Table 4. Clinical response

Clinical response	Level 1 (n = 9)	Level 2 (n = 6)	Level 3 (n = 6)	Level 4 (n = 6)	Total
Complete response	4	1	4	3	12
Partial response	1	2	0	1	4
No change	0	0	0	0	0
Progressive disease	0	0	2	1	3
Not evaluable	4	3	0	1	8
Response rate (%)	100 (5/5)	100 (3/3)	67 (4/6)	80 (4/5)	84 (16/19)

two cases of diarrhea, one in level 1 and one in level 4 and one febrile neutropenia in level 4 matched the dose-limiting toxicity criteria (d) and (b). Other than these toxicities, alopecia was the most frequently observed toxicity: 85% (23/27) of patients developed grade 2 alopecia during all courses of chemotherapy. Grade 2/3 hypersensitivity and any grade renal toxicity (rise of serum creatinine) were not observed during the study. It was noteworthy that grade 2/3 sensory neuropathy was not observed during the first course of chemotherapy and only one patient [4% (1/27)] developed grade 2 sensory neuropathy during all courses of chemotherapy.

Clinical Response

Eight patients had no measurable disease at entry. In the other 19 patients with two-dimensionally measurable disease, the response to chemotherapy was evaluated (Table 4). Twelve patients achieved complete response and four achieved partial response. The overall response rate was 84% (16/19) among patients with measurable disease. The remaining three patients had progressive disease. The response rate at dose levels 1–4 was 100, 100, 67 and 80%, respectively, suggesting no correlation between the dose level and response rate.

Recommended Dose

Table 5 summarizes the characteristics of chemotherapy at each level. In level 4, the majority of cycles [91% (30/33)] required G-CSF support and more than 30% of chemotherapy cycles required some modification in the dose or starting date of chemotherapy. However, chemotherapy could be continued until the planned cycle was completed or disease progression in most cases [83% (5/6)]. Moreover, 93.4% of the planned doses of agents could be administered at level 4. Considering all the factors, such as hematological and non-hematological toxicities, clinical responses and actual dose deliveries at dose level 4, RD for further study was decided as dose level 4 consisting of 110 mg/m^2 of PTX, 50 mg/m^2 of DOX and 75 mg/m^2 of CDDP.

DISCUSSION

In this study, we evaluated the safety and efficacy of a combination regimen of PTX, DOX and CDDP (TAP) as first-line

Table 5. Summary of chemotherapies

	Level 1	Level 2	Level 3	Level 4
No. of cycles administered	48	36	32	33
Percentage of cycles required				
G-CSF use	60 (29/48)	72 (26/36)	66 (21/32)	91 (30/33)
Dose reduction	13 (5/39)	7 (2/30)	15 (4/26)	33 (9/27)
Treatment delay	21 (8/39)	20 (6/30)	8 (2/26)	30 (8/27)
Percentage of patients who completed chemotherapy*	67 (6/9)	100 (6/6)	100 (6/6)	83 (5/6)
Average drug administration				
PTX(mg/m^2)	106	108	107	103
DOX(mg/m^2)	19	29	39	47
CDDP(mg/m^2)	72	74	73	70
Percentage of actual/planned doses	96.4	98.2	97.2	93.4

*All six cycles of chemotherapy were completed or chemotherapy was discontinued because of disease progression.

chemotherapy for AOC. Because of the bone marrow toxicity of both CBDCA and DOX, CDDP seems to be safer than CBDCA to combine with DOX as a platinum analog. On the other hand, the combination of CDDP and PTX may produce severe and irreversible neurotoxicity (2,16,17). To avoid this adverse effect and to reduce cardiac toxicity, PTX was administered in a 24 h continuous infusion (18). The PTX dose was set at 110 mg/m^2 as the minimum dose at which sufficient response could be expected, because there is no dose–response relationship in a range of 110 mg/m^2 or more (19). The dose of CDDP was decided as the standard dose of 75 mg/m^2 (20). The DOX dose was increased from 20 to 50 mg/m^2 and was expected to improve efficacy over the standard combination of PTX and platinum. To avoid excessive toxicity, PTX was administered following DOX (21,22) and CDDP was administered following PTX (23). The regimen therefore consisted of 20–50 mg/m^2 increasing doses of DOX followed by 24 h infusion of 110 mg/m^2 of PTX followed by 75 mg/m^2 of CDDP.

Concerning the safety of the regimen, the three-drug combination regimen seemed to be sufficiently safe to use as first-line chemotherapy for patients with ovarian cancer. The major toxicities observed in our study were neutropenia and leukopenia. Grade 4 neutropenia and leukopenia were observed in 85% (23/27) and 44% (12/27) in the first course of chemotherapy. However, these toxicities rarely lasted long enough to be counted as DLT and were not cumulative in the 2nd to 6th courses of chemotherapy. Thus, these hematological toxicities seemed manageable. Moreover, non-hematological toxicities were generally mild or moderate. The grade 3 toxicities observed were nausea and vomiting in 11% (3/27), diarrhea in 11% (3/27) and febrile neutropenia in 22% (6/27), during all courses

of chemotherapy. Grade 3 sensory neuropathy was not observed during all courses of chemotherapy. To our knowledge, seven phase I or I/II studies (10,24–29), evaluating the value of anthracyclines in a taxane and platinum-based regimen for previously untreated AOC, have been published. The major toxicities observed throughout the studies were hematological toxicities, such as neutropenia, leukopenia and thrombocytopenia. In particular, neutropenia was reported in 100% in some studies (25,27,28). However, the toxicity was readily managed using G-CSF and was rarely complicated with serious infection or sepsis. Non-hematological toxicities, excluding nausea, vomiting and alopecia, were generally mild and manageable. No severe cardiac toxicity or neuropathy was observed throughout the previous studies.

As for the efficacy of the triplet combination in our study, a response rate (RR) of 84% (16/19), including 63% (12/19) complete response (CR), was observed. Even in level 1, 100% RR was achieved and there was no correlation between the dose level and response rate. In the previous studies, that using docetaxel (DOC) as the taxane (28) showed a relatively lower response rate of 36%, but studies using PTX as the taxane showed a higher response rate of 83–100%. In studies using PTX, there were no apparent differences in the response rate between studies using CDDP (86–100%) (25,26) and those using CBDCA (83–100%) (10,24,29) as platinum compound and between studies using DOX (100%) (24,25) and those using EpiDOX (83–86%) (10,26,29) as anthracycline.

In summary, the combination regimen of DOX with PTX and CDDP is highly active and hematological toxicities are readily manageable and non-hematological toxicities, including cardiac toxicity and sensory neuropathy, were mild or moderate. From our study and previous studies, we conclude that the addition of anthracyclines to PTX plus a platinum-based regimen may provide an effective and safe regimen for patients with untreated ovarian cancer. However, the hematological toxicities seem to be relatively severe compared with those reported with a PTX/CBDCA combination (3,30,31). At present, AGO–GINECO (Arbeitsgemeinschaft Gynäkologische Onkologie–Groupe d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens) (32) and NSGO–EORTC–NCIC CTG (Nordic Society of Gynecological Oncology–European Organization for Research and Treatment of Cancer–National Cancer Institute of Canada Clinical Trials Group) (33) are conducting phase III studies comparing epirubicin/paclitaxel/carboplatin vs. paclitaxel/carboplatin. To assess the usefulness of anthracyclines, the results of these studies are awaited.

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References

1. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996;334:1–6.
2. Piccart MJ, Bertelsen K, James K, Cassidy J, Mangioni C, Simonsen E, et al. Randomized intergroup trial of cisplatin–paclitaxel versus cisplatin–cyclophosphamide in women with advanced epithelial ovarian cancer: three-year results. *J Natl Cancer Inst* 2000;92:699–708.
3. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 2003;21:3194–200.
4. Nguyen HN, Averette HE, Hoskins W, Sevin BU, Penalver M, Steren A. National survey of ovarian carcinoma. VI. Critical assessment of current International Federation of Gynecology and Obstetrics staging system. *Cancer* 1993;72:3007–11.
5. Omura GA, Bundy BN, Berek JS, Curry S, Delgado G, Mortel R. Randomized trial of cyclophosphamide plus cisplatin with or without doxorubicin in ovarian carcinoma: a Gynecologic Oncology Group study. *J Clin Oncol* 1989;7:457–65.
6. Cyclophosphamide plus cisplatin versus cyclophosphamide, doxorubicin and cisplatin chemotherapy of ovarian carcinoma: a meta-analysis. The Ovarian Cancer Meta-Analysis Project. *J Clin Oncol* 1991;9:1668–74.
7. Fanning J, Bennett TZ, Hilgers RD. Meta-analysis of cisplatin, doxorubicin and cyclophosphamide versus cisplatin and cyclophosphamide chemotherapy of ovarian carcinoma. *Obstet Gynecol* 1992;80:954–60.
8. A'Hern RP, Gore ME. Impact of doxorubicin on survival in advanced ovarian cancer. *J Clin Oncol* 1995;13:726–32.
9. Tobinai K, Kohno A, Shimada Y, Watanabe T, Tamura T, Takeyama K, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. *Jpn J Clin Oncol* 1993;23:250–7.
10. du Bois A, Luck HJ, Bauknecht T, Meier W, Richter B, Kuhn W, et al. First-line chemotherapy with epirubicin, paclitaxel and carboplatin for advanced ovarian cancer: a phase I/II study of the Arbeitsgemeinschaft Gynäkologische Onkologie Ovarian Cancer Study Group. *J Clin Oncol* 1999;17:46–51.
11. Siddiqui N, Boddy AV, Thomas HD, Bailey NP, Robson L, Lind MJ, et al. A clinical and pharmacokinetic study of the combination of carboplatin and paclitaxel for epithelial ovarian cancer. *Br J Cancer* 1997;75:287–94.
12. Bolis G, Scarfone G, Villa A, Acerboni S, Siliprandi V, Guarnerio P. A phase I trial with fixed-dose carboplatin and escalating doses of paclitaxel in advanced ovarian cancer. *Semin Oncol* 1997;24, No 1, Suppl 2:23–5.
13. Huizing MT, van Warmerdam LJ, Rosing H, Schaefers MC, Lai A, Helmerhorst TJ, et al. Phase I and pharmacologic study of the combination paclitaxel and carboplatin as first-line chemotherapy in stage III and IV ovarian cancer. *J Clin Oncol* 1997;15:1953–64.
14. ten Bokkel Huinink WW, Veenhof C, Huizing M, Rodenhuis S, Helmerhorst T, Dubbelman R, et al. Carboplatin and paclitaxel in patients with advanced ovarian cancer: a dose-finding study. *Semin Oncol* 1997;24, No 1, Suppl 2:31–3.
15. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;47:207–14.
16. Rowinsky EK, Chaudhry V, Forastiere AA, Sartorius SE, Ettinger DS, Grochow LB, et al. Phase I and pharmacologic study of paclitaxel and cisplatin with granulocyte colony-stimulating factor: neuromuscular toxicity is dose-limiting. *J Clin Oncol* 1993;11:2010–20.
17. Chaudhry V, Rowinsky EK, Sartorius SE, Donehower RC, Cornblath DR. Peripheral neuropathy from taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies. *Ann Neurol* 1994;35:304–11.
18. Smith RE, Brown AM, Mamounas EP, Anderson SJ, Lembersky BC, Atkins JH, et al. Randomized trial of 3-hour versus 24-hour infusion of high-dose paclitaxel in patients with metastatic or locally advanced breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-26. *J Clin Oncol* 1999;17:3403–11.
19. Rowinsky EK, Mackey MK, Goodman SN. Meta analysis of paclitaxel dose–response and dose–intensity in recurrent or refractory ovarian cancer. *Proc Am Soc Clin Oncol* 1996;15:Meeting Abstract.

20. Kaye SB, Paul J, Cassidy J, Lewis CR, Duncan ID, Gordon HK, et al. Mature results of a randomized trial of two doses of cisplatin for the treatment of ovarian cancer. Scottish Gynecology Cancer Trials Group. *J Clin Oncol* 1996;14:2113–9.
21. Sledge GW Jr, Robert N, Sparano JA, Cobeligh M, Goldstein LJ, Neuberg D, et al. Paclitaxel (taxol)/doxorubicin combinations in advanced breast cancer: the Eastern Cooperative Oncology Group experience. *Semin Oncol* 1994;21:15–8.
22. Sledge GW Jr, Robert N, Sparano JA, Cogleigh M, Goldstein LJ, Neuberg D, et al. Eastern Cooperative Oncology Group studies of paclitaxel and doxorubicin in advanced breast cancer. *Semin Oncol* 1995;22:105–8.
23. Rowinsky EK, Gilbert MR, McGuire WP, Noe DA, Grochow LB, Forastiere AA, et al. Sequences of taxol and cisplatin: a phase I and pharmacologic study. *J Clin Oncol* 1991;9:1692–703.
24. Hill M, Macfarlane V, Moore J, Gore ME. Taxane/platinum/anthracycline combination therapy in advanced epithelial ovarian cancer. *Semin Oncol* 1997;24, No 1, Suppl 2:34–7.
25. Naumann RW, Alvarez RD, Omura GA, Segars E, Kilgore LC, Partridge EE. A phase I study of paclitaxel, doxorubicin and cisplatin in patients with previously untreated epithelial ovarian cancer. *Gynecol Oncol* 1998;71:450–3.
26. Papadimitriou CA, Moulopoulos LA, Vlahos G, Voulgaris Z, Kiosses E, Georgoulias N, et al. Paclitaxel, cisplatin and epirubicin first-line chemotherapy in stage III and IV ovarian carcinoma: long-term results of a phase II study. *Cancer* 2000;89:1547–54.
27. Gregory RK, Hill ME, Moore J, A'Hern RP, Johnston SR, Blake P, et al. Combining platinum, paclitaxel and anthracycline in patients with advanced gynaecological malignancy. *Eur J Cancer* 2000;36:503–7.
28. O'Neill VJ, Kaye SB, Reed NS, Paul J, Davis JA, Vasey PA. A dose-finding study of carboplatin–epirubicin–docetaxel in advanced epithelial ovarian cancer. *Br J Cancer* 2002;86:1385–90.
29. Romanini A, Tanganelli L, Carnino F, Fanucchi A, Lionetto R, Pastorino S, et al. First-line chemotherapy with epidoxorubicin, paclitaxel and carboplatin for the treatment of advanced epithelial ovarian cancer patients. *Gynecol Oncol* 2003;89:354–9.
30. Neijt JP, Engelholm SA, Witteveen PO, Tuxen MK, Sorensen PG, Hansen M, et al. Paclitaxel (175 mg/m^2 over 3 h) with cisplatin or carboplatin in previously untreated ovarian cancer: an interim analysis. *Semin Oncol* 1997;24, No 5, Suppl 15:36–9.
31. du Bois A, Luck HJ, Meier W, Mobus V, Costa S, Richter B, et al. Carboplatin/paclitaxel versus cisplatin/paclitaxel as first-line chemotherapy in advanced ovarian cancer: an interim analysis of a randomized phase III trial of the Arbeitsgemeinschaft Gynakologische Onkologie Ovarian Cancer Study Group. *Semin Oncol* 1997;24, No 5, Suppl 15: 44–52.
32. du Bois A, Weber B, Pfisterer J, Goupil A, Wagner U, Barats J, et al. Epirubicin/paclitaxel/carboplatin (TEC) vs. paclitaxel/carboplatin (TC) in first-line treatment of ovarian cancer FIGO stages IIb/IV. Interim results of an AGO/GINECO Intergroup Phase III trial. *Proc Am Soc Clin Oncol* 2001;20:Abstract 805.
33. Kristensen G, Vergote I, Stuart G, Izqiuerdo Delso M, Mirza MR, Aavall-Lundquist E, et al. First line treatment of ovarian cancer FIGO stages IIb–IV with paclitaxel/epirubicin/carboplatin (TEC) vs. paclitaxel/carboplatin (TC). Interim results of an NSGO–EORTC–NCIC CTG Gynecological Cancer Intergroup phase III trial. *Proc Am Soc Clin Oncol* 2002;21:Abstract 805.

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Clinical Trial Note

Feasibility Study of Neoadjuvant Chemotherapy Followed by Interval Cytoreductive Surgery for Stage III/IV Ovarian, Tubal and Peritoneal Cancers: Japan Clinical Oncology Group Study JCOG0206

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A feasibility study was started in January 2003 on neoadjuvant chemotherapy (NAC) followed by interval cytoreductive surgery (ICS) and postoperative chemotherapy for stage III/IV müllerian carcinomas such as ovarian, tubal and peritoneal carcinomas. The purpose is to assess the safety and efficacy of the treatment starting with NAC and also to know whether we can accurately diagnose these advanced carcinomas by imaging studies, cytologic findings and tumor markers without staging laparotomy or laparoscopy. Fifty-six patients with advanced müllerian carcinomas will be recruited to the study. After confirmation of diagnosis by laparoscopic inspection and biopsies, patients undergo four cycles of chemotherapy as NAC, followed by ICS and an additional four cycles of post-surgical chemotherapy. The primary endpoint is proportion of clinical complete remission after accomplishment of the protocol treatment, while the major secondary endpoint is positive predictive value of diagnosis before laparoscopy regarding tumor origin, histology and stage. Based on the results of this study, we will conduct a phase III study to compare the treatment starting with NAC and primary cytoreductive surgery followed by post-surgical chemotherapy.

Key words: ovarian neoplasms – laparoscopy – neoadjuvant therapy – interval cytoreductive surgery

INTRODUCTION

Prognosis of patients with advanced epithelial ovarian, tubal and peritoneal carcinomas is known to be poor. Even using platinum compound regimens, the 5-year survival rate of stage III/IV ovarian cancer is still around 20% (1). The current standard treatment for advanced ovarian cancer is primary cytoreductive surgery followed by post-surgical chemotherapy. However, optimal cytoreduction in primary surgery can be achieved only in 40% of stage III/IV ovarian cancer patients (2). An alternative to primary surgical cytoreduction in patients with unresectable bulky tumors or poor performance status is the use of chemotherapy in the neoadjuvant setting. Recent retrospective analyses (3–6) have revealed that progression-free and overall survival were comparable between patients treated with neoadjuvant chemotherapy (NAC) followed by interval cytoreductive surgery (ICS) and those treated by primary cytoreductive surgery, though the former group was older and had a poorer performance status. Phase II and III trials have not been performed on the role of neoadjuvant-setting treatment for advanced ovarian, tubal and peritoneal cancers. Therefore, we started a phase II study to assess the safety and efficacy of NAC followed by ICS and post-surgical chemotherapy before comparing with the current standard treatment including primary cytoreductive surgery in randomized controlled trial. Neoadjuvant setting has the advantage of earlier treatment start and lower invasiveness. However, according to the current general rules for the management of ovarian cancer, it is neces-

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sary to confirm the origin, histology and stage before starting treatment by staging laparotomy or laparoscopy. Thus, we also determine whether we can omit the 'extra procedure' of staging laparotomy or laparoscopy before the neoadjuvant-setting treatment in the majority of patients with advanced ovarian, tubal or peritoneal cancer.

The study protocol was designed by Gynecologic Cancer Study Group (GCSG) of the Japan Clinical Oncology Group (JCOG), approved by the Clinical Trial Review Committee of JCOG on December 6, 2002, and activated on January 14, 2003.

PROTOCOL DIGEST OF THE JCOG0206

PURPOSE

The purposes are to assess the safety and efficacy of the treatment starting with NAC with paclitaxel and CBDCA for phase III study, comparing NAC therapy with current standard procedure, and to know whether we can accurately diagnose these advanced carcinomas by imaging studies, cytologic findings and tumor markers without staging laparotomy or laparoscopy.

STUDY SETTING

A multi-institutional (26 centers) non-randomized phase II trial.

RESOURCES

Health Sciences Research Grants for Clinical Research for Evidenced Based Medicine and Grants-in Aid for Cancer Research (nos 14S-4, 14-12), from the Ministry of Health, Labor and Welfare, Japan.

ENDPOINTS

Primary endpoint is proportion of clinical complete remission (%cCR) among all stage III or IV müllerian carcinoma confirmed by laparoscopic inspection and histopathology of biopsy specimens. Clinical complete remission is defined as disappearance of all lesions by computed tomography (CT) or magnetic resonance imaging (MRI), no pleural effusions by chest radiography and normal serum CA125 level (<20 U/ml) after completion of the protocol treatment.

Secondary endpoints are as follows: (i) positive predictive value (PPV) of pre-laparoscopic diagnosis concerning the origin and histology—proportion of the patients diagnosed as müllerian carcinoma by laparoscopic inspection and histopathology of biopsy specimen among those diagnosed by pre-laparoscopic findings; (ii) PPV of prelaparoscopic diagnosis concerning clinical stage—proportion of the patients diagnosed as stage III or IV by laparoscopic inspection among those diagnosed by pre-laparoscopic findings; (iii) PPV of overall pre-laparoscopic diagnosis—proportion of the patients diagnosed as stage III or IV müllerian carcinoma by laparoscopic inspection and histopathology of biopsy specimen among those diagnosed by pre-laparoscopic findings.

Other secondary endpoints are: (iv) response rate to NAC among patients whose clinical diagnosis is confirmed by laparoscopy; (v) proportion of patients who received ICS among patients whose clinical diagnosis is confirmed by laparoscopy; (vi) progression-free survival among patients whose clinical diagnosis is confirmed by laparoscopy; (vii) operative morbidity among all enrolled patients; (viii) adverse events among all enrolled patients: and (ix) overall survival among all enrolled patients.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

The study subjects are patients diagnosed as stage III or IV müllerian carcinoma by pre-laparoscopic clinical findings including imaging studies (CT, MRI or ultrasonography) and cytology of ascites, pleural effusions or fluids obtained by tumor centesis. Malignancies of other origins, such as breast and digestive tract, should be excluded by endoscopy, opaque enema or ultrasonography when these malignancies are suspected from symptoms, physical examination or imaging diagnosis. To rule out malignancy of digestive tract origin, criteria for tumor markers are set to be CA125 >200 U/ml and CEA <20 ng/ml.

Further inclusion criteria are: (i) clinically deemed to be a candidate for debulking surgery without evidence of brain, bone, bone marrow metastases, multiple lung or multiple liver metastases; (ii) presence of at least one measurable lesion; (iii) previously untreated for these malignancies and no history of treatment with chemotherapy nor radiotherapy even for other diseases; (iv) age 20–75 years; (v) Eastern Cooperative Oncology Group (ECOG) performance status of 0–3; (vi) adequate bone marrow, hepatic, renal, cardiac and respiratory functions; and (vii) written informed consent.

EXCLUSION CRITERIA

These are: (i) synchronous or metachronous (within 5 years) malignancy other than carcinoma in situ; (ii) pregnant or nursing; (iii) severe mental disorders; (iv) systemic and continuous use of steroidal drugs; (v) active infections; (vi) uncontrolled hypertension; (vii) diabetes mellitus, uncontrolled or controlled with insulin; (viii) history of cardiac failure, unstable angina, myocardial infarction within 6 months prior to the registration; (ix) liver cirrhosis or bleeding tendency contraindicating debulking surgery; (x) intestinal occlusion necessary for surgical treatment; and (xi) hypersensitivity to alcohol.

TREATMENT METHODS

DIAGNOSTIC LAPAROSCOPY

After enrolment, diagnostic laparoscopy is performed within 2 weeks. To confirm pre-laparoscopic clinical diagnosis of origin, histology and stage, inspection of peritoneal cavity and biopsy from the main tumor or metastatic tumors are per-

formed. Resection of any organs or tumors attempting to reduce tumor volume is not allowed.

NEOADJUVANT CHEMOTHERAPY (NAC)

Four cycles of combination of paclitaxel (175 mg/m^2, day 1) and carboplatin (AUC = 6, day 1) are administered every 3 weeks. NAC is initiated within 1 week after laparoscopy.

INTERVAL CYTOREDUCTIVE SURGERY (ICS)

ICS is performed in 4–7 weeks after administration of the fourth cycle of NAC unless disease progression occurs during NAC. Standard procedures of ICS consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed, but not included in standard procedures.

POST-SURGICAL CHEMOTHERAPY

An additional four cycles of chemotherapy (same regimen as NAC) is administered (eight cycles of chemotherapy in total). Post-surgical chemotherapy is initiated within 3 weeks after ICS.

STUDY DESIGN AND STATISTICAL METHODS

The study is planned as a single-stage safety and efficacy study. Sample size calculation was primarily based on binominal test for the primary endpoint, %cCR. Forty-four eligible patients are required when expected %cCR of 40% and an acceptable lowest %cCR of 20% with alpha error level of 0.05 and beta error level of 0.1. Additionally, PPV is to be confident enough to omit laparoscopy before NAC in the following phase III study. It is not possible to use sensitivity or specificity to evaluate accuracy of clinical diagnoses, because laparoscopy is performed only in patients diagnosed as stage III/IV müllerian carcinomas by clinical findings in this study setting. Thus, Bayesian monitoring PPV is planned, which requires 56 patients to have the 10% or lower Bayesian posterior probability that PPV is <90% in case of three false positive patients assuming prior distribution of beta (9,1). The target sample size was determined to be 56, which also can be expected sufficient for primary endpoint. The planned accrual period is 1 year and the follow-up period is set as 3 years after the completion of accrual.

STUDY MONITORING

In-house interim monitoring is performed by the JCOG Data Center to ensure data submission, patient eligibility, protocol compliance, safety and on-schedule study progress according to the JCOG standard procedures. The monitoring reports are submitted to the JCOG Data and Safety Monitoring Committee every 6 months.

PARTICIPATING INSTITUTIONS

Hokkaido University, Sapporo Medical University, Tohoku University, University of Tsukuba, Gunma Prefectural Cancer Center, Shinshu University, National Defense Medical College, Saitama Cancer Center, National Cancer Center Hospital, The Jikei University School of Medicine, Cancer Institute Hospital, University of Tokyo, Juntendo University, Nagaoka Red Cross Hospital, Aichi Cancer Center, National Nagoya Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Kinki University, Niigata Cancer Center, Kure National Hospital (Chugoku District Cancer Center), National Shikoku Cancer Center, National Kyushu Cancer Center, University of Kurume, Kyushu University, Saga Medical School and Kagoshima City Hospital.

References

1. Nguyen HN, Averette HE, Hoskins W, Sevin BU, Penalver M, Steren A. National survey of ovarian carcinoma. VI. Critical assessment of current International Federation of Gynecology and Obstetrics staging system. *Cancer* 1993;72:3007–11.
2. Covens AL. A critique of surgical cytoreduction in advanced ovarian cancer. *Gynecol Oncol* 2000;78:269–74.
3. Jacob JH, Gershenson DM, Morris M, Copeland LJ, Burke TW, Wharton JT. Neoadjuvant chemotherapy and interval debulking for advanced epithelial ovarian cancer. *Gynecol Oncol* 1991;42:146–50.
4. Onnis A, Marchetti M, Padovan P, Castellan L. Neoadjuvant chemotherapy in advanced ovarian cancer. *Eur J Gynaecol Oncol* 1996;17:393–6.
5. Schwartz PE, Rutherford TJ, Chambers JT, Kohorn EI, Thiel RP. Neoadjuvant chemotherapy for advanced ovarian cancer: long-term survival. *Gynecol Oncol* 1999;72:93–9.
6. Vergote I, De Wever I, Tjalma W, Van Gramberen M, Decloedt J, van Dam P. Neoadjuvant chemotherapy or primary debulking surgery in advanced ovarian carcinoma: a retrospective analysis of 285 patients. *Gynecol Oncol* 1998;71:431–6.

Vascular Endothelial Growth Factor C and Vascular Endothelial Growth Factor Receptor 2 Are Related Closely to the Prognosis of Patients with Ovarian Carcinoma

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BACKGROUND. The vascular endothelial growth factor (VEGF) family and VEGF receptors (VEGFR) play an essential role in the angiogenesis of both pathologic and nonpathologic conditions. However, the prognostic significance of VEGF and VEGFR expression in ovarian carcinoma is unclear.

METHODS. The tissue expression levels of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 in 80 specimens of ovarian carcinoma were examined immnohistochemically. The results obtained were analyzed clinicopathologically.

RESULTS. VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were expressed both in tumor cells and in adjacent endothelial cells of blood and lymph vessels. The tissue expressions of VEGF-C and VEGFR-2 were correlated significantly with tumor extension, including peritoneal metastases outside the pelvic cavity ($P = 0.0010$ and $P = 0.0008$, respectively), lymph node metastases ($P = 0.0030$ and $P = 0.0018$, respectively), and positive ascitic cytology ($P = 0.025$ and $P = 0.0016$, respectively). Conversely, there was no significant correlation between VEGF-A and VEGFR-3 expression and clinicopathologic features of ovarian carcinoma. Logistic regression analysis revealed that the expressions of VEGF-C and VEGFR-2 also were independent risk factors for peritoneal and lymph node metastases. Survival curves determined by the Kaplan–Meier method and in univariate analysis demonstrated that high expression levels of VEGF-C and VEGFR-2 were associated with the 5-year survival rate. In multivariate analysis, high expression levels of VEGF-C and VEGFR-2 emerged as independent indicators for disease-specific survival.

CONCLUSIONS. High tissue expression of VEGF-C and VEGFR-2 reflects the aggressiveness of the spread of tumor in ovarian carcinoma. Thus, both have predictive value for identifying high-risk patients who have a poor prognosis. *Cancer* **2004; 101:1364–74.** © *2004 American Cancer Society.*

KEYWORDS: vascular endothelial growth factor, ovarian carcinoma, immunohistochemistry, prognosis.

Neoplastic angiogenesis and lymphangiogenesis are essential for the growth of tumor tissue in both primary and metastatic sites. Vascular endothelial growth factor (VEGF) is now accepted as a powerful angiogenic agent in neoplastic tissues as well as in normal tissues. Under the influences of some cytokines and other growth factors, the VEGF family appears in tumor tissue and adjacent stroma, and it plays an essential role in the new proliferation of blood and lymph vessels.[1–3] Among the VEGF family, VEGF-A, VEGF-B, and VEGF-C induce new blood vessel proliferation, and VEGF-C and

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VEGF-D relate to lymphangiogenesis. These factors act with their own VEGF receptors (VEGFRs).[4–9]

Ovarian carcinoma has the poorest prognosis among malignancies in the gynecologic field, and surgical staging according to the International Federation of Gynecology and Obstetrics (FIGO) system is regarded as the most important prognostic factor.[10] When the tumor is confined to the ovary, a > 90% chance of 5-year survival is expected. If the disease extends to the peritoneal cavity, however, the prognosis is limited to ≤ 30%.[11] Histologic classification and grading also relate to the patient's prognosis.[12–16] Cytotoxic chemotherapy and maximal debulking are conventional prognostic methods of controlling ovarian carcinoma on which the treatment policy is based. Despite the current progress of cytotoxic chemotherapy and surgical techniques, the consequence of ovarian carcinoma is unchanged. Therefore, new management strategies against the disease are required. To evaluate the prognostic significance of the neoplastic angiogenic factors, tissue expression of VEGF-A, VEGF-C, and their receptors were examined in ovarian tumors.

MATERIALS AND METHODS

Patients and Specimens

Clinical records and preserved specimens from 80 patients with ovarian carcinoma who underwent surgical treatment at Kurume University Hospital between 1997 and 2002 were examined consecutively. All patients submitted informed consent and agreed with the use of their tissues in this study. All patients underwent a staging laparotomy, including total hysterectomy, bilateral salpingooophorectomy, partial omentectomy, peritoneal cytology, and/or pelvic and paraaortic lymphadenectomy. No patient had received any preoperative treatment. The patients were staged in accordance with FIGO criteria and included 33 patients with Stage I disease, 5 patients with Stage II disease, 34 patients with Stage III disease, and 8 patients with Stage IV disease. Tumors were grouped according to the World Health Organization (WHO) histologic typing system and were graded according to the FIGO grading system.[17] Grading criteria offered by Silverberg et al.[15,16] also were applied. During the follow-up, which ranged from 11 weeks to 359 weeks (mean, 132.8 weeks), there were 34 disease recurrences and 24 disease-related deaths. The mean age of our patients at surgery was 54.4 years (range, ≈ 23–79 years). Surgically resected tissues from 10 tumors with low potential malignancy (LPM) and 22 benign cystadenomas also were examined for the purposes of comparison.

Immunohistochemistry

Formalin fixed, paraffin embedded serial sections (4 μm) that were mounted on 3-aminopropyltriethoxysilane-coated slides (Matsunami Glass Ind., Ltd. Japan) were deparaffinized in xylene alcohol and graded alcohol. Rabbit polyclonal anti-VEGF-C (Zymed Laboratories Inc., San Francisco, CA), anti-VEGF-A, anti-VEGFR-3 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and anti-VEGFR-2 (Upstate Cell Signaling Solutions, VA) antibodies were used at concentrations of 1.6 μg/mL, 2.0 μg/mL, 2.0 μg/mL, and 5.0 μg/mL, respectively. Mouse monoclonal anti-CD31 antibody (1:40 dilution, DAKO, Ltd., Copenhagen, Denmark) also was used. Immunohistochemistry was performed by using a catalyzed signal-amplification system (DAKO, Ely, United Kingdom) according to the manufacture's protocol. The sections were treated with 0.3% hydrogen peroxide (H_2O_2) in water for 10 minutes to quench the endogenous peroxidase activity within the tissue. Nonspecific binding sites were blocked with 20% heat-inactivated nonserum protein for 10 minutes at room temperature. The sections were incubated for 15 minutes in the presence of the primary antibody. Slides were then washed in phosphate buffered saline (PBS) containing 0.1% Tween 20 (PBS/Tween) for 15 minutes by changing the solution 3 times before the application of the secondary biotinylated antibody. The slide was incubated with secondary antibody for 15 minutes at room temperature before it was washed for 15 minutes in PBS/Tween, which was changed 3 times. The sections were then incubated for 15 minutes with avidin-biotinylated-horseradish peroxidase complex, and the reaction was visualized with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (Nichirei, Tokyo, Japan) as a chromogen in Tris-HCl buffer, pH 7.6, containing 0.03% H_2O_2. Hematoxylin was used to counterstain of the nuclei. For each antibody, a negative control study was performed by using normal rabbit serum instead of the primary antibody. For positive controls, formalin fixed, paraffin embedded sections of human placenta were stained for VEGF-A and VEGF-C, and sections of normal human umbilical cord were stained for VEGFR-2 and VEGFR-3 using the same procedure.

Microscopic Assessment of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 Expressions and Microvessel Density

Two pathologists (H.Y. and N.N.) independently evaluated and interpreted the results of immunostaining without knowledge of the clinical data for each patient. VEGF-A and VEGF-C were stained both in tumor cells and in adjacent endothelial cells from blood and lymph vessels. The staining results in tumor cells were

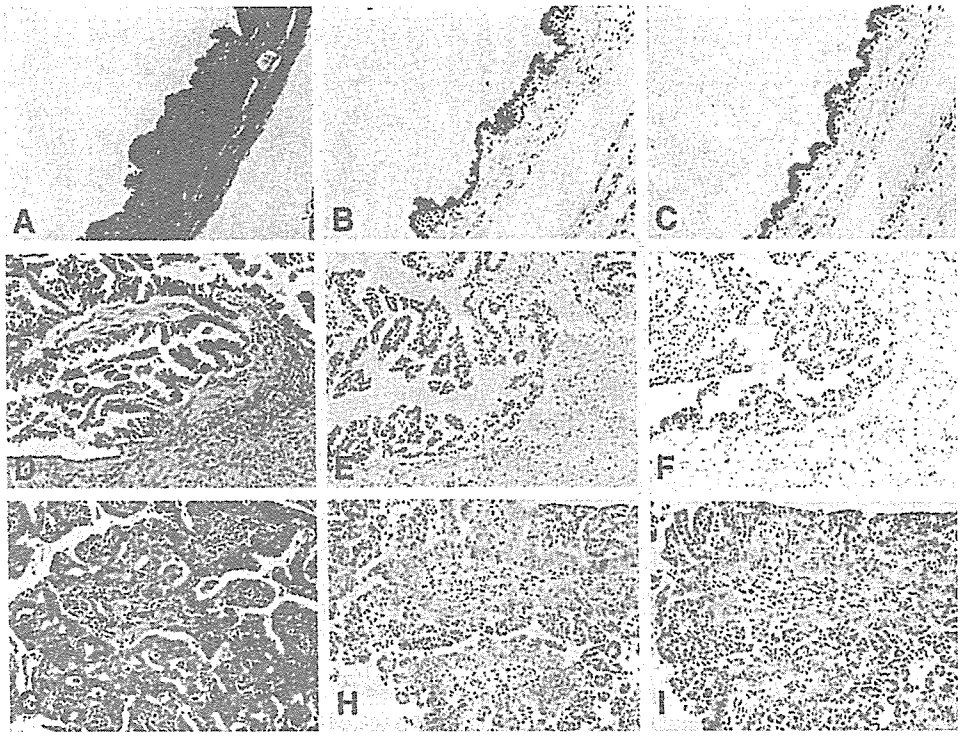

FIGURE 1. Immunohistochemical staining of vascular endothelial growth factor C (VEGF-C) and VEGF receptor 3 (VEGFR-3) in ovarian carcinoma. Samples of serous cyst adenoma (A), serous tumors with low potential malignancy (LPM) (D), and serous adenocarcinoma (G) were stained with hematoxylin and eosin. Serous cyst adenoma was negative for both VEGF-C (B) and VEGFR-3 (C). VEGF-C was positive in serous LPM (E) and in serous adenocarcinoma (H). VEGFR-3 was positive in serous LPM (F) and in serous adenocarcinoma (I).

classified into 3 levels: negative expression when immunostain-positive tumor cells accounted for $< 10\%$ of the tumor area on the section, low expression when the positive cells accounted for from 10% to $< 50\%$ of the tumor area, and high expression when the positive cells accounted for $> 50\%$ of the tumor area. We used such criteria, because the median proportion immunostain-positive tumor cells in the whole sample was $\approx 50\%$. Faint or equivocal immunoreactions were not regarded as positive. The specimens were considered positive to VEGF-A and/or VEGF-C when expression levels were $\geq 10\%$. VEGFR-2 and VEGFR-3 also were stained both in tumor cells and in adjacent endothelial cells from blood and lymph vessels. VEGFR-2 and VEGFR-3 staining in endothelial cells was considered positive when at $\geq 5\%$ of endothelial cells in the area were strongly immunoreactive, as seen in the positive control cells. Microvessel density (MVD) was assessed by counting CD31-positive microvessels under $\times$ 200 magnification in a grid area of 0.16 mm^2 according to the criteria of Weidner.[18] Five areas of high vascular density (hot spots) were selected, and microvessels were counted on each section.

Statistical Analysis

All statistical calculations were performed by using Stat View software (version 5; SAS Institute Inc., Cary, NC). The significance of correlations between the expression of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3; MVD; and clinicopathologic factors was evaluated by using univariate analysis (chi-square test) and logistic regression analysis. Survival rates were calculated by using the Kaplan–Meier method, and the statistical significance of differences in the cumulative survival curves between the groups was evaluated by using the log-rank test. Multivariate survival analysis was conducted by using the Cox proportional hazard method. Other statistical analyses were carried out with the Mann–Whitney U test. Results were deemed significant at $P < 0.05$.

RESULTS

VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were expressed in the cytoplasm of both tumor cells and endothelial cells from the blood and lymph vessels in the stroma adjacent to tumor nests (Figs. 1, 2). The frequency of VEGF-C expression was found in 72.5% of adenocarcinomas (58 of 80 patients), in 40% of LPM tumors (4 of 10 patients), and in only 9.1% of benign cystadenomas (2 of 22 patients); whereas VEGFR-3 expression was found in 18.1% (4 of 22 patients), 50% (5 of 10 patients), and 72.5% (58 of 80 patients), respectively. Significant differences were observed in VEGF-C expression levels between benign cystadenomas and LPM tumors ($P < 0.04$; chi-square test), LPM tumors and adenocarcinomas ($P < 0.04$), and benign cystadenomas and adenocarcinomas ($P < 0.0001$) and in the VEGFR-3 expression levels between benign cys-

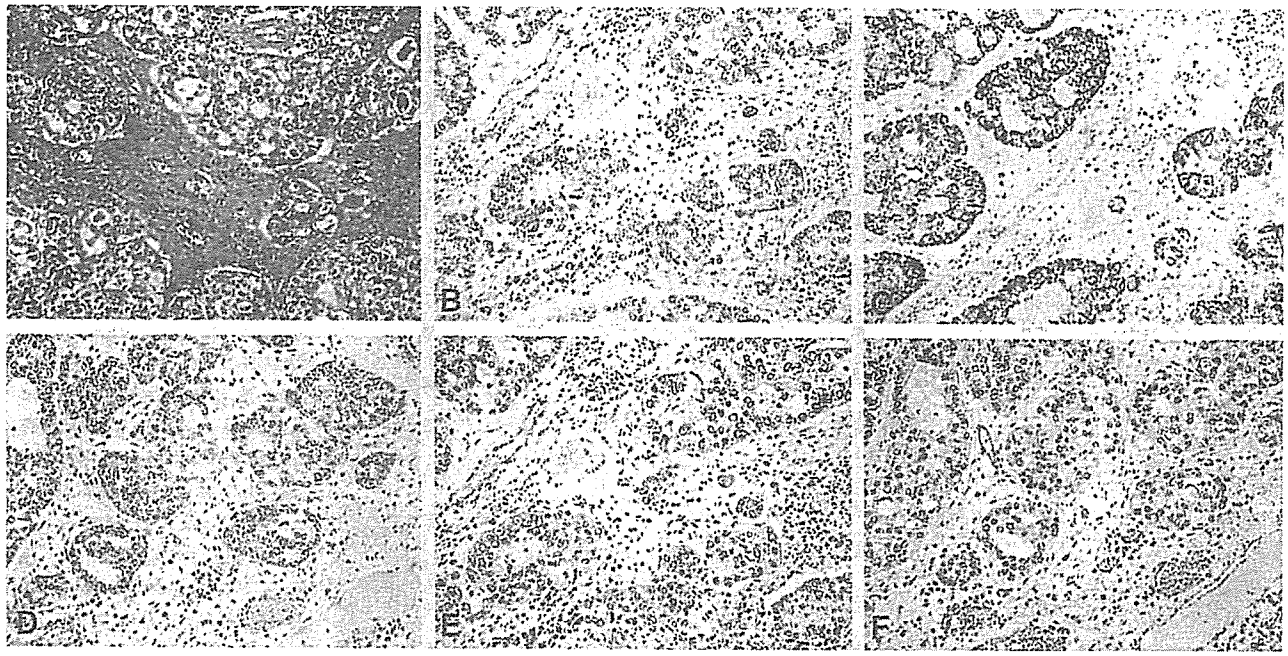

FIGURE 2. Immunohistochemical staining of vascular endothelial growth factor A (VEGF-A), VEGF-C, VEGF receptor 2 (VEGFR-2), and VEGFR-3 in ovarian carcinomas. A sample of clear cell adenocarcinoma (A) was stained with hematoxylin and eosin. Both tumor cells and adjacent endothelial cells were stained for VEGF-A (B), VEGF-C (C), VEGFR-2 (D), and VEGFR-3 (E). CD31 was stained in endothelial cells from blood and lymph vessels in the tumor stroma. (F).

tadenomas and adenocarcinomas ($P < 0.0001$). Expression levels of VEGF-C and VEGFR-3 increased along with the increase in malignant potential.

The histologic tumor types are listed in Table 1. Expression levels of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were lower in mucinous carcinoma than in the other tumor types, but the differences were not significant.

Among the 80 tumors, 20 tumors were negative for VEGFR-2, and 60 tumors were positive for VEGFR-2. Twelve of 20 VEGFR-2-negative tumors (60.0%) were positive for VEGF-A, whereas 49 of 60 VEGFR-2-positive tumors (81.7%) also were positive for VEGF-A ($P < 0.05$; chi-square test). Regarding the relation between VEGFR-2 expression and VEGF-C expression, 8 of 20 VEGFR-2-negative tumors (40.0%) were positive for VEGF-C, and 50 of 60 VEGFR-2-positive tumors (83.3%) also were positive for VEGF-C ($P = 0.0002$). Conversely, VEGFR-3 expression was negative in 22 tumors and positive in 58 tumors, 12 of 22 VEGFR-3-negative tumors (54.5%) were positive for VEGF-C, and 46 of 58 VEGFR-3-positive tumors (79.3%) also were positive for VEGF-C ($P < 0.03$). These results showed that receptor-positive tumors have a strong propensity to exhibit the corresponding growth factor.

The correlations between clinicopathologic features and the expression of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 are summarized in Table 2.

TABLE 1
Expression of Vascular Endothelial Growth Factor A (VEGF-A), VEGF-C, VEGF Receptor 2 (VEGFR-2), and VEGFR-3 in Ovarian Carcinomas

	No. of patients (%): Histologic type			
Expression	Serous (n = 36)	Mucinous (n = 11)	Endometrioid (n = 18)	Clear cell (n = 15)
VEGF-A				
Negative	7 (19.4)	5 (45.4)	3 (16.7)	5 (33.3)
Low	24 (66.7)	2 (18.2)	10 (55.5)	7 (46.7)
High	5 (13.9)	4 (36.4)	5 (27.8)	3 (20.0)
VEGF-C				
Negative	7 (19.4)	6 (54.5)	4 (22.2)	5 (33.3)
Low	18 (50.0)	2 (18.2)	3 (16.7)	4 (26.7)
High	11 (31.6)	3 (27.3)	11 (66.1)	6 (40.0)
VEGFR-2				
Negative	5 (13.9)	7 (63.6)	2 (11.1)	6 (40.0)
Low	17 (47.2)	2 (18.2)	7 (38.9)	5 (33.3)
High	14 (38.9)	2 (18.2)	9 (50.0)	4 (26.7)
VEGFR-3				
Negative	13 (36.2)	4 (36.4)	3 (16.7)	2 (13.3)
Low	17 (47.2)	6 (54.5)	9 (50.0)	6 (40.0)
High	6 (16.6)	1 (9.1)	6 (33.3)	7 (46.7)

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained.

TABLE 2
Relation between the Expression of Vascular Endothelial Growth Factor A (VEGF-A), VEGF-C, VEGF Receptor 2 (VEGFR-2), and VEGFR-3 and Clinicopathologic Factors in 80 Women with Ovarian Carcinoma

		No. of patients (%)			
Variable	Total	VEGF-A	VEGF-C	VEGFR-2	VEGFR-3
Peritoneal metastasis outside the pelvic cavity					
Not present	38	28 (73.7)	21 (55.3)	22 (57.9)	27 (71.1)
Present	42	33 (78.6)	37 (88.1)	38 (90.5)	31 (73.8)
P value[a]	—	0.61	0.0010	0.0008	0.78
Lymph node metastasis					
Not present	44	33 (75.0)	26 (59.1)	27 (61.4)	31 (70.5)
Present	36	28 (77.8)	32 (88.9)	33 (91.7)	27 (75.0)
P value[a]	—	0.77	0.0030	0.0018	0.65
Ascitic cytology in early stage					
Negative	11	6 (54.5)	3 (27.3)	2 (18.2)	6 (54.5)
Positive	27	22 (81.5)	18 (66.7)	20 (74.1)	21 (77.8)
P value[a]	—	0.087	0.025	0.0016	0.15
Age					
≤ 60 yrs	55	44 (80.0)	40 (72.7)	41 (74.5)	41 (74.5)
> 60 yrs	25	17 (68.0)	18 (72.0)	19 (76.0)	17 (68.0)
P value[a]	—	0.24	0.95	0.89	0.54
Silverberg grade					
1	12	10 (83.3)	9 (75.0)	8 (66.7)	9 (75.0)
2	49	37 (75.5)	35 (71.4)	35 (71.4)	36 (73.5)
3	19	14 (73.7)	14 (73.7)	17 (89.5)	13 (68.4)
P value[b]	—	0.63	0.99	0.17	0.70
FIGO grade					
1	23	18 (78.2)	16 (69.6)	15 (65.2)	17 (73.9)
2	33	24 (72.7)	23 (69.7)	25 (75.8)	23 (69.7)
3	24	19 (79.1)	19 (79.1)	20 (83.3)	18 (75.0)
P value[b]	—	0.94	0.49	0.18	0.93

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively: VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; FIGO: International Federation of Gynecology and Obstetrics.
[a] Chi-square test
[b] Mann-Whitney *U* test.

The frequency of peritoneal metastasis outside the pelvic cavity was significantly greater in patients with tumors that expressed VEGF-C ($P = 0.0010$) or VEGFR-2 ($P = 0.0008$), so did the frequency of lymph node metastasis in VEGF-C ($P = 0.0030$) and VEGFR-2 positive tumors ($P = 0.0018$). Positive results of ascitic cytology related to the expression of VEGF-C ($P = 0.025$) and VEGFR-2 ($P = 0.0016$). Conversely, tumors with peritoneal metastasis outside the pelvic cavity and lymph node metastases and tumors with positive ascitic cytology tended to have greater positivity in the expressions of VEGF-A and VEGFR-3, although the differences were not significant.

Age at surgery did not correlate to VEGF expression, although patients age ≤ 60 years tended to have higher rates of positive VEGF-A, VEGF-C, and VEGFR-3 expression. There was no significant difference between the expression of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3 and high FIGO or Silverberg grade.

VEGF expression levels also were examined as independent risk factors for peritoneal metastasis outside the pelvic cavity, lymph node metastasis, and positive ascitic cytology (Table 3). VEGFR-2 expression was related most closely to lymph node metastasis ($P = 0.027$; risk ratio, 5.11; 95% confidence interval [95%CI], 1.208–21.630), peritoneal metastasis outside the pelvic cavity ($P = 0.038$; risk ratio, 4.79; 95%CI, 1.087–21.079), and positive ascitic cytology ($P = 0.018$; risk ratio, 15.55; 95%CI, 1.592–151.884). VEGF-C expression also was an independent risk factor of peritoneal metastasis ($P = 0.026$; risk ratio, 4.82; 95%CI, 1.203–19.297) and lymph node metastasis ($P = 0.045$; risk ratio, 4.30; 95%CI, 1.031–17.957).

The disease-free and 5-year survival rates were determined using the Kaplan–Meier method (Table 4, Fig. 3). According to the staining level classification for VEGF-C expression, the 5-year survival rate was 90.9% (20 of 22 patients) for the negative-expression group, 81.5% (22 of 27 patients) for the low-expression group,

TABLE 3
Independent Risk Factors Associated with Metastasis

Variable	Coefficient	SE	Chi-square statistic	*P* value	RR	95% CI
Peritoneal metastasis outside the pelvic cavity						
VEGF-A	−1.03	0.77	1.83	0.18	0.36	0.079–1.592
VEGF-C	1.57	0.71	4.94	0.026	4.82	1.203–19.297
VEGFR-2	1.63	0.74	4.92	0.027	5.11	1.208–21.630
VEGFR-3	−0.57	0.67	0.73	0.39	0.56	0.151–2.100
MVD	0.034	0.014	5.54	0.019	1.04	1.006–1.064
Lymph node metastasis						
VEGF-A	−0.98	0.74	1.75	0.19	0.38	0.089–1.599
VEGF-C	1.46	0.73	4.01	0.045	4.30	1.031–17.957
VEGFR-2	1.57	0.76	4.29	0.038	4.79	1.087–21.079
VEGFR-3	−0.30	0.64	0.22	0.64	0.74	0.214–2.584
MVD	0.025	0.013	3.50	0.061	1.03	0.999–1.052
Ascitic cytology						
VEGF-A	0.64	1.03	0.39	0.53	1.91	0.254–14.306
VEGF-C	1.16	1.01	1.34	0.25	3.19	0.446–22.886
VEGFR-2	2.74	1.16	5.57	0.018	15.55	1.592–151.884
VEGFR-3	−0.33	1.06	0.098	0.75	0.72	0.090–5.701
MVD	−0.033	0.029	1.37	0.24	1.38	0.914–1.023

SE: standard error; RR: risk ratio; 95% CI: 95% confidence interval; VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; MVD: microvessel density.

and 45.2% (14 of 31 patients) for the high-expression group: The 5-year survival rate was significantly higher in patients with VEGF-C-negative tumors and in patients with low-expression tumors ($P = 0.0018$; log-rank test). Similarly, with regard to VEGFR-2 expression, the 5-year survival rate was 85.0% (17 of 20 patients) for the VEGFR-2-negative group, 83.9% (26 of 31 patients) for the low-expression group, and 44.8% (13 of 29 patients) for the high-expression group: The 5-year survival rate was significantly higher in patients with VEGFR-2-negative tumors and in patients with low-expression tumors ($P = 0.019$; log-rank test). Conversely, VEGF-A expression and VEGFR-3 expression were not related to the 5-year survival rate.

With regard to VEGFR-2 expression, the disease-free survival rate was 28.6% (8 of 29 patients) for the high-expression group, 67.7% (21 of 31 patients) for the low-expression group, and 85.0% (17 of 20 patients) for the negative-expression group. Recurrence rates increased along with increases in expression levels ($P = 0.0002$; log-rank test). With regard to the expression of VEGF-C and VEGFR-3, patients in the high-expression group tended to have higher recurrence rates, although there were no significant differences ($P = 0.25$ and $P = 0.054$, respectively; log-rank test). Patients who had tumors with high VEGF-A expression tended to have higher 5-year and disease-free survival rates.

Other factors that were included in the univariate analysis were peritoneal metastasis outside the pelvic cavity, lymph node metastasis, patient age at surgery, Silverberg grade, and FIGO grade. The presence or absence of peritoneal metastasis outside the pelvic cavity was related significantly to disease-free survival ($P = 0.042$; log-rank test) and 5-year survival ($P = 0.0013$; log-rank test). Lymph node metastasis also was related significantly to 5-year survival ($P = 0.0029$; log-rank test). Other factors did not show significant correlations.

A Cox regression analysis was used to examine the correlations between disease-specific and disease-free survival and VEGF-A expression, VEGF-C expression, VEGFR-2 expression, VEGFR-3 expression, FIGO stage, histology, age at surgery, FIGO grade, and MVD (Table 5). The results showed that the death rate in the high-VEGF-C-expression group was 5.0 times higher compared with the death rate in the low VEGF-C-expression group, and the death rate in the high VEGFR-2-expression group was 4.2 times higher compared with the death rate in the low VEGFR-2-expression group. The disease recurrence rate in the high VEGFR-2-expression group was 3.3 times higher compared with the disease recurrence rate in the low VEGFR-2-expression group.

CD31 was expressed in endothelial cells from blood and lymph vessels in the tumor stroma (Fig. 2F). Figure 4 summarizes the correlations between MVD and peritoneal metastasis outside the pelvic cavity ($P = 0.0054$), lymph node metastasis ($P = 0.027$), VEGF-A expression ($P = 0.0051$), and VEGF-C expression ($P = 0.042$; Mann–

TABLE 4
Univariate Analysis of Patients who Attained Disease-Free Survival or 5-Year Survival

		Disease-free survival		5-year survival	
Factor	No. of patients	No. (%)	*P* value	No. (%)	*P* value
Age					
≤ 60 yrs	55	28 (50.9)	0.16	39 (70.9)	0.62
> 60 yrs	25	18 (72.0)	—	17 (68.0)	—
Peritoneal metastasis outside the pelvic cavity					
Not present	38	27 (71.1)	0.042	35 (92.1)	0.0013
Present	42	19 (45.2)	—	21 (50.0)	—
Lymph node metastasis					
Not present	44	28 (63.6)	0.25	38 (86.4)	0.0029
Present	36	18 (50.0)	—	18 (50.0)	—
VEGF-A expression					
Negative	20	11 (55.0)	0.47	13 (65.0)	0.46
Low	43	24 (55.8)	—	30 (69.8)	—
High	17	11 (64.7)	—	13 (76.5)	—
VEGF-C expression					
Negative	22	14 (63.6)	0.25	20 (90.9)	0.0018
Low	27	17 (62.9)	—	22 (81.5)	—
High	31	15 (48.4)	—	14 (45.2)	—
VEGFR-2 expression					
Negative	20	17 (85.0)	0.0002	17 (85.0)	0.019
Low	31	21 (67.7)	—	26 (83.9)	—
High	29	8 (28.6)	—	13 (44.8)	—
VEGFR-3 expression					
Negative	22	18 (81.8)	0.054	16 (72.7)	0.99
Low	38	20 (52.6)	—	27 (71.1)	—
High	20	8 (40.0)	—	13 (65.0)	—
Silverberg grade					
1	12	9 (75.0)	0.24	10 (83.3)	0.055
2	49	29 (59.2)	—	32 (65.3)	—
3	19	8 (42.1)	—	14 (73.7)	—
FIGO grade					
1	23	15 (65.2)	0.84	18 (78.3)	0.17
2	33	20 (60.6)	—	20 (60.6)	—
3	24	11 (45.8)	—	18 (75.0)	—

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively: VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained; FIGO: International Federation of Gynecology and Obstetrics.

Whitney *U* test). Conversely, MVD was not an independent risk factors of peritoneal metastasis outside the pelvic cavity ($P = 0.014$; risk ratio, 1.04; 95%CI, 1.006–1.064) (Table 3). MVD showed no significant correlation with either the disease recurrence rate ($P = 0.84$) or the 5-year survival rate ($P = 0.36$).

DISCUSSION

The prognostic value of neoplastic angiogenic factors is controversial. In the gynecologic oncology field, Kaku et al. emphasize the prognostic significance of both angiogenic factors and MVD for patients with endometrial carcinoma[19] and cervical adenocarcinoma[20]; whereas there are contrary opinions with regard to this correlation in patients with ovarian carcinoma.[21–24] There also are differing reports regarding VEGF-A expression as a poor prognostic factor[23] or as a factor that has no effect on some histologic tumor types.[24] These controversies suggest that the growth of ovarian carcinoma does not depend only on VEGF-A but also on the presence of other factors that affect the proliferation and infiltration of epithelial cancer cells. In the current study, the role of VEGF-C, as the other angiogenic factor and the corresponding receptor, VEGFR-3, was observed; and the tissue expression of VEGFR-2, as the common receptor for both VEGF-A and VEFF-C, also was examined.

The expression levels of VEGF-C and VEGFR-3 increased along with the progression of malignant potential from benign cystadenoma, to LPM tumor, and to adenocarcinoma. This finding agreed with the report of Yokoyama et al.[25] The expression of VEGF-C,

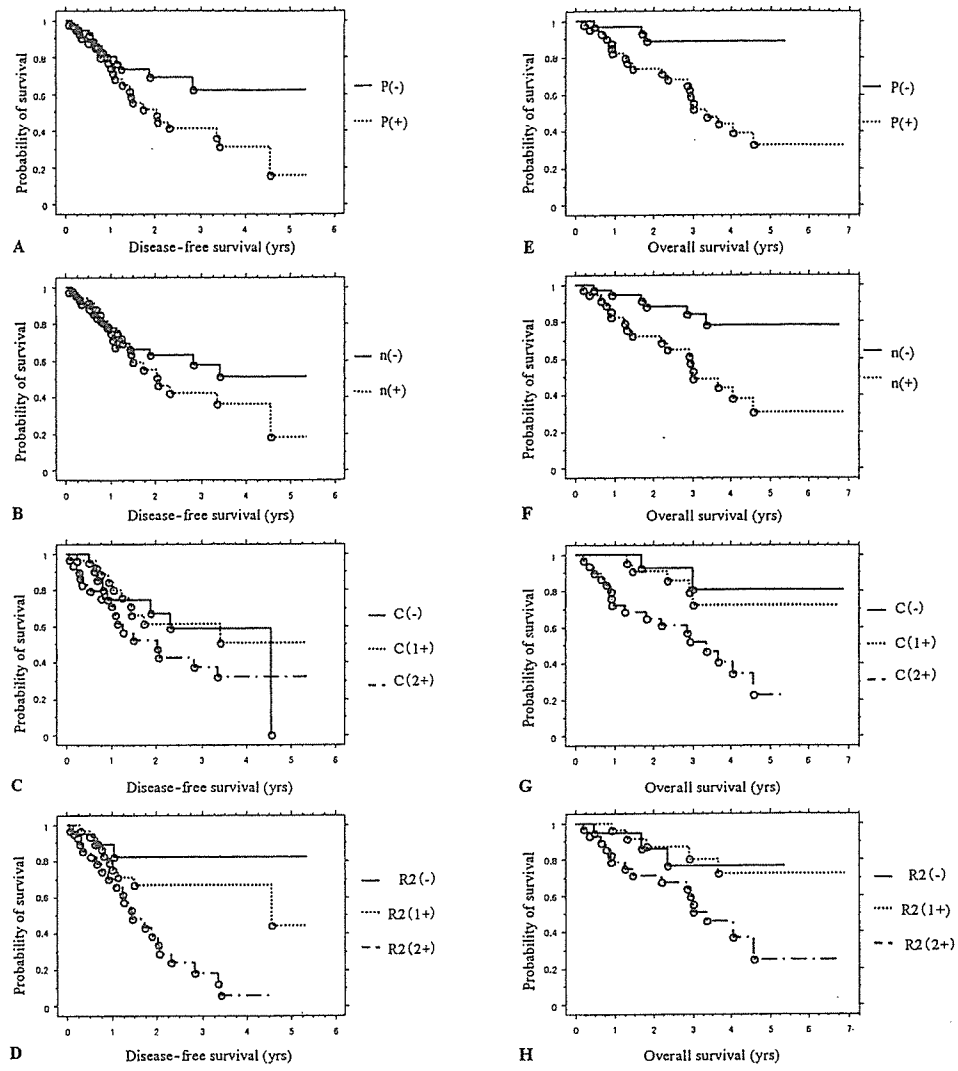

FIGURE 3. Kaplan–Meier analysis of overall survival and disease-free survival of patients after surgery for ovarian carcinoma according to the presence or absence of peritoneal metastasis (P) outside the pelvis (A,E), lymph node metastasis (n) (B,F), vascular endothelial growth factor C (C) expression (C,G), and VEGF receptor 2 (R2) expression (D,H).

VEGFR-3, VEGF-A, and VEGFR-2 was not related to the histologic type of ovarian carcinoma (Table 1). However, this result should be interpreted with caution, because the number of patients in each group was small.

In angiogenesis and lymphangiogenesis, it is known that VEGFR-1 and VEGFR-2 act as receptors for VEGF-A and that VEGFR-2 and VEGFR-3 act as receptors for VEGF-C.[4–9] The current study in patients with ovarian carcinoma also showed a significant correlation between expression of the two angiogenesis factors and their corresponding receptors and expression of the two angiogenesis factors and MVD (Fig. 4). These findings suggest that VEGF-A and VEGF-C act on their corresponding receptors and initiate angiogenesis in ovarian carcinoma.

It has been reported that VEGF-A and VEGF-C are expressed not only in tumor cells but also in endothelial cells from blood and lymph vessels and on fibrous connective tissues from tumor stroma.[26–28] In the current study, we found that their receptors also are expressed both in tumor cells and in endothelial cells from blood and lymph vessels. This indicates that there are both paracrine and autocrine mechanisms: VEGF-A and VEGF-C produced by tumor cells act on their corresponding receptors on endothelial cells through a paracrine mechanism, promote angiogenesis and lymphangiogenesis, and may affect hematogenous and lymphogenous metastases; the factors also may act on receptors on the tumor cells through an autocrine mechanism and promote tumor proliferation (Tables 2, 3).

In the FIGO grading system, in which tumor histology was graded according to the ratio of solid components, there were no significant differences of any other factor. According to the Silverberg grade, which is the overall evaluation of architectural pattern, nu-

TABLE 5
Multivariate Cox Regression Analysis for Disease-Free Survival or Carcinoma-Specific Survival

	Disease-free survival			Carcinoma-specific survival		
Variable	OR	95% CI	*P* value	OR	95% CI	*P* value
Age						
≤ 60 yrs	1.21	0.380–3.833	0.75	0.74	0.181–3.007	0.67
> 60 yrs	1.00			1.00		
Histology						
Clear cell	1.29	0.396–5.423	0.73	6.01	0.920–39.232	0.061
Endometrioid	1.27	0.426–3.788	0.67	1.77	0.458–6.865	0.41
Mucinous	0.945	0.004–0.502	0.012	1.11	0.131–9.424	0.92
Serous	1.00			1.00		
Stage						
I/II	0.60	0.202–1.760	0.35	0.072	0.009–0.572	0.013
III/VI	1.00			1.00		
FIGO grade						
1	1.36	0.440–4.213	0.59	3.53	0.753–16.095	0.11
2	2.01	0.745–5.436	0.17	4.17	1.124–15.456	0.033
3	1.00			1.00		
VEGF-A expression						
Negative	7.16	1.719–29.826	0.0070	2.83	0.497–16.095	0.24
Low	2.03	0.631–6.543	0.23	4.06	0.872–18.887	0.074
High	1.00			1.00		
VEGF-C expression						
Negative	1.76	0.531–5.843	0.36	0.29	0.043–1.937	0.20
Low	0.73	0.256–2.103	0.56	0.20	0.051–0.812	0.035
High	1.00			1.00		
VEGFR-2 expression						
Negative	0.33	0.068–1.593)	0.17	2.96	0.462–18.992	0.25
Low	0.30	0.105–0.859	0.025	0.24	0.066–0.892	0.033
High	1.00			1.00		
VEGFR-3 expression						
Negative	0.22	0.044–1.130	0.84	1.02	0.203–5.094	0.98
Low	1.11	0.391–3.162	0.23	1.86	0.536–6.452	0.32
High	1.00			1.00		
MVD	1.01	0.992–1.036	0.84	1.01	0.987–1.038	0.36

OR: odds ratio; 95% CI: 95% confidence interval; FIGO: International Federation of Gynecology and Obstetrics; VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained; MVD: microvessel density.

clear pleomorphism, and mitotic activity,[15,16] there also were no significant differences for other factors. These results show that histologic level and tumor cell atypism are not related to angiogenesis or lymphangiogenesis.

Prognostic factors, such as peritoneal metastasis outside the pelvic cavity and lymph node metastasis, are determined by surgical staging. Examination of these prognostic factors and their correlation with VEGF-A, VEGF-C, VEGFR-2, VEGFR-3, and MVD revealed that VEGF-C and VEGFR-2 have significant effects on prognosis (Tables 2, 3).

Patients who had tumors with high expression of VEGF-C and VEGFR-2 had a significantly high death rate, which also indicates that these factors are related to patient prognosis. Yokoyama et al. examined the relation between VEGF-C expression and death rates and showed that VEGF-C was related to the 10-year survival rate.[25] Patients in the current study were followed for a much shorter period (mean, 132.8 weeks); however, high expression levels of VEGF-C and VEGFR-2 were related to death from disease. Conversely, no significant correlation was observed in the current study between negative expression or low expression levels of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3 and the recurrence rate or death rate: This may be attributable to our short follow-up.

It is known that the VEGF family and VEGFRs affect the prognosis of patients with adenocarcinoma that develops in the uterus[19] and ovaries[25,29] and in patients with gastric[30,31] and colorectal carcinomas,[32,33] breast carcinoma,[34,35] lung carcinoma,[36–38] head and neck squamous cell carcinoma,[39] Kaposi

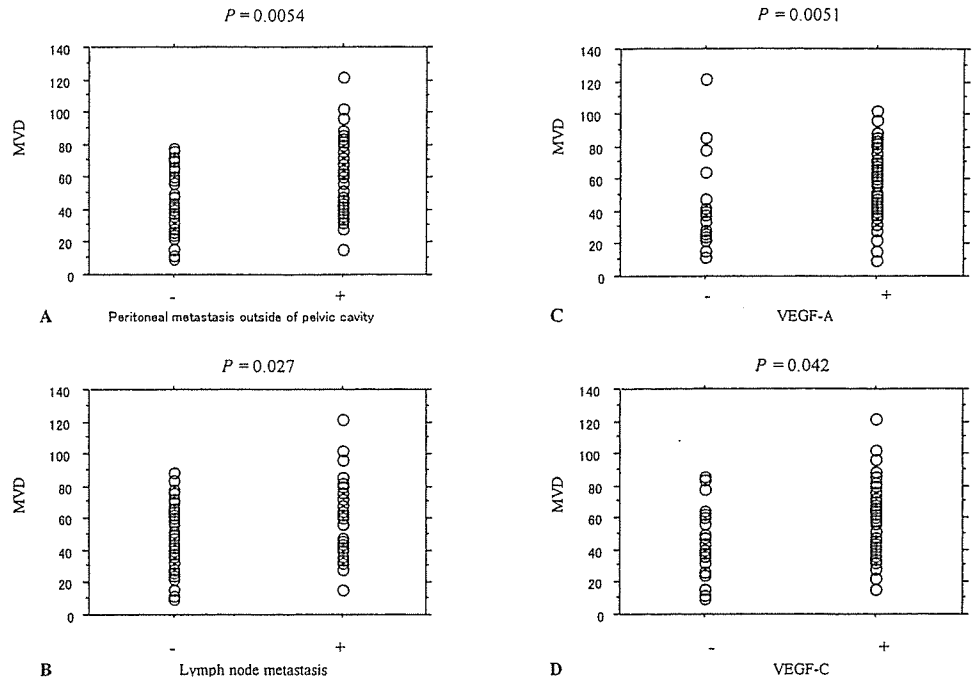

FIGURE 4. Plot of microvessel density (MVD) in relation to peritoneal metastasis outside the pelvis (A), lymph node metastasis (B), vascular endothelial growth factor A (VEGF-A) expression (C), and VEGF-C expression (D). The *P* value shown in each figure represents the significant level of correlation (Mann–Whitney *U* test).

sarcoma,[40] and malignant mesothelioma.[41] The relation between ovarian carcinoma and VEGF-C expression was examined in a previous study[25]; however, in the current study, we investigated the correlation between malignant potential and the expression of VEGFR-2 and VEGFR-3 (the receptor of VEGF-C) and MVD in patients with ovarian carcinoma for the first time. Our findings show that VEGF-C and VEGFR-2 expression levels relate to peritoneal metastasis and lymph node metastasis. The expression of VEGF-C and VEGFR-2 may be used to predict the metastatic spread of ovarian carcinoma cells and to identify patients prospectively who are at a high risk of a poor outcome. The suppression of VEGF-C and VEGFR-2 using angiogenesis suppressors or receptor inhibitors may suppress not only angiogenesis in tumor but also growth of the tumor itself.

REFERENCES

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst.* 1990;82:4–6.
2. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med.* 1995;1:27–31.
3. Folkman J. Clinical applications of research on angiogenesis. *N Engl J Med.* 1995;333:1757–1763.
4. Veikkoa T, Karkkainen M, Claesson-Welsh L, Alitalo K. Regulation of angiogenesis via vascular endothelial growth factor receptors. *Cancer Res.* 2000;60:203–212.
5. Orlandini M, Marconcini L, Ferruzzi R, Oliviero S. Identification of a *c-fos*-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family. *Proc Natl Acad Sci USA.* 1996;93:11675–11680.
6. Joukov V, Pajusola K, Kaipainen A, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J.* 1996;15:290–298.
7. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* 1999;13:9–22.
8. Mandriota SJ, Jussila L, Jeltsch M, et al. Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J.* 2001;20:672–682.
9. Rafii S, Skobe M. Splitting vessels: keeping lymph apart from blood. *Nat Med.* 2003;9:166–168.
10. Heinz AP, Odisino F, Maisoneuve P, et al. Carcinoma of the ovary. *J Epidemiol Biostat.* 2001;6:107–138.
11. Greenlee RT, Murray T, Bolden S, Wingo PA, Harris AL, Fox SB. Cancer statistics. *Cancer J Clin.* 2000;50:7–33.
12. Malkasian GD Jr., Melton LJ III, O'Brien PC, Greene MH. Prognostic significance of histologic classification and grading of epithelial malignancies of the ovary. *Am J Obstet Gynecol.* 1984;149:272–284.
13. Friedlander ML, Dembo AJ. Prognostic factors in ovarian cancer. *Semin Oncol.* 1991;18:205–212.
14. Omura GA, Brady MF, Homesley HD, et al. Long-term follow up and prognostic factor analysis in advanced ovarian carcinoma: the Gynecologic Oncology Group experience. *J Clin Oncol.* 1991;9:1138–1150.
15. Silverberg SG. Prognostic significance of pathologic features of ovarian carcinoma. *Curr Topics Pathol.* 1989;78:85–109.
16. Shimizu Y, Kamoi S, Amada S, Akiyama F, Silverberg SG. Toward the development of a universal grading system for ovarian epithelial carcinoma. *Cancer.* 1998;82:893–901.
17. International Federation of Gynecology and Obstetrics. Classification and staging malignant tumors in the female pelvis. *Acta Obstet Gynecol Scand.* 1971;50:1–7.
18. Weidner N. Tumor angiogenesis: review at current applications in tumor prognostication. *Semin Diagn Pathol.* 1993; 10:302–313.

19. Kaku T, Kamura T, Kinukawa N, et al. Angiogenesis in endometrial adenocarcinoma. *Cancer.* 1997;80:741–747.
20. Kaku T, Hirakawa T, Kamura T, et al. Angiogenesis in adenocarcinoma of the uterine cervix. *Cancer.* 1998;83:1384–1390.
21. Hollingsworth HC, Kohn EC, Steinberg SM, Rothenberg ML, Merino MJ. Tumor angiogenesis in advanced stage ovarian carcinoma. *Am J Pathol.* 1995;147:33–41.
22. Abulafia O, Triest WE, Sherer DM. Angiogenesis in primary and metastatic epithelial ovarian carcinoma. *Am J Obstet Gynecol.* 1997;177:541–547.
23. Yamamoto S, Konishi I, Mandai M, et al. Expression of vascular endothelial growth factor in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. *Br J Cancer.* 1997; 76:1221–1227.
24. Ogawa S, Kaku T, Kobayashi H, et al. Prognostic significance of microvessel density, vascular endothelial growth factor expression in ovarian carcinoma: special review for clear cell adenocarcinoma. *Cancer Lett.* 2002;176:111–118.
25. Yokoyama Y, Charnock-Jones DS, Licence D, et al. Vascular endothelial growth factor D is an independent prognostic factor on epithelial ovarian carcinoma. *Br J Cancer.* 2003;88: 237–224.
26. Gunningham SP, Currie MJ, Han C, et al. The short form of the alternatively spliced flt-4 but not its ligand vascular endothelial growth factor C is related to lymph node metastasis in human breast cancers. *Clin Cancer Res.* 2000;6:4278–4286.
27. Boocock CA, Charnock-Jones DS, Sharkey AM, et al. Expression of vascular endothelial growth factor and its receptors flt and kdr in ovarian carcinoma. *J Natl Cancer Inst.* 1995; 87:506–516.
28. Brown LF, Berse B, Jackman RW, et al. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. *Am J Pathol.* 1993;143:1225–1262.
29. Sowter HM, Corps AN, Evans AL, Clark DE, Charnock-Jones DS, Smith SK. Expression and localization of the vascular endothelial growth factor family in ovarian epithelial tumors. *Lab Invest.* 1997;77:607–614.
30. Amioka T, Kitadai Y, Tanaka S, et al. Vascular endothelial growth factor-C expression predicts lymph node metastasis of human gastric carcinomas invading the submucosa. *Eur J Cancer.* 2002;38:1413–1419.
31. Yonemura Y, Endo Y, Fujita H, et al. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. *Clin Cancer Res.* 1999;5:1823–1829.
32. Andre T, Kotelevets L, Vaillant JC, et al. Vegf, Vegf-B, Vegf-C and their receptors KDR, FLT-1 and FLT-4 during the neoplastic progression of human colonic mucosa. *Int J Cancer.* 2000;86:174–181.
33. George ML, Tutton MG, Janssen F, et al. VEGF-A, VEGF-C, and VEGF-D in colorectal cancer progression. *Neoplasia.* 2001;3:420–427.
34. Skobe M, Hawighorst T, Jackson DG, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. *Nat Med.* 2001;7:192–198.
35. Kurebayashi J, Otsuki T, Kunisue H. et al. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. *Jpn J Cancer Res.* 1999;90:977–981.
36. Decaussin M, Sartelet H, Robert C, et al. Expression of vascular endothelial growth factor (VEGF) and its two receptors (VEGF-R1-Flt1 and VEGF-R2-Flk1/KDR) in non-small cell lung carcinomas (NSCLCs): correlation with angiogenesis and survival. *J Pathol.* 1999;188:369–377.
37. Kajita T, Ohta Y, Kimura K, et al. The expression of vascular endothelial growth factor C and its receptors in non-small cell lung cancer. *Br J Cancer.* 2001;85:255–260.
38. Niki T, Iba S, Tokunou M, Yamada T, Matsuno Y, Hirohashi S. Expression of vascular endothelial growth factor A, B, C, and D and their relationships to lymph node status in lung adenocarcinoma. *Clin Cancer Res.* 2000;6:2431–2439.
39. O-charoenrat P, Rhys-Evans P, Eccles SA. Expression of vascular endothelial growth factor family members in head and neck squamous cell carcinoma correlates with lymph node metastasis. *Cancer.* 2001;92:556–568.
40. Jussila L, Valtola R, Partanen TA, et al. Lymphatic endothelium and Kaposi sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. *Cancer Res.* 1998;58:1599–1604.
41. Ohta Y, Shridhar V, Bright RK, et al. VEGF and VEGF type C play an important role in angiogenesis and lymphangiogenesis in human malignant mesothelioma tumours. *Br J Cancer.* 1999;81:54–61.

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The effect of single weekly paclitaxel in heavily pretreated patients with recurrent or persistent advanced ovarian cancer

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Abstract

Objectives. We have reported that single weekly paclitaxel has moderate activity in heavily pretreated ovarian cancer patients and is associated with a favorable toxicity profile. The purpose of this study was to reconfirm the effect of weekly paclitaxel in more number of cases.

Methods. Although 39 patients were enrolled, 37 patients with recurrent or persistent ovarian cancer previously treated with between one and three chemotherapeutic regimens containing platinum were eligible. Patients had measurable or assessable disease defined by clinical exam, radiographic studies, or serum CA 125. One cycle of treatment consisted of paclitaxel 80 mg/m^2/week in 1-h infusion, 3 weeks on, 1 week off, and repeated at least twice. Two patients were withdrawn because of refusal of further treatment for neuropathy after the first cycle. Clinical responses were defined by established criteria.

Results. Thirty-seven patients were included in this intent-to-treat study. The overall clinical response rate was 45.9% (5 complete responses, 12 partial responses). The clinical response rate in patients with measurable tumor was 25.0% (2 complete responses, 1 partial response), while that in patients without measurable tumor and with assessable CA 125 levels was 56.0% (3 complete responses, 11 partial responses). Clinical response rate in patients with chemotherapy-free interval more than 6 months had about twice higher than that in patients with chemotherapy-free interval less than 6 months. The clinical response rate by number of prior regimens revealed that as number of prior regimens increases, the response rate decreases.

Conclusion. Weekly paclitaxel has significant antitumor activity in heavily pretreated patients with recurrent or persistent ovarian carcinoma and warrants as second or third line chemotherapy in such setting.
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Keywords: Weekly paclitaxel; Ovarian cancer; Second line chemotherapy; CA 125

Introduction

Ovarian cancer is the fourth leading cause of cancer death in the female population and the most fatal gynecologic malignancy. The disease is surgically curable when localized (stage I to II). However, the majority of patients present, initially and at relapse, with bulky intra-abdominal disease that is not surgically resectable. Systemic cisplatin-based chemotherapy in combination with debulking surgery has become the standard for initial therapy, with reported response rates that range from 50% to 80% [1]. Unfortunately, the majority of patients eventually die of disease persistence or recurrence, with the abdominal cavity being the most common site of recurrence. The management of tumor recurrence remains a clinical challenge, since the

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chance of response to a secondary treatment is currently less than 20% [2], especially if the disease is platinum-resistant [3]. To improve this outcome, several clinical trials are now exploring the possibility of incorporating new drugs into the first-line chemotherapy regimen [4]. Furthermore, new biological agents and molecularly targeted therapies aimed to overcome drug resistance with less toxic effects are under investigation [5].

Paclitaxel, a unique antimicrotubule agent, has been one of the most promising drugs to enter into clinical trials in the setting of cisplatin-refractory ovarian cancer. Responses have been reported in both heavily and minimally pretreated ovarian cancer patients (20% to 37%) [6,7]. However, myelotoxicity was found to be a major concern even with granulocyte colony-stimulating factor (G-CSF) support. In order to minimize toxicity, paclitaxel can be given weekly instead of triweekly [8,9]; this results in a higher dose intensity of the drug [10]. Two non-randomized trials [11,12] have suggested that the activity of paclitaxel in epithelial ovarian cancer is dose-dependent, and a randomized trial [10] has shown reduced toxicity with weekly scheduling without detriment to efficacy. We have reported that single weekly paclitaxel has moderate activity in heavily pretreated ovarian cancer patients, and 80 mg/m^2 of paclitaxel was recommended as the phase II dose for outpatients [13]. When 80 mg/m^2 of paclitaxel was given, the dose intensity may not be greater than every triweekly. However, continuous low-dose paclitaxel so-called metronomic chemotherapy has been reported to result in antiangiogenic effects and tumor dormancy [14,15]. Thus, we attempted to determine effects of single weekly paclitaxel in heavily pretreated patients with recurrent or persistent ovarian cancer.

Patients and methods

Eligibility criteria

Eligible patients had recurrent or persistent ovarian cancer that was histologically proven at primary diagnosis. All patients had either measurable or assessable disease. Disease was classified as measurable if the patient had bidimensionally measurable disease by computed tomography (CT). Assessable disease was only used in patients with no measurable disease and was defined as a CA 125 $\geq$ 75 U/ml. Eligibility criteria required the patients to have a baseline leukocyte count >2500, absolute neutrophil count >1500, platelet count >75 000, serum creatinine <1.5 mg/dl, serum bilirubin <2.5 mg/dl, and liver function tests <3 times the laboratory standard value. Patients were required to have a life expectancy of at least 2 months and any Gynecologic Oncology Group (GOG) performance score was acceptable for enrollment in this study. Thirty-seven of 39 patients enrolled were eligible for this study. Twenty-three and 14 patients had chemotherapy-free interval ≥6 months and <6 months, respectively. Patients must have had one or more previous chemotherapy regimens (Table 1).

Exclusion criteria included borderline histology, pregnancy, fertility, diagnosis of another malignancy within the past 5 years, prior treatment with weekly paclitaxel, active infection, hepatitis, gastrointestinal bleeding, congestive heart failure, unstable angina, or myocardial infarction in the past 6 months.

Study design

This study was a nonparametric multicenter study of weekly paclitaxel. The investigative sites involved were National Defense Medical College in Saitama and Jichi Medical School in Tochigi, Japan. All investigative sites obtained institutional review board approved and all patients provided signed informed consent.

Treatment plan

Eligible patients who signed informed consent underwent a complete history and physical exam. Pretreatment laboratory tests included a complete blood count (CBC), chemistry panel to include glucose, electrolytes, BUN, creatinine, SGOT, SGPT, bilirubin, alkaline phosphatase, CA 125 level,

Table 1
Patient characteristics

Characteristic	No. of patients	%
Patients		
Enrolled	39	
Eligible	37	
Median age (range)	59 (42–74)	
Original FIGO stage		
Ia	1	2.7
Ic	2	5.4
IIIa	2	5.4
IIIc	23	62.2
IV	9	24.3
Histological type		
Serous	26	70.3
Clear	3	8.1
Mucinous	2	5.4
Endometrioid	2	5.4
Others	4	10.8
Chemotherapy-free interval[a]		
≥6 months	23	62.2
<6 months	14	37.8
Prior regimens		
1	19	51.4
2	14	37.8
3	4	10.8

[a] Interval from prior chemotherapy to start of weekly paclitaxel.

chest X-ray, EKG, and CT scan or magnetic resonance imaging (MRI).

On days 1, 8, and 15 of each 28-day cycle (1 cycle), patients received intravenous infusions of paclitaxel at 80 mg/m^2. Paclitaxel was given as a 1-h intravenous infusion via non-PVC tubing and connectors. Premedications consisted of diphenhydramine (50 mg), cimetidine (300 mg), and dexamethasone (20 mg) intravenously given 30 min before paclitaxel infusion. A minimum of six doses (two cycles) were administered at weekly intervals. Chemotherapy was withheld for white cell counts below 2500/mm^3 or absolute neutrophil counts below 1500/mm^3 and for platelet counts below 75 000 mm^3. Toxicity was assessed by using the GOG scoring system [16]. In patients with progression of disease, chemotherapy was either stopped or changed to another agent. In patients with stable disease or a clinical response, weekly paclitaxel was continued until disease progression or adverse effects necessitated removal from the study. Withdrawal from the study at patient request was allowed at any time.

Response assessment

Although most of the patients had elevated CA 125 levels, many did not have measurable disease on CT, MRI, or clinical exam. Hence, the criteria for response was based on declining CA 125 levels as described by Rustin et al. [17]. Partial response was defined by reduction of CA 125 by more than 50% after two samples or greater than 75% serial reduction over three consecutive samples, with the final sample taken at least 28 days after the previous sample. This has been correlated to standard response criteria as defined by the Gynecologic Oncology Group (GOG) in patients with measurable disease [18]. Partial response by CT scan was defined as a 50% reduction in the sum of the two perpendicular diameters of all measurable tumors for at least 1 month. Complete response was defined as total disappearance of all clinically or radiologically measurable tumors with normalization of CA 125 levels (<35) for at least 1 month. Progression of disease was defined as appearance of new lesions or an increase of more than 50% in the sum of two perpendicular diameters of any existing lesion or increase in CA 125 levels on two consecutive measurements. The term stable disease was used for any response that fell in between progression and a partial response. For statistical comparison, the Mann–Whitney two-sample test and Fisher's Exact Test have been used. Time to progression (TTP) was measured as interval from prior chemotherapy to start of the weekly paclitaxel for progression. Survival was measured from start of the weekly paclitaxel to the date of death or last contact if the date of death is unknown.

Table 2
Clinical response (N = 37 evaluable patients)

Response	No. of patients	%
Complete response	5	13.5
Partial response	12	32.4
Stable disease	16	43.2
Progression	4	10.8

Table 3
Response with tumor regression (N = 12 evaluable patients)

Response	No. of patients	%
Complete response	2	16.7
Partial response	1	8.3
Stable disease	6	50.0
Progression	3	25.0

All patients had morphologically measurable tumor.

Results

From April 1999 to September 2002, 39 patients were enrolled in this prospective trial and received weekly paclitaxel therapy. Two patients were withdrawn because of refusal of further treatment for neuropathy after the first cycle. Demographics for the 37 evaluable patients are listed in Table 1. Twenty-three patients (62.2%) had chemotherapy-free interval ≥6 months. All of 14 (37.8%) patients with chemotherapy-free interval <6 months had platinum-based chemotherapy. The number of patients with one prior chemotherapy regimen was 19, that with two prior regimens was 14, and that with three prior regimens was 4. Primary chemotherapy consisted of 30 patients with combination chemotherapy by cisplatin, Adriamycin, and cyclophosphamide (CAP), 4 patients with combination chemotherapy by paclitaxel and carboplatin (TJ), and 3 patients with combination chemotherapy by carboplatin and cisplatin (JP). Performance status (GOG) of all patients enrolled was 0 or 1.

All 37 patients were evaluable for response. Five patients (13.5%) showed a complete response, 12 (32.4%) showed a partial response. Total response rate was 45.9% (Table 2).

Two (16.7%) out of 12 patients with measurable tumor had complete response and 1 (8.3%) had partial response. The response rate was 25.0% (Table 3). Regarding response based on CA 125 levels, 3 (12.0%) of 25 patients had complete response and 11 (44.0%) had partial response. The

Table 4
Response based on CA 125 levels (N = 25)

Response	No. of patients	%
Complete response	3	12.0
Partial response	11	44.0
Stable disease	10	40.0
Progression	1	4.0

No patient had morphologically measurable tumor.