						
FOV-041 (A31/A33)	Lck-90	na	27	19	758	0	0	88	4	4	5	PD	12.5	AWD
	Lck-449		106	96	13170	189	0	164						
	CypB-129		18	18	24099	0	0	0						
	WHSC2-103		111	101	193	429	133	0						
FOV-042 (A31/A33)	SART3-511	na	141	65	64	0	0	46	7	7	7	PD	9.5	AWD
	Lck-90		542	407	327	0	0	0						
	CypB-129		115	98	16114	0	0	0						
	WHSC2-103		160	149	524	0	79	0						
FOV-043 (A11)	SART3-511	na	0	268	16065	0	0	0	4	6	4	PD	12.2	AWD
	SART3-734		375	1075	7041	0	191	0						
	Lck-449		134	364	3980	0	0	215						
	PAP-248		154	136	4479	0	0	0						
	WHSC2-103		141	89	0	0	0	0						
FOV-044 (A24/A31)	SART2-93	na	47	80	194	0	368	0	9	7	16	PD	12.2	AWD
	SART2-161		0	129	1728	0	0	0						
	Lck-486		17	3983	30429	0	0	0						
	Lck-488		30	399	52865	0	454	0						
	SART3-511		0	0	116	0	0	0						
	Lck-449		45	0	21890	0	221	0						
	WHSC2-103		40	0	1000	0	0	0						
FOV-045 (A11/A24)	SART2-93	na	15	11	na	0	0	na	7	8	na	PD	8.3	DOD
	SART3-109		17	14	na	0	513	na						
	Lck-488		29	25	na	0	45	na						
	SART3-511		61	61	na	0	0	na						
	SART3-734		71	51	na	0	0	na						
FOV-046 (A11/A24)	SART2-93	na	103	8781	na	59	851	na	4	4	na	PD	6.6	DOD
	Lck-488		17	143	na	0	649	na						
	Lck-449		21	15066	na	0	388	na						
	WHSC2-103		32	0	na	0	0	na						
FOV-047 (A24/A33)	SART2-93	na	167	154	192	0	0	85	16	14	21	PD	8.4	AWD
	MRP3-1293		37	33	52	0	0	0						
	Lck-486		49	34	13420	0	0	79						
	Lck-488		159	133	18116	0	0	0						
FOV-048 (A2/A24)	EGF-R-800	na	162	155	na	0	0	na	21	18	na	PD	4.7	AWD
	Lck-488		599	4943	na	0	0	na						
	PSMA-624		198	297	na	0	0	na						
	PTHrP-102		138	141	na	0	0	na						
FOV-049 (A24)	SART2-93	na	236	282	3995	0	0	0	7	10	10	PD	10.8	AWD
	Lck-486		32	37	322	0	0	0						
	Lck-488		69	81	408	0	0	0						
	PSMA-624		24	40	798	0	0	0						

CR, complete response; SD, stable disease; PD, progress disease; AWD, alive with disease; DOD, died of disease; and na, not avairable.

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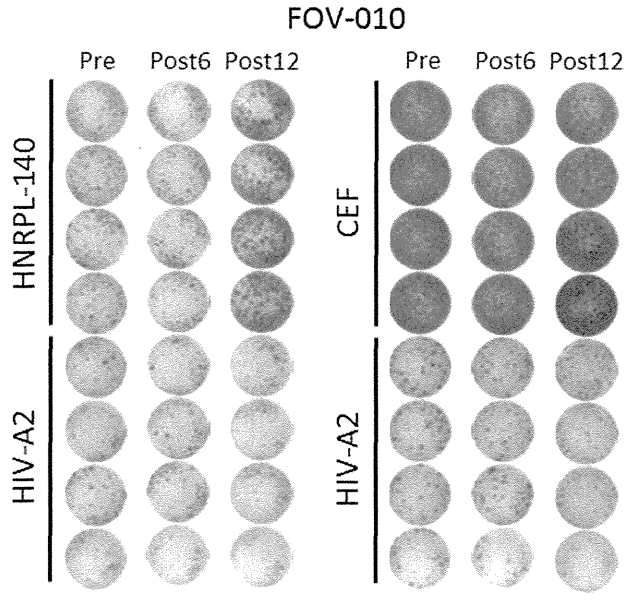

Figure 2. Representative well images of ELISPOT assay. HIV-A2 peptide was used as a negative control. CEF is a cocktail of CMV-, EBV- and Flu-peptides and used as a positive control.

plus chemotherapy. No patient had a partial response. Three patients (FOV-002, -006 and -015) had stable disease (SD). The remaining 21 patients had progressive disease (PD). The median survival time (MST) of the 42 recurrent patients was 19.1 months. Among them, the MST values of the 17 platinum-sensitive and 25 platinum-resistant recurrent cases were 39.3 and 16.2 months, respectively (Figure 4a). The MST values of PPV monotherapy or PPV in combination with any chemotherapy during the 1st to 12th vaccination for the total 42 cases were 20 and 19.1 months (Figure 4b), for the platinum-sensitive cases were 39.3 and 32.2 months (Figure 4c) and for the platinum-resistant cases were 16.8 and 16.1 months, respectively (Figure 4d).

Under these circumstances, the Cox proportional hazards model was used to determine whether immunological responses could be prognostic factors for OS (Table 5). The frequency of lymphocytes at the sixth vaccination and ES were significantly prognostic of OS ($p = 0.0029$ and $p = 0.0135$, respectively). Neither an increase in CTL nor an increase in IgG responses was significantly correlated to the OS, although augmentation of the IgG response was observed in each of the three SD and one CR cases and augmentation of the CTL response was observed in one CR and one SD case. For a better understanding of the involvement of these factors, a log-rank test was also used for the statistical analysis, and the patients with higher lymphocyte frequency or with ES at the sixth vaccination showed longer OS, respectively (Figure 5a and b). As a consequence, ED was inversely correlated with OS (ED + versus ED−, $p = 0.0797$; ED + versus ES, $p = 0.0247$) (Figure 5c and d). In contrast, age, PS and the number of previous regimens were not significantly prognostic of OS.

Discussion

The prognosis of recurrent ovarian cancer remains very poor, with an MST of 18–30 months in platinum-sensitive cases and 8–12 months in platinum-resistant cases[25–28]. Therefore, new innovative therapies are needed for the treatment of recurrent ovarian cancer. We conducted a phase II study of PPV for recurrent ovarian cancer from the viewpoint of OS. This study showed that the MST values of 17 cases of platinum-sensitive and 25 cases of platinum-resistant recurrent ovarian cancer treated with PPV were 39.3 and 16.2 months, respectively. These MST values were longer than the historical control values for recurrent ovarian cancer patients treated in our institution (the historical MST values for platinum-sensitive and -resistant cases were 23 and 8 months, respectively). These results suggest that PPV has the potential to prolong the OS of both platinum-sensitive and platinum-resistant cases.

Thirty-one of 37 cases in this study showed PD at the 12th vaccination, suggesting that PPV did not shrink the tumors but rather delayed the tumor progression, in agreement with the previously conducted PPV for patients with advanced cancers other than ovarian cancer[17–20].

Our previously conducted trials of PPV in various types of cancers also confirmed its safety[21]. PPV toxicity consisted mainly of skin reactions at the injection sites. Although PPV is considered to be feasible, one severe adverse event associated with vaccination was observed. This patient underwent pelvic lymphadenectomy for primary debulking surgery. Lower-limb lymphedema appeared along with a skin reaction at the vaccination sites. Thereafter, infection occurred. We concluded that the infection was associated with PPV. It would thus be better to avoid vaccination of the lower limbs in patients who have undergone pelvic lymphadenectomy. We also investigated the therapeutic potential of the combination of PPV plus chemotherapy. The MST values of PPV monotherapy or PPV in combination with any chemotherapy during the 1st to 12th vaccination of the platinum-sensitive cases were 39.3 and 32.2 months, and those of platinum-resistant cases were 16.8 and 16.1 months, respectively. The patients who could not tolerate concomitant chemotherapy received PPV monotherapy, and most of these patients underwent chemotherapy after completion of PPV. The boosting of immune responses began to be apparent at the 12th vaccination in the vast majority of patients in this study. Therefore, PPV monotherapy for the 1st to 12th vaccination followed by chemotherapy in platinum-sensitive cases could be recommended not only from a clinical but also an immunological point of view. In the platinum-resistant cases, we did not observed such a clear difference between the PPV monotherapy and the PPV in combination with chemotherapy. Therefore, in platinum-resistant cases, the combination of PPV plus chemotherapy might not contribute to better prognosis, and might increase adverse events. These issues, however, should be addressed in the next step of a clinical trial with relatively large numbers of patients.

Since only some of the patients showed clinical benefit from the peptide-based cancer vaccine, the identification of biomarkers to predict the OS is an important issue[29–32]. Increased IgG responses were observed in 38.1% and 96.7% of patients at the 6th and 12th vaccinations, suggesting that 12 vaccinations would be required to obtain the peptide-specific immunity by PPV. On the other hand, increased CTL responses were observed in 42.9% and 63.3% of patients at

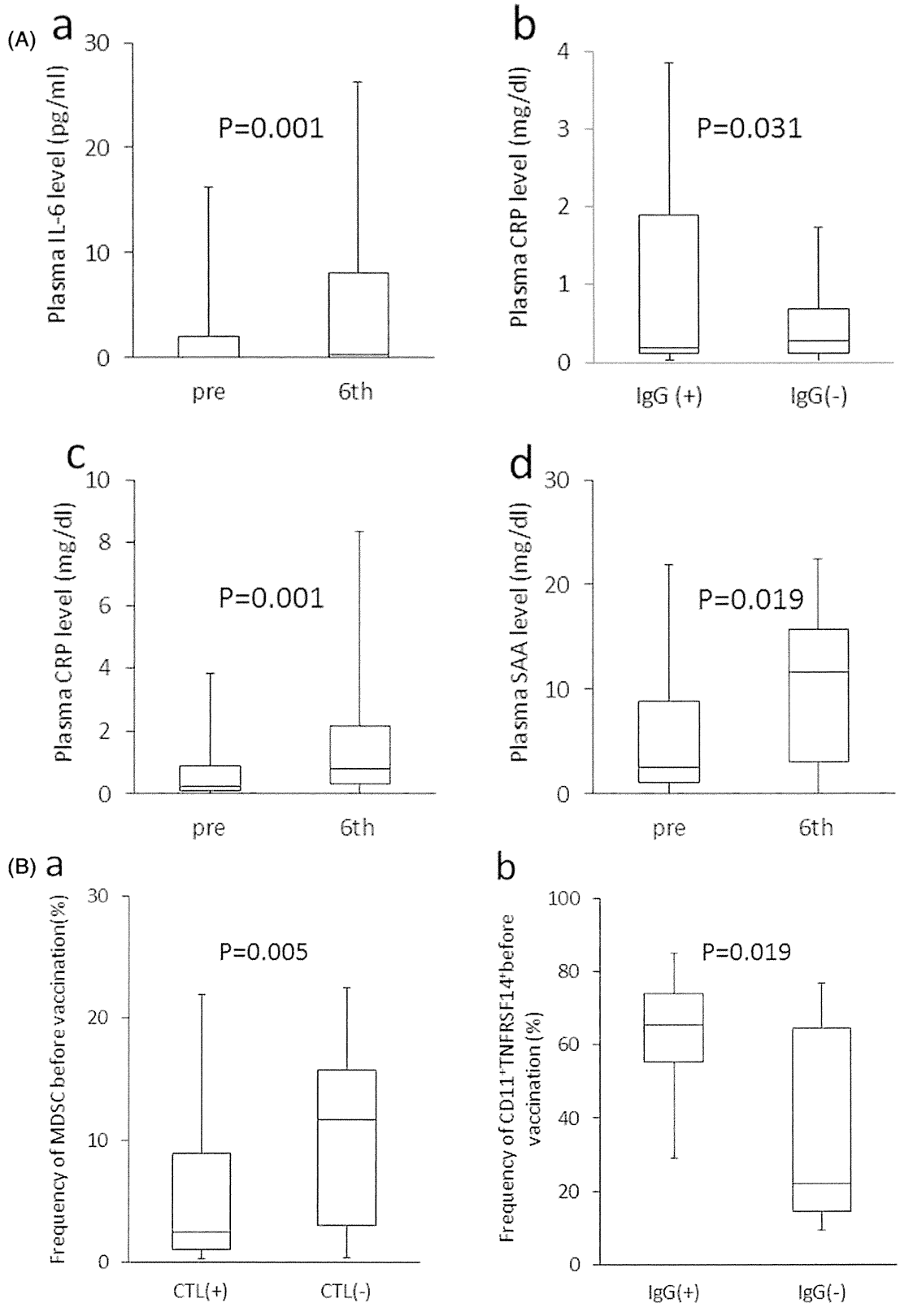

Figure 3. (A) Plasma levels of inflammatory cytokines. There were significant increases in IL-6, CRP, and SAA levels at the time of the 6th vaccination ($p = 0.001$, $p = 0.001$ and $p = 0.01$). Plasma CRP levels before vaccination were higher in the group that showed an augmentation of peptide-specific IgG response at the sixth vaccination ($p = 0.031$). (B) Flow-cytometric analysis of PBMCs. The frequency of MDSC in prevaccination PBMCs was lower in the group that showed an augmentation of CTL response at the sixth vaccination ($p = 0.005$) (a). The frequency of CD11 + TNFRSF14+ before vaccination was higher in the group that showed an augmentation of IgG response at the sixth vaccination ($p = 0.019$) (b).

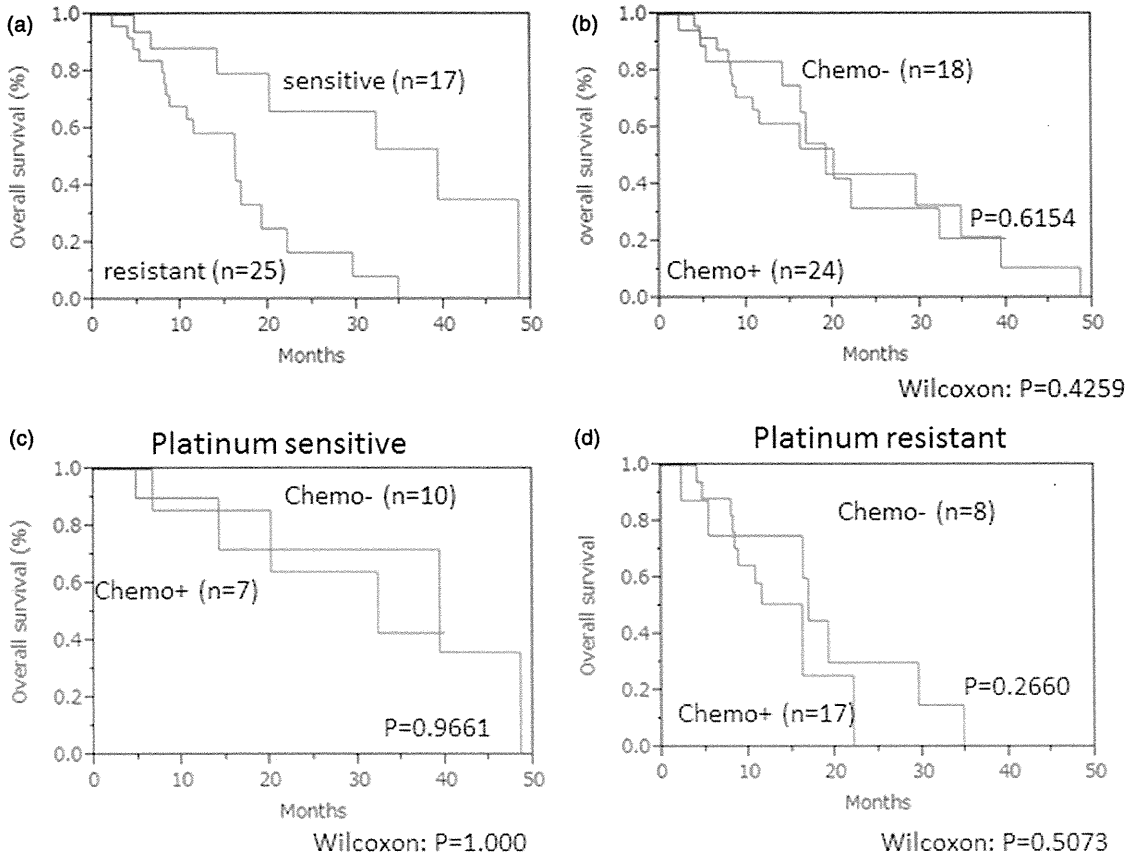

Figure 4. Kaplan–Meier analysis of overall survival. (a) The median survival times of platinum-sensitive (blue) and -resistant (red) recurrent cases were 1179 and 483 days, respectively. (b) The overall survival times of patients who underwent PPV with (red) and without (blue) chemotherapy were not significantly different ($p = 0.0941$, Log-rank test). (c) The overall survival times of platinum-resistant recurrent patients who underwent PPV with (red) and without (blue) chemotherapy were not significantly different ($p = 0.3497$, Log-rank test). (d) The overall survival times of platinum-sensitive recurrent patients who underwent PPV with (red) and without (blue) chemotherapy were not significantly different ($p = 0.2032$, Log-rank test).

Table 5. Univariate and multivariate analyses with clinical and immunological data and OS ($n = 42$).

	Univariate analysis	
Factors	Hazard ratio (95% CI)	p Value
Lymphocyte frequency (%) at pre-vaccination	0.967 (0.927–1.007)	0.1083
Lymphocyte frequency (%) at sixth vaccination	0.927 (0.874–0.976)	0.0029
Skin reaction at the injection	0.458 (0.163–1.474)	0.1771
Increase in CTL responses	0.659 (0.261–1.553)	0.3450
Increase in IgG responses	0.868 (0.241–2.509)	0.8045
epitope spreading	0.299 (0.095–0.789)	0.0135

CI, confidence interval and CTL, cytotoxic T lymphocytes.

the 6th and 12th vaccinations, respectively. The CTL response was less augmented at the 12th vaccination than the IgG response. This might reflect immunological anergy or suppression through MDSC or T-cell checkpoint molecules such as PD-1 or CTLA-4[11,33]. Indeed, we showed that MDSC could be involved in suppression of CTL induction. Repeated vaccination by the same epitope peptides may also induce T-cell exhaustion. We are currently conducting a clinical study in which different peptide sets will be used for each cycle of vaccination to determine whether the T cell exhaustion can be prevented by such a regimen.

Interestingly, ES was correlated with IgG and CTL responses at the 6th vaccination. Furthermore, ES was a prognostic factor by univariate analysis. These results indicated that PPV induced not only peptide-specific immunological boosting in response to the vaccinated peptides but also promoted the spreading of immune responses to the other TAA-derived peptides, which together resulted in the prolongation of OS. In contrast, ED was negatively correlated with OS. These results suggest that T-cell responses to large numbers of TAAs could be better than those to small numbers of TAAs. Further studies with large numbers of patients will be needed to confirm this point. It should be noted that IgGs to the CTL-epitope peptides may not reflect IgGs to the parental protein in most cases, since the peptide-specific IgG recognized a linear epitope but not a conformational epitope, and most of the linear epitopes are conformationally hidden within the molecules.

Although multivariate analysis was not performed due to the limited number of cases, univariate analysis revealed that the frequency of lymphocytes at the 6th vaccination and ES were correlated with unfavorable and favorable OS, respectively. More data still need to be collected to validate these findings. Evaluation of the identified factors could be useful for predicting whether individual patients with recurrent ovarian cancer would benefit from cancer vaccines. These factors might not necessarily be unique to the vaccinated patients.

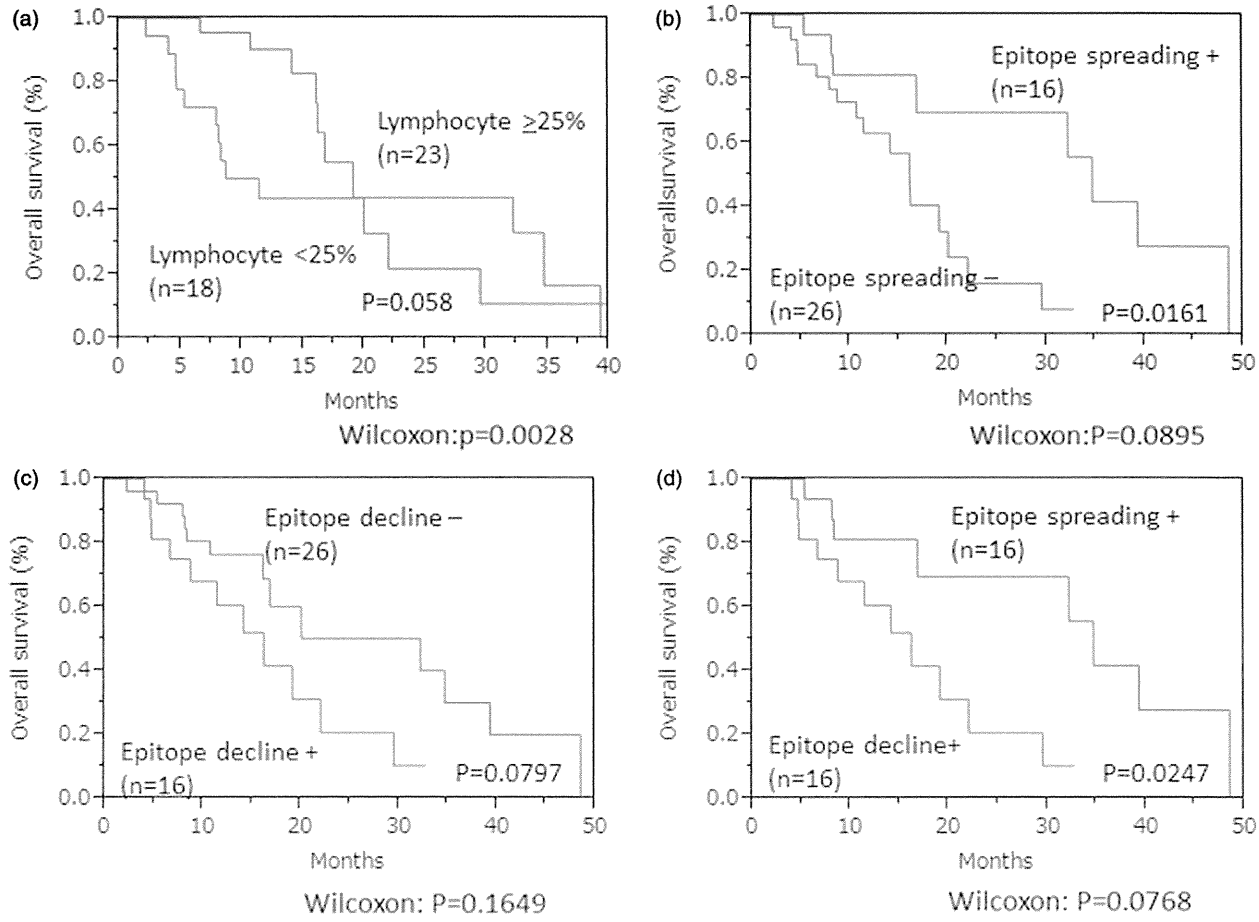

Figure 5. Post-vaccination biomarker analysis. The patients with a lymphocyte frequency >25% tended to have longer overall survival, although this association was not statistically significant ($p = 0.058$) (a). The patients who demonstrated epitope spreading at the sixth vaccination showed longer overall survival ($p = 0.0164$, Log-rank test) (b). The patients with epitope decline at the sixth vaccination showed shorter overall survival as compared to those without epitope decline ($p = 0.0798$, Log-rank test) (c) or those with epitope spreading ($p = 0.0247$, Log-rank test) (d).

Based on these findings on the safety, immune responses and possible prolongation of OS, the next stage of a clinical trial of PPV without chemotherapy during the 1st to 12th vaccination could be recommended for recurrent ovarian cancer patients.

Conclusion

A phase II study of PPV for recurrent ovarian cancer patients was performed to evaluate the efficacy from the point of view of OS. Boosting of CTL or IgG responses specific for the peptides used for vaccination was observed in the majority of patients without any vaccine-related systemic severe adverse events. The MST of the PPV monotherapy group was significantly longer than that of the PPV with chemotherapy group. Because of the safety and possible prolongation of OS, a clinical trial of PPV without chemotherapy during the 1st to 12th vaccination in recurrent ovarian cancer patients is merited.

Declaration of interest

Yamada is a part-time executive of the Green Peptide Co. All other authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Original Article

Validity of Intraoperative Diagnosis at Laparoscopic Surgery for Ovarian Tumors

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ABSTRACT **Study Objective:** To evaluate the accuracy and usefulness of intraoperative diagnosis of ovarian tumor during laparoscopic surgery.
Design: Retrospective cohort study (Canadian Task Force classification II-3).
Setting: Tertiary care university hospital.
Patients: We reviewed the cases of 262 patients who underwent laparoscopic surgery at our institution between January 2005 and December 2011 in whom a benign ovarian tumor was diagnosed intraoperatively.
Interventions: Intraoperative pathologic assessment of frozen sections.
Measurements and Main Results: Intraoperative diagnosis of ovarian tumors demonstrated sensitivity of 80%, specificity of 99.6%, positive predictive value of 80%, and diagnostic accuracy of 99.2%. Mucinous tumors diagnosed intraoperatively showed differing intraoperative and final pathologic diagnoses significantly more frequently than did other types of tumors.
Conclusion: Intraoperative pathologic assessment of benign ovarian tumors during laparoscopic surgery is reliable. However, clinicians should recognize that it is possible to make an incorrect diagnosis in some situations and should exercise caution accordingly. Journal of Minimally Invasive Gynecology (2014) 21, 576–579 © 2014 AAGL. All rights reserved.

Keywords: Frozen section; Intraoperative assessment; Laparoscopy; Ovary

Use of laparoscopic surgery to treat gynecologic disorders has increased because it has many advantages compared with laparotomy, including less postoperative pain, better cosmetics results, and shorter hospital stay. Benign ovarian tumors are considered an indication for laparoscopy. However, some ovarian tumors are ultimately diagnosed as malignant or borderline malignant, although before surgery they were expected to be benign.

Intraoperative diagnosis using frozen sections is expected to decrease the discrepancy between preoperative and final diagnoses. Although the accuracy of intraoperative diagnosis using frozen sections in laparotomy has been demonstrated in many studies [1], there are few reports of its use in laparoscopic surgery to treat ovarian tumors [2,3]. We retrospectively investigated the accuracy and usefulness of intraoperative diagnosis of benign ovarian tumors during laparoscopic surgery.

Patients and Methods

We retrospectively reviewed the medical records of patients who underwent laparoscopic surgery at Kurume University Hospital from January 2005 to December 2011 in whom a benign ovarian tumor was diagnosed via intraoperative assessment using frozen sections. Written informed consent was obtained from all patients. As our usual institutional protocol, 1 camera port (12 mm in diameter) at the

The authors declare no conflicts of interest.

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navel and 2 or 3 forceps ports (5 mm in diameter) at the lower abdomen were inserted. The harvested ovarian tumor or adnexa was removed through the navel port using an endo-bag. In the endo-bag, the tumor was cut into small pieces so that it could be removed from the patient after aspiration of the content fluid. The intraoperative diagnosis was made using frozen sections of 1 or 2 samples from the area that seemed most malignant at macroscopic examination by the attending surgeon.

Pathologic diagnoses of frozen sections and the final diagnoses were made by pathologists. Radiologic assessments of preoperative computed tomography scans or magnetic resonance images were performed by radiologists.

We compared the results of the intraoperative diagnosis using frozen sections and the final pathologic diagnosis to estimate the accuracy of the intraoperative assessment of benign ovarian tumor. The pathologic diagnoses were classified as benign, borderline malignant, and malignant. Mucinous borderline malignant tumors, serous borderline malignant tumors, and atypical endometriosis were classified as the borderline group, and these were further classified as malignant tumors in the analyses between benign vs malignant tumors. We calculated the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the intraoperative diagnoses. Patient age, tumor diameter, and the intraoperative diagnosis were analyzed to determine which groups were prone to incorrect intraoperative diagnosis.

Statistical analysis was performed using commercially available software (StatView version 5.0; SAS Institute, Cary, NC). The χ^2 test was used to compare pairs of groups. Significance was considered at $p < .05$.

Results

Patient Characteristics

From January 2005 to December 2011, 265 patients underwent laparoscopic surgery at our institution because of a preoperative diagnosis of benign ovarian tumor. Of the 265 cases, 216 ovarian cystectomies and 46 adnexectomies were included. The cases of 3 patients who did not undergo intraoperative assessment were excluded from the study. The median patient age was 32.0 years (range, 11–76 years), and the median diameter of the tumors was 61 mm (range, 27–160 mm). All patients underwent computed tomography or magnetic resonance imaging preoperatively to rule out a malignant tumor. Thirteen laparoscopic surgical procedures were performed as emergent operations.

Coordination of Intraoperative and Final Diagnoses

Accuracy of the intraoperative diagnosis for each tumor subtype is given in Table 1. The most frequent result was mature cystic teratoma, followed by endometriotic cyst. Intraoperative diagnostic accuracy was verified in most cases except in the following 3 histologic subtypes: endometriotic cyst, mucinous cystadenoma, and mucinous borderline malignant tumor.

Table 1

Accuracy of intraoperative diagnosis

Intraoperative diagnosis	Accuracy of diagnosis, no. (%)
Mature cystic teratoma	108/108 (100)
Endometriotic cyst	103/104 (99.0)
Serous cystadenoma	22/22 (100)
Mucinous cystadenoma	12/13 (92.3)
Mucinous borderline malignant tumor	1/2 (50)
Serous borderline malignant tumor	1/1 (100)
Atypical gland, endometriosis	1/1 (100)
Brenner tumor	1/1 (100)
Endometrioid adenocarcinoma	1/1 (100)
No malignancy	9/9 (100)
Total	259/262 (98.9)

Insofar as the diagnosis of benign or malignant tumors, 5 tumors were intraoperatively diagnosed as potentially malignant. At histologic analysis, 4 of these were diagnosed as malignant, and the fifth was diagnosed as benign. In contrast, 1 tumor that was diagnosed intraoperatively as benign was histologically diagnosed as borderline malignant. We found that for the intraoperative diagnosis of malignancy, sensitivity was 80%, specificity was 99.6%, positive predictive value was 80%, and negative predictive value was 99.6%. Accuracy for the preoperative and intraoperative diagnoses of malignancy was 98.1% and 99.2%, respectively.

Patient age (<32 years or >32 years cut-off) did not affect the rate of intraoperative diagnostic accuracy of malignancy. No significant relationship was found between the surgical procedure (e.g., cystectomy or adnexectomy) or tumor size and the accuracy rate of diagnosis of malignancy.

In the 15 patients with an intraoperative diagnosis of mucinous tumor that included mucinous borderline malignant tumor, intraoperative diagnostic accuracy was 86.7%. This was a significantly lower rate than for the other subtypes ($p = .01$).

Intraoperative and Postoperative Treatment

Data for the 6 patients with a diagnosis of borderline malignant or malignant tumor in the intraoperative or final diagnosis are given in Table 2. In 5 of these patients the intraoperative diagnosis was borderline malignant or malignant tumor. Two procedures were converted to laparotomy to perform a staging surgery; 3 were not converted to laparotomy; and the remaining patient had a postoperative diagnosis of benign tumor. All 5 lesions with a final diagnosis of borderline malignant or malignant tumor were FIGO stage I. Only 1 patient was diagnosed as having an endometrioid adenocarcinoma, both intraoperatively and at final pathology. Because

Table 2

Cases with malignancy in intraoperative or final diagnosis

Patient	Patient age, yr	Parity	Tumor size, mm	Intraoperative diagnosis	Conversion	Final histologic diagnosis	Restaging surgery	Adjuvant chemotherapy
1	24	G0P0	40	Endometrioid adenocarcinoma	Yes	Endometrioid adenocarcinoma G2	No	Paclitaxel and carboplatin
2	27	G0P0	85	Mucinous borderline malignant tumor	Yes	Mucinous borderline malignant tumor	No	No
3	34	G2P2	93	Mucinous borderline malignant tumor	No	Mucinous cystadenoma	No	No
4	40	G3P3	65	Serous borderline malignant tumor	No	Serous borderline malignant tumor	BSO, PLND, OMT, TAH	No
5	38	G1P1	41	Atypical gland, endometriosis	No	Atypical endometriosis	BSO, PLND, OMT, TAH	No
6	36	G0P0	41	Mucinous cystadenoma	No	Mucinous borderline malignant tumor	LSO, OMT, PLND	No

BSO = bilateral salpingo-oophorectomy; OMT = omentectomy; PLND = pelvic lymph node dissection; TAH = total abdominal hysterectomy.

this patient was aged 24 years and no part of the tumor was enhanced with use of a radiopaque agent at magnetic resonance imaging, the tumor was preoperatively assumed to be benign. Only this patient received adjuvant chemotherapy.

Discussion

Many studies have demonstrated the reliability of intraoperative diagnosis during laparotomic surgery to treat ovarian tumors [1,4,5]. In a meta-analysis of 18 studies of frozen-section diagnosis of adnexal masses, the authors concluded that accuracy was good, with sensitivity of 65% to 97% and specificity of 97% to 100% [1]. Likewise, the present study achieved sufficiently accurate results for intraoperative diagnosis during laparoscopic surgery, i.e., 80% sensitivity and 99.6% specificity for extracting malignant or borderline malignant ovarian tumors. The accuracy of intraoperative identification of a malignant tumor was 99.2%.

To our knowledge, only 2 reports have focused on intraoperative diagnosis during laparoscopic surgery to treat ovarian tumors [2,3]. Although our study was limited to cases with tumors assumed to be benign, we achieved a good accuracy rate of intraoperative diagnosis. The present report included the largest number of patients.

Intraoperative diagnosis has been considered more helpful in ruling out malignant tumors when compared with preoperative tumor markers or ultrasonography [6]. Nevertheless, low accuracy has been reported in the diagnosis of borderline malignant tumors and mucinous tumors [7–10]. In a review of 140 ovarian borderline malignant tumors including 80 serous, 47 mucinous, 11 mixed, and two endometrioid types, the concordance rate between intraoperative diagnosis and final diagnosis was 60% for all borderline malignant tumors [10]. Underdiagnosis was more likely in the nonserous types, tumors >20 cm, and tumors confined to the ovaries [5]. Storms et al [11] examined 73 mucinous tumors and found that the concordance rate between intraoperative and final diagnosis was 66% and that borderline malignant and benign mucinous tumors were particularly difficult to differentiate. In the present study, too, the concordance rate between the intraoperative and final diagnoses of mucinous tumors was significantly lower than that for other tumors. Greater tumor size and the frequent coexistence of benign, borderline malignant, and malignant components have been suggested as the sources of difficulty in diagnosing mucinous tumors intraoperatively [7,10]. Biopsy of 3 sections was recommended for mucinous ovarian tumors for intraoperative diagnoses because of the low accuracy rate of frozen-section diagnosis in large mucinous ovarian tumors [12].

Ideally, all tumors diagnosed intraoperatively as malignant or borderline malignant should be staged at surgery to avert a secondary surgery. However, in the present study, 3 of 5 tumors diagnosed as potentially malignant did not undergo staging laparotomy despite being diagnosed as

potentially malignant because the tumors were strongly suspected to be benign in macroscopic appearance. In one patient, the intraoperative diagnosis of borderline malignant tumor was changed to benign in the final diagnosis.

To avert overtreatment, we should recognize the limitations of frozen sections, in particular in the diagnosis of mucinous tumors, and the importance of gross findings when the treatment strategy is being planned. A gynecologist should inform the patient and her family about the possibility of a false-negative result obtained via frozen-section biopsy which could result in a planned secondary surgery, especially in the case of mucinous tumors.

In conclusion, the results of the present study confirm the usefulness of intraoperative diagnosis during laparoscopic surgery to treat benign ovarian tumors. If economically feasible, intraoperative diagnosis should be considered in all laparoscopic surgical procedures. However, it should be remembered that in specific tumor subtypes, there are limitations to intraoperative diagnosis.

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Prevention of lymphocele development in gynecologic cancers by the electrothermal bipolar vessel sealing device

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Objective: A number of new techniques have been developed to prevent lymphocele formation after pelvic lymphadenectomy in gynecologic cancers. We assessed whether the electrothermal bipolar vessel sealing device (EBVSD) could decrease the incidence of postoperative lymphocele secondary to pelvic lymphadenectomy.

Methods: A total of 321 patients with gynecologic cancer underwent pelvic lymphadenectomy from 2005 to 2011. Pelvic lymphadenectomy without EBVSD was performed in 134 patients, and pelvic lymphadenectomy with EBVSD was performed in 187 patients. We retrospectively compared the incidence of lymphocele and symptoms between both groups.

Results: Four to 8 weeks after operation, 108 cases of lymphocele (34%) were detected by computed tomography scan examination. The incidence of lymphocele after pelvic lymphadenectomy was 56% (75/134) in the tie ligation group, and 18% (33/187) in the EBVSD group. We found a statistically significant difference in the incidence of lymphocele between both groups ($p<0.01$). To detect the independent risk factor for lymphocele development, we performed multivariate analysis with logistic regression for three variables (device, number of dissected lymph nodes, and operation time). Among these variables, we found a significant difference ($p<0.001$) for only one device.

Conclusion: Use of the EBVSD during gynecological cancer operation is useful for preventing the development of lymphocele secondary to pelvic lymphadenectomy.

Keywords: Electrothermal bipolar vessel sealing device, Gynecologic cancer, LigaSure, Lymphocele, Pelvic lymphadenectomy, Venous thrombosis

INTRODUCTION

Pelvic lymphocele, one of the sequelae of pelvic lymphadenectomy, is defined as a collection of lymphatic fluid without distinct epithelial lining, resulting from the transection of afferent lymphatic channels [1]. Lymphocele is caused by leakage of lymph from afferent lymphatic channels as the result of tissue trauma or operation. Pelvic lymphadenectomy is a crucial step in gynecologic cancer operation. The most frequent postoperative complication of pelvic lymphadenectomy is lymphocele, also known as lymphocyst, and it is a consequence of surgical dissection and inadequate closure of afferent lymphatic vessels. In literature, the reported incidences of clinically detected lymphocele after pelvic lymphadenectomy range from 1% to 49% [2-11]. The risk factors of lymphocele include extensive pelvic lymphadenectomy, number of lymph nodes (LNs) removed, lack of ligation of lymphatic vessels, preoperative or postoperative radiation therapy, presence of metastasis to the LNs, use of retroperitoneal suction drainage, and administration of low-dose heparin for thromboembolic prophylaxis [12,13].

Most lymphoceles are asymptomatic; thus, they are found

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incidentally. However, large lymphoceles may sometimes be symptomatic, resulting from compression of surrounding structures. Associated symptoms include pelvic pain, leg edema and pain, hydronephrosis, and deep vein thrombosis (DVT). Furthermore, if the lymphocele becomes infected, an abscess may form, and possibly cause sepsis.

To prevent postoperative lymphocele formation, a number of techniques have been challenged so far, including the non-closure of the pelvic peritoneum, absence of retroperitoneal drainage, omentoplasty, and fibrin application. Although these techniques have been developed, little has been reported on whether they lead to a significant reduction in postoperative lymphocele after pelvic lymphadenectomy [7,8,10,11,14-18].

The electrothermal bipolar vessel sealing device (EBVSD) has been designed to aid in coagulation and dissection with less thermal spread than conventional electrocautery. We introduced EBVSD to gynecologic cancer operation in 2007. Considering the ability of this method to firmly seal the lymphatic vessels, we hypothesized that EBVSD could decrease the incidence of postoperative lymphocele secondary to pelvic lymphadenectomy. To our knowledge, there are only a few up-to-date studies focusing on lymphocele development after the use of EBVSD post pelvic lymphadenectomy in patients with gynecologic cancers. The aim of this study was to clarify whether EBVSD contributed to a decrease in the incidence of postoperative lymphocele secondary to pelvic lymphadenectomy.

MATERIALS AND METHODS

1. patients

A total of 321 patients with gynecologic cancer underwent surgical procedures including pelvic lymphadenectomy, at the Department of Obstetrics and Gynecology of Kurume University Hospital, between 2005 and 2011. These surgeries were performed on patients with cervical cancer (n=126), endometrial cancer (n=119), ovarian cancer (n=70), and other types of gynecologic cancers (n=6). Pelvic lymphadenectomy was performed with total abdominal hysterectomy, radical hysterectomy, or modified radical hysterectomy, with para-aortic LN sampling, with or without bilateral salpingo-oophorectomy.

We did a retrospective analysis of the incidence of lymphocele after pelvic lymphadenectomy, with or without EBVSD in patients with gynecologic cancer. Patients were classified into two groups; the tie ligation and EBVSD groups. Respectively, these groups were compared for each factor, i.e., primary lesion, International Federation of Gynecology and Obstetrics (FIGO) stage, age, bleeding during operation, operation time, number of dissected LNs, LN metastasis, adjuvant radiotherapy, lymphocele formation, and diameter of lymphocele.

2. Surgical procedure

Since 2007, we have used the EBVSD (BiClamp, Elektromedizin GmbH, Tubingen, Germany; LigaSure, Covidien, Boulder, CO, USA) in the operation of gynecologic cancer.

Pelvic lymphadenectomy was defined as the excision of all fibro-fatty tissue along the external iliac vein, including the bifurcation of the common iliac artery together with fibro-fatty tissue within the obturator fossa. During pelvic lymphadenectomy, we paid special attention to seal or ligate lymphatic vessels: (1) at the level of the femoral canal, on the ventral walls of external iliac vessels; (2) at the level of obturator fossa, where numerous channels are connected with the lateral parametria; and (3) at the bifurcation of common iliac vessels, cranially to the internal iliac vessels, and medial to the external iliac vessels (**Fig. 1**).

In this series, we did not suture the retroperitoneum in all patients. From 2005 to 2008, a Penrose drain was placed in each paravesical space and led outward through the vagina. Since 2009, a closed suction drain was placed through the abdominal wall into each paravesical space. The volume of fluid from each drain was measured daily. The drains were removed when the fluid drainage was <100 mL/day. All patients received intraoperative antibiotic prophylaxis, which was continued postoperatively for 3 days. The dissected LNs were counted and submitted for histopathological examination.

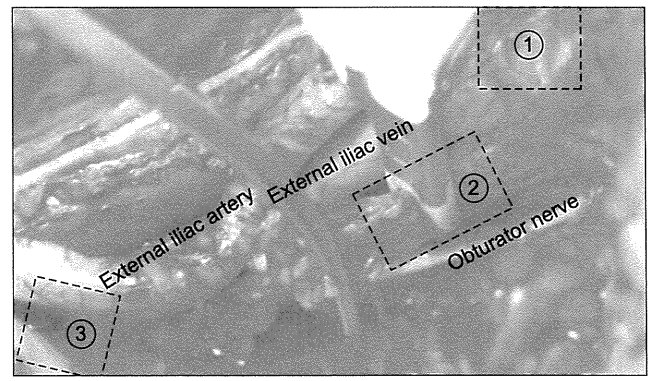

Fig. 1. Boxed parts indicate: (1) the level of the femoral canal, on the ventral walls of external iliac vessels; (2) the level of obturator fossa, where numerous channels are connected with the lateral parametria; and (3) the bifurcation of common iliac vessels, cranially to the internal iliac vessels, and medial to the external iliac vessels. We performed double sealing of the lymphatic vessel edge at the level of the obturator fossa by LigaSure small jaw.

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We checked for lymphocele by computed tomography (CT) between 4 and 8 weeks postoperatively. We also checked routinely for lymphocele and recurrent disease by CT at 6 months postoperatively. In the event that lymphocele was detected during postoperative visits, we started close follow-up by CT or ultrasonography. All findings were checked by a radiologist. The finding of a smooth and thin-walled cavity filled with a water-equivalent fluid, which was sharply demarcated from its surroundings on CT, was interpreted as lymphocele. In this series, we diagnosed a symptomatic lymphocele if the patient had at least one grade 1, 2, or 3 adverse effects according to the Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0. Main symptoms were lower abdominal pain, back pain, prolonged ileus, leg pain, edema, or fever. We also retrospectively compared the incidence and diameter of symptomatic lymphocele between groups: the tie ligation and the EBVSD groups.

3. Statistical analysis

For parametric analyses, we used the Student t-test and Welch t-test, while for nonparametric analyses we used the Mann-Whitney U-test. Statistical analysis was performed with the chi-square test in a contingency table. Simultaneous analysis of all possible influential variables was performed with logistic regression. A $p<0.05$ was regarded as statistically significant. The data collected were analyzed using JMP ver. 8.0.2 (SAS Institute Inc., Cary, NC, USA).

Table 1. Comparison of patient characteristics between tie ligation and electrothermal bipolar vessel sealing device groups

Characteristic	Tie ligation group (n=134)	EBVSD group (n=187)
Age (yr)	52±12	53±12
Primary lesion		
Cervical	58 (43)	68 (36)
Endometrial	42 (31)	77 (41)
Ovarian	33 (25)	37 (20)
Others	1 (1)	5 (3)
FIGO stage		
I	68 (51)	97 (52)
II	23 (17)	27 (15)
III	41 (31)	60 (32)
IV	2 (1)	3 (1)

Values are presented as mean±SD or number (%).
EBVSD, electrothermal bipolar vessel sealing device; FIGO, International Federation of Gynecology and Obstetrics.

RESULTS

1. Characteristics of patients

Three hundred twenty-one patients underwent pelvic lymphadenectomy in Kurume University Hospital between 2005 and 2011. From 2005 to 2007, 134 patients underwent pelvic lymphadenectomy without EBVSD (tie ligation group). From 2007 to 2011, 187 patients had pelvic lymphadenectomy with EBVSD (EBVSD group). Comparison of patient characteristics between the two groups was shown in **Table 1**. Between the two groups, there were no statistically significant differences in bleeding during the operation, number of dissected pelvic LNs, LN metastasis, or adjuvant radiotherapy. The operation time was longer in the EBVSD group ($p=0.014$) (**Table 2**).

2. Detection of lymphocele

Four to 8 weeks after operations, in 108 of the 315 patients, lymphocele (34%) was detected by CT scan. The incidence of lymphocele after pelvic lymphadenectomy was 56% (75/134) in the tie ligation group, while only 18% (33/187) in the EBVSD group. We found a notable decrease in the incidence of lymphocele in the EBVSD group ($p<0.01$). The mean±SD diameter of the lymphocele was 3.8±1.6 cm in the tie ligation group, and 3.8±2.1 cm in the EBVSD group. There were no significant differences between both groups (**Table 2**).

3. Incidence of symptomatic lymphocele

The total incidence of symptomatic lymphocele was 9% (29/321), while asymptomatic lymphocele was 25% (79/321). We found that the incidence of symptoms was in proportion to the diameter of the lymphocele. The mean±SD diameter of lymphoceles was 5.2±2.0 cm in symptomatic lymphoceles,

Table 2. Comparison of treatment characteristics between tie ligation and electrothermal bipolar vessel sealing device groups

Variable	Tie ligation group (n=134)	EBVSD group (n=187)	p-value
Bleeding (mL)	1,004±898	973±878	0.80
Operation time (min)	311±92	340±95	0.01
No. of dissected LNs	23±9	22±9	0.93
Positive LN metastasis	38 (28)	52 (28)	0.91
Adjuvant radiotherapy	17 (13)	13 (7)	0.08
Lymphocele formation	75 (56)	33 (18)	<0.01
Diameter of lymphocele (cm)	3.8±1.6	3.8±2.1	0.54

Values are presented as mean±SD or number (%).
EBVSD, electrothermal bipolar vessel sealing device; LN, lymph node.

and 3.3±1.3 cm in asymptomatic lymphoceles (p<0.01). The main presenting symptoms were lower abdominal pain, fever, and ileus, which developed from 2 to 6 weeks after the operation. Symptomatic lymphoceles included 21% (6/29) with grade 1 adverse effect (light low abdominal pain without fever), and 79% (23/29) with grades 2 and 3 adverse effect (fever, pelvic abscess, ileus, or hydronephrosis) in CTCAE ver. 4.0.

A notable difference (p<0.001) in the incidence of symptomatic lymphocele was found between the tie ligation group (14%, 19/134) and the EBVSD group (5.3%, 10/187). However, in terms of the ratio of the two groups in 108 cases with lymphocele, there was no statistical difference in the incidence of symptomatic lymphocele (tie ligation group: 25%, 19/75; EBVSD group: 30%, 10/33; p=0.59). All lymphocele diminished within ten months without surgical treatment. In the EBVSD group, there was no difference in the postoperative drainage volume between a closed suction drain and a Penrose drain (open; p=0.9).

4. Risk factors of lymphocele development

We performed univariate analysis of all possible influential variables that could influence the development of lymphocele (device [tie ligation vs. EBVSD], primary lesion, FIGO stage, age [<50 vs. ≥50], bleeding, operation time, number dissected of LNs, LN metastasis, and adjuvant radiotherapy) (**Table 3**). There was significant difference in device (p<0.01). Additionally, there seemed to be some difference in operation time (p=0.071) and number dissected of LNs (p=0.10). On the other hand, there were no significant difference in primary lesion, FIGO stage, age, bleeding, LN metastasis, and adjuvant radiotherapy. Furthermore, we performed multivariate analysis with logistic regression on three variables (device, operation time, and number dissected of LNs). We found that there was only a significant difference for device (p<0.001) among these variables. A high odds ratio (OR) 5.61 was observed in the tie ligation group, while a low OR 0.18 was found in the EBVSD group (**Table 3**).

DISCUSSION

The presentation of lymphocele may be quite diverse in pelvic cancer operation. The rate of asymptomatic lymphocele may approach 50% to 60% [19]. Symptomatic patients may present with ileus, pain, fever, lower extremity edema, or urinary complaints [20]. Symptomatic or clinically significant lymphocele was reported in 1.6% to 3.5% of patients who underwent pelvic lymphadenectomy for prostate cancer [21,22]. In our research, we found that 34% of patients with gynecologic cancer undergoing pelvic lymphadenectomy at our hospital between 2005 and 2011 developed lymphocele.

The formation of lymphocele may be promoted by several factors: extensive pelvic lymphadenectomy, the number of LNs removed, lack of ligation of lymph vessels, the presence of metastasis to LNs, postoperative radiation therapy, the pres-

Table 3. Univariate and multivariate analysis of risk factors of lymphocele development

Factor	Category	Univariate		Multivariate*	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Device	Tie ligation	3.17 (2.25–4.47)	<0.01†	5.61 (3.38–9.47)	<0.001
	EBVSD	0.17 (0.10–0.28)		0.18 (0.11–0.30)	
Primary lesion			0.98†		
FIGO stage			0.66†		
Age (yr)	<50	0.92 (0.66–1.28)	0.62†		
	≥50	1.09 (0.78–1.50)			
Bleeding		0.99 (0.99–1.00)	0.711†		
Operation time		0.99 (0.99–1.00)	0.071†	1.00 (0.99–1.00)	0.28
No. of dissected LNs		1.02 (0.99–1.05)	0.10§	1.02 (0.99–1.05)	0.15
LN metastasis	Positive	1.47 (0.88–2.44)	0.13†		
	Negative	0.78 (0.57–1.07)			
Adjuvant radiotherapy	+	0.54 (0.26–1.06)	0.12†		
	–	0.69 (0.45–1.05)			

CI, confidence interval; EBVSD, electrothermal bipolar vessel sealing device; FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; OR, odds ratio.
*Logistic regression. †Chi-square test for independence. ‡Mann-Whitney U-test. §Student t-test.

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ence of drainage, and the prophylactic use of anticoagulants [1,7,8,10,11,13-18,23-28]. However, none of these potential factors has been proven to be significant.

A retrospective study reported the risk factors of lymphocele in 264 patients who underwent pelvic lymphadenectomy during gynecologic cancer operations [29]. Among these patients, body mass index and number of resected LNs were higher in the lymphocele group, and the use of postoperative radiotherapy was associated with a higher risk of lymphocele. In the present series, we could not detect a significant difference in the effect of number of dissected of LNs and adjuvant radiotherapy on the formation of lymphocele. In terms of extensive lymphadenectomy, patients in this series underwent pelvic lymphadenectomy with para-aortic LN sampling. Therefore, we did not check the influence of para-aortic LN dissection on the formation of lymphocele in this series. A complete understanding of the formation of lymphocele remains to be elucidated.

Lymphocele incidence can be minimized by meticulous surgical techniques and attention to sealing the lymphatic vessels during node dissection, by blocking lymphatic drainage from lower extremities and preventing lymph accumulation in the pelvic cavity. In particular, treatment-associated morbidity can be reduced when all lymphatic channels lateral to the external artery are saved or clamped [5,7,8,15]. In the reported series, different techniques for the prevention of lymphocele have been described [14,16-18]. The intraoperative application of fibrin glue did not reduce the rate of lymphocele after pelvic lymphadenectomy [18]. Moreover, closed suction drainage or omental flaps have been proposed to prevent the occurrence of pelvic lymphadenectomy-related complications. However, a widely agreed-upon solution is yet to be found [9,14,15,17].

The development of lymphocele is an issue for patients when it leads to sequelae relevant to their health. Additionally to secondary infection of lymphocele, sequelae comprise mainly thromboembolic events due to compression of pelvic vessels. Development of DVT secondary to lymphocele has been reported [30,31]. The reported rate of DVT in patients with gynecologic cancer ranges from 11% to 18%, with a rate of pulmonary embolism (PE) between 1% and 2.6%. Among patients with ovarian cancer, the postoperative rate of PE is as high as 7.2% [24,32-36]. Fortunately, these events are rare in association with pelvic lymphadenectomy, though they represent a significant cause of operative and perioperative mortality.

In this series, we detected five cases of DVT preoperatively. All the patients with DVT were administered intravenous heparin and put on an inferior vena cava (IVC) filter preoperatively. These patients also received intravenous heparin postoperatively until removal of the IVC filter. None of the patients developed DVT postoperatively.

Since 2007, we introduced EBVSD for sealing lymphatic vessels during pelvic lymphadenectomy, in order to prevent the development of lymphocele postoperatively. The introduction of energy-based vessel sealing technologies has expanded the arsenal of potential techniques available for transoperative hemostasis. These devices allow a rapid sequential tissue and vessel sealing, coagulation, and transection. There are several EBVSD currently in use. In our series, we used the bipolar sealing devices LigaSure and BiClamp. LigaSure employs a unique combination of pressure and energy to create vessel fusion. This optimized combination of pressure and energy melts the collagen and elastin in the vessel walls, reforming it into a permanent, plastic-like seal [37]. LigaSure has the highest burst pressure and fastest sealing time compared to other similar devices, and it was the highest rated overall [38]. In this series, we mainly used LigaSure small jaw, which has a suitably sized body (18.8 cm) for lymph vessel sealing and has an appropriate angled jaw (28°) for pelvic floor procedures.

Due to the lack of smooth muscle cells in the wall of lymphatic vessels, a low concentration of clotting factors, and a lack of thrombocytes in lymphatic fluids, it is possible that an EBVSD is less effective in sealing only the lymphatic vessels. Therefore, it is recommended to seal surrounding connective tissue together with the lymphatic vessels to reinforce the sealing effect (**Fig. 1**). Additionally, we performed double sealing of lymphatic vessel edges, especially the three major lymphatic channels 1 to 3 as mentioned in the Materials and Methods. These ingenuities may contribute to a decrease in the lymphocele development compared with previous reports [2-11].

Before introducing EBVSD, the prevention of lymphocele formation was achieved by simply tie-ligating the edge of lymphatic vessels. Using only tie ligation, we experienced lymphocele in 56% (75/134) of cases between 2005 and 2007. However, since the introduction of EBVSD on 2007, the incidence of lymphocele dramatically decreased to 18% (33/187). Our findings suggest that the development of lymphocele notably decreases by using EBVSD in pelvic lymphadenectomy.

Symptomatic lymphocele led to considerable delays in the introduction of adjuvant chemotherapy. In the case of patients needing adjuvant chemotherapy, symptomatic lymphocele can account for a significant amount of morbidity and cost because these patients are more susceptible to repeated infections by the bone marrow suppression caused by adjuvant chemotherapy.

In our series, all patients who had grade 2 and 3 adverse effects of symptomatic lymphocele (n=23) were administered

intravenous antibiotics for 3 to 5 days. All these patients responded adequately to antibiotic treatment alone, and we did not enforce other treatment strategies, such as percutaneous needle aspiration, percutaneous catheter drainage, sclerotherapy or open drainage by laparotomy, or laparoscopy.

However, in 93% of patients (14/15) with symptomatic lymphocele who had indication of adjuvant chemotherapy, the induction of first cycle of chemotherapy was delayed. Considering these delays, especially for patients who need adjuvant chemotherapy, we may suggest attempting other treatment strategies, such as percutaneous needle aspiration in addition to the administration of intravenous antibiotics.

The difference in the size of lymphocele between the tie ligation group and the EBVSD group was not discernable. Compared with the tie ligation group, a considerable amount of diminutive cases of symptomatic lymphocele formation were observed in the EBVSD group, while there was no difference in the ratio of symptomatic to asymptomatic lymphocele between groups. Therefore, the introduction of EBVSD could decrease the development of symptomatic lymphocele by decreasing the total number of lymphocele themselves.

The limitation of this study was its retrospective design. Therefore, further prospective studies of technique comparisons, such as EBVSD versus tie ligation versus clip, are needed to improve the selection process of proper devices to prevent lymphocele formation.

In conclusion, the use of EBVSD during pelvic lymphadenectomy could be beneficial in the prevention of lymphocele development in patients with gynecological cancer.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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ORIGINAL ARTICLE

A phase I study of irinotecan and pegylated liposomal doxorubicin in recurrent ovarian cancer (Tohoku Gynecologic Cancer Unit 104 study)

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Abstract
Purpose A phase I clinical study was conducted to determine the maximum tolerated dose (MTD) and the recommended dose (RD) of irinotecan hydrochloride (CPT-11) in CPT-11/pegylated liposomal doxorubicin (PLD) combination therapy, a novel treatment regimen for platinum- and taxane-resistant recurrent ovarian cancer.
Methods Pegylated liposomal doxorubicin was administered intravenously on day 3 at a fixed dose of 30 mg/m^2. CPT-11 was administered intravenously on days 1 and 15, at a dose of 50 mg/m^2 on both days. One course of chemotherapy was 28 days, and patients were given a maximum of six courses, with the CPT-11 dose being increased in increments of 10 mg/m^2 (level 1, 50 mg/m^2; level 2, 60 mg/m^2; level 3, 70 mg/m^2; level 4, 80 mg/m^2) to determine MTD and RD.
Results During the period from April 2010 to March 2013, three patients were enrolled for each level. In the first course, no dose-limiting toxicity occurred in any of the patients. Grade 4 neutropenia was observed in two of three patients at level 4. At level 4, the antitumor effect was a partial response (PR) in two of the three patients and stable disease (SD) in one. At level 3, one of the three patients showed PR and two had SD. At level 4, the start of the next course was postponed in two of three patients. In addition, one patient at level 4 experienced hemotoxicity that met the criteria for dose reduction in the next course. The above results suggested that administration of CPT-11 at dose level 5 (90 mg/m^2) would result in more patients with severe neutropenia and in more patients requiring postponement of the next course or a dose reduction. Based on the above, the RD of CPT-11 was determined to be 80 mg/m^2.
Conclusions The results suggest that CPT-11/PLD combination therapy for recurrent ovarian cancer is a useful treatment method with a high response rate and manageable adverse reactions. In the future phase II study, the safety and efficacy of this therapy will be assessed at 80 mg/m^2 of CPT-11 and 30 mg/m^2 of PLD.

Keywords Recurrent ovarian cancer · Chemotherapy · CPT-11 · PLD

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Introduction

The standard initial chemotherapy for advanced ovarian cancer is paclitaxel plus carboplatin (TC) combination therapy [1–3]. However, no treatment regimen for second-line chemotherapy has yet been established against recurrence after TC therapy. Various attempts are currently being made using, as criteria, the type of recurrence and the period from the last treatment until recurrence. Since recurrent

ovarian cancer with a treatment-free interval (the period from the end of the initial chemotherapy until recurrence) of <6 months is considered to be platinum resistant, it will be essential to select drugs not showing cross-resistance with the initial therapy. In the United States and Europe, the type I DNA topoisomerase inhibitor topotecan [4], pegylated liposomal doxorubicin (PLD) [5], and gemcitabine [6] are used against platinum-resistant recurrent ovarian cancer. In a Japanese phase II study involving patients with ovarian cancer previously treated with chemotherapy including platinum-based agents, PLD was reported to achieve an overall response rate of 21.9 % (27.3 % [3/11] in the platinum-sensitive group and 21.0 % [13/62] in the platinum-resistant group) [7]. In a phase III non-inferiority study comparing PLD with topotecan, it was reported that in patients treated with PLD, the response rate was 19.7 %, median progression-free survival (PFS) was 16.1 weeks, and mean survival time (MST) was 60.0 weeks and, in patients with platinum-resistant tumors in particular, the response rate was 12.3 %, median PFS was 9.1 weeks, and MST was 35.6 weeks [8], suggesting that PLD would be a promising therapeutic agent for recurrent ovarian cancer. On the other hand, irinotecan hydrochloride (CPT-11), an anticancer agent developed in Japan, acts by inhibiting topoisomerase I. In a study in which CPT-11 (100 mg/m^2) alone was administered to patients with platinum-resistant recurrent ovarian cancer, the response rate was 29 %, the tumor growth inhibition rate (complete response [CR] + partial response [PR] + not changed) was 61 %, median time to progression was 17 weeks, and MST was 8 months, exhibiting favorable results [9]. Sugiyama et al. [10] reported that CPT-11/cisplatin therapy was effective as second-line chemotherapy for recurrent ovarian cancer after treatment with a platinum agent, raising the expectation that CPT-11 may be effective against platinum- and taxane-resistant tumors.

Herein, we conducted a phase I clinical study to determine the maximum tolerated dose (MTD) and the recommended dose (RD) of CPT-11 in CPT-11/PLD combination therapy, a novel treatment regimen for platinum- and taxane-resistant recurrent ovarian cancer, with the aim of improving the outcomes of ovarian cancer patients.

Subjects and methods

Study population

Upon receiving approval from the intramural ethics committee of each study center, a multicenter clinical study was conducted in patients with recurrent ovarian cancer who met the following criteria and were enrolled in the study during the period from April 2010 to March 2013: (1) ovarian cancer confirmed by histological or cytological diagnosis, (2) recurrence less than 6 months after previous chemotherapy, (3) containing a measurable or evaluable lesion (including CA-125 level), (4) ECOG performance status (PS) 0–2, (5) 20–75-year old, (6) expected survival time of at least 2 months, (7) major organs remained functional (white blood cell count $\geq$3,000/mm^3, neutrophil count $\geq$1,500/mm^3, platelet count $\geq$10,0000/mm^3, total bilirubin $\leq$1.5 mg/dL), and (8) informed consent provided. Exclusion criteria were (1) serious complication(s), (2) evident pulmonary fibrosis or interstitial pneumonitis, (3) pleural or cardiac effusion necessitating prompt local treatment, (4) brain metastasis requiring prompt treatment, (5) diarrhea (watery stool), (6) intestinal paralysis or intestinal obstruction, (7) active infection requiring treatment with antimicrobial agents, and (8) patients considered inappropriate as subjects by the physician in charge for any other reason.

Protocol

Pegylated liposomal doxorubicin was administered intravenously at a fixed dose of 30 mg/m^2 on day 3. CPT-11 was administered intravenously on days 1 and 15. One course of chemotherapy was 28 days, and as a general rule, patients were given at least 2 courses, 6 courses at the maximum.

Method for dose escalation

CPT-11 was started at level 1 (50 mg/m^2) and then increased up to level 4 (80 mg/m^2) (Table 1). A group of three patients were given the same dose level of CPT-11, and if no dose-limiting toxicity (DLT) was observed in any of them, the dose was increased to the next level. If DLT was observed in one of the three patients at the same level, three additional patients were treated at the same dose level, and if there was no observable DLT in at least three of the total six patients, the dose was increased to the next level. If DLT was observed in at least three of the total six patients, the dose was judged to be MTD. If DLT was observed in two of three patients at any level, this dose level was judged to be MTD. The dose that was 1 level below MTD was determined to be RD. DLT was defined as (1) grade

Table 1 Dose escalation schema

	CPT-11 (mg/m^2)	PLD (mg/m^2)
Level 0	40	30
Level 1	50	30
Level 2	60	30
Level 3	70	30
Level 4	80	30

4 leukopenia or neutropenia lasting for at least 4 days, (2) grade 3 or higher leukopenia or neutropenia accompanied by pyrexia of ≥38 °C, (3) grade 4 or higher thrombocytopenia or thrombocytopenia requiring platelet transfusion, or (4) grade 3 or higher nonhematological toxicity (except nausea/vomiting, anorexia, and general malaise). Adverse events were evaluated according to NCI-CTCAE ver. 3, and MTD was determined during the first course.

Criteria for changing dosing schedule

If any of the following applied, CPT-11 administration on day 15 was to be postponed and the drug was to be administered on day 22 upon confirming recovery from the condition: (1) white blood cell count ≤2,000/mm^3, (2) neutrophil count ≤1,000/mm^3, (3) platelet count ≤75,000/mm^3, or (4) grade 1 or higher diarrhea. If recovery from the condition was not seen on day 22, the second CPT-11 administration was to be skipped (not to be administered on day 29). The criteria for proceeding to the second and subsequent courses were (1) white blood cell count ≥3,000/mm^3, (2) neutrophil count ≥1,500/mm^3, (3) platelet count ≥100,000/mm^3, (4) total bilirubin ≤1.5 mg/dL, (5) diarrhea grade 0, and (6) grade 1 or lower hand-and-foot syndrome and stomatitis. If the patient met any of the above criteria, administration was to be performed after waiting for recovery for a maximum of 14 days. If recovery from these conditions was not seen after 14 days, the treatment was to be discontinued. If the severity of hand-and-foot syndrome or stomatitis remained at grade 2 or higher after a 14-day postponement, PLD on day 3 in the next course was to be skipped.

Criteria for dose reduction

The doses of CPT-11 and PLD in the next course were reduced according to the severity of adverse reactions that occurred in the previous course. If grade 4 leukopenia, grade 4 neutropenia, or grade 3 thrombocytopenia were observed in the previous course, CPT-11 was reduced by 10 mg/m^2, and PLD by 7.5 mg/m^2. If grade 2 or higher diarrhea, spasmodic abdominal pain, or watery stool were observed, the CPT-11 dose was reduced by 10 mg/m^2. If grade 3 hand-and-foot syndrome or stomatitis was observed, the PLD dose was reduced by 7.5 mg/m^2 regardless of whether or not these conditions improved before the start of the next course.

Evaluation of antitumor effect

The antitumor effect was evaluated by imaging at the end of every two courses. For the evaluation of the antitumor effect, the best response rate was calculated according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline.

Results

Patient background characteristics

Table 2 shows the background characteristics of 12 patients enrolled in this study during the period from April 2010 through March 2013. All patients had been treated with taxane- or platinum-based agents as a part of the previous therapy.

Adverse events

Three patients were enrolled for each level, and none of them experienced DLT in the first course. No grade 3 or higher neutropenia was observed at level 1. Grade 4 leukopenia was observed in one patient each at level 2 and level 3, and in two patients at level 4. No grade 3 or higher thrombocytopenia was observed at level 1 or 2. At level 3, grade 3 thrombocytopenia was observed in one patient, and at level 4, two patients developed grade 2 thrombocytopenia, while no grade 3 or higher thrombocytopenia

Table 2 Patients characteristics (N=12)

Age	
Median	56
Range	40–65
PS	
0	11
1	1
FIOG stage	
I	2
II	1
III	8
IV	1
Histological type	
Serous	8
Mucinus	0
Clear cell	3
Endometrioid	1
Previous regimens	
1	4
2	3
3≤	5
Last regimen	
TC	8
TP	1
DP	1
CDDP/VP16	1
PTX	1

TC paclitaxel/carboplatin, *TP* paclitaxel/cisplatin, *DP* docetaxel/cisplatin, *CDDP* cisplatin, *PTX* paclitaxel