O-[^{18}F]fluoromethyl-L-tyrosine is a potential tracer for monitoring tumour response to chemotherapy using PET: an initial comparative in vivo study with deoxyglucose and thymidine

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Abstract. *Purpose:* To compare the utility of a new artificial amino acid, *O*-[^{18}F]fluoromethyl-L-tyrosine ([^{18}F]FMT), for monitoring cancer chemotherapy with deoxyglucose and thymidine.
Methods: [^{18}F]FMT, [^{14}C]deoxyglucose ([^{14}C]DG) and [6-^{3}H]thymidine ([^{3}H]Thd) were applied in this study. A 2.5 mg/kg dose of mitomycin (MMC) was administered to AH272 rat hepatoma-bearing Donryu rats. Tumour uptake of each tracer was measured just before (baseline) and on days 1, 3, 5 and 7 after the MMC administration, 1 h after a mixture of [^{18}F]FMT, [^{14}C]DG and [^{3}H]Thd had been injected, and was shown as DURs (% injected dose/gram tissue normalised for the rat body weight). Dual-tracer macroautoradiographs with [^{18}F]FMT and [^{14}C]DG were also prepared.
Results: The tumour uptake for each tracer decreased earlier than did the tumour size. DURs (mean±SD) at baseline and on days 1, 3, 5 and 7 were as follows: [^{18}F]FMT: 4.68±0.72, 3.34±0.66, 3.13±0.72, 3.42±0.45, 3.01±0.32; [^{14}C]DG: 3.26±0.40, 3.09±0.55, 3.01±0.97, 2.28±0.35, 1.70±0.72; and [^{3}H]Thd: 2.23±0.46, 1.54±0.45, 1.28±0.37, 1.35±0.20, 0.94±0.12. Decrease in [^{18}F]FMT uptake compared with baseline was significant from day 1 ($p<0.01$), and the decrease in [^{3}H]Thd uptake was also significant on day 1 ($p<0.05$) and days 3–7 ($p<0.01$). However, decrease in [^{14}C]DG uptake was only significant from day 5 ($p<0.01$). Macroautoradiography suggested that the influence of inflammatory cells on the accumulation of [^{18}F]FMT in tumours is smaller than that on the accumulation of [^{14}C]DG.
Conclusion: [^{18}F]FMT uptake shows a rapid and sensitive response to chemotherapy, comparable to that of [^{3}H]Thd, suggesting that it may be applied as a powerful tracer for monitoring of proliferative activity after cancer chemotherapy using PET.

Keywords: *O*-[^{18}F]fluoromethyl-L-tyrosine – [^{18}F]fluorodeoxyglucose – [^{3}H]thymidine – PET – Cancer chemotherapy

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Introduction

During the past decade, advances in positron emission tomography (PET) have facilitated the acquisition of information on metabolism in neoplasms to such an extent that it has become an essential tool in the management of cancer patients. The glucose analogue ^{18}F-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most frequently used tracer in PET studies in oncology, its application being based on increased glucose uptake in tumour cells owing to increased glycolysis [1]. [^{18}F]FDG PET has been successfully used to image various kinds of malignant tumour, for staging and restaging [2], and for monitoring the efficacy of cancer treatment [3–7]. However, [^{18}F]FDG is well known to exhibit non-specific uptake in inflammatory cells and granulation tissue [8], and this is a disadvantage in monitoring the results of therapy.

Increased amino acid metabolism is also a well-known characteristic of malignant tumours [9], both amino acid

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transport and protein synthesis rates being enhanced in malignancies. Therefore, clinical interest in imaging protein metabolism with the aid of radiolabelled amino acids is expected to increase [10]. L-[*S*-methyl-^{11}C] methionine ([^{11}C]Met) is one of the most widely used agents for this purpose because of its simple labelling procedure [11] and high uptake in a number of tumours [12–16]. However, ^{18}F-fluorinated amino acids may have practical benefits for tumour diagnosis in PET studies, because the half-life of ^{18}F (109 min) is more suitable for whole-body tumour imaging than that of ^{11}C (20 min) [17].

^{18}F-labelled amino acids that enter the protein synthesis pathway, such as 2-[^{18}F]fluoro-L-tyrosine ([^{18}F]TYR) [18], have been developed as protein synthesis tracers but suffer from the disadvantage of also reflecting the maintenance of cells and not only cell proliferation [10, 19]. More recently, ^{18}F-labelled artificial amino acids, such as *O*-[^{18}F]fluoroethyl-L-tyrosine ([^{18}F]FET) [20] and [^{18}F]fluorine-α-methyl-tyrosine [21], have been developed, and these are amino acid transport tracers that are thought to be more directly related to cell proliferation [10]. In contrast to glucose, amino acids play only a minor role in the metabolism of inflammatory cells [22], so they might be expected to serve as more specific tracers for monitoring cancer treatment, especially chemotherapy, than [^{18}F]FDG. Since there have been no experimental in vivo studies to compare the utility of different radioactive tracers for monitoring chemotherapeutic effects, the present comparative investigation was performed.

O-[^{18}F]fluoromethyl-L-tyrosine ([^{18}F]FMT) is an amino acid transport tracer, developed in our institute [23, 24], which features ease of synthesis and a satisfactorily high radiochemical yield. In the present study, it was compared with glucose and nucleic acid metabolic tracers, using mitomycin chemotherapy of the AH272 rat hepatoma transplanted into Donryu rats as a suitable model.

Materials and methods

Radiopharmaceuticals

[^{18}F]FMT was synthesised according to the method of Iwata et al. with a radiochemical purity higher than 98% [23]. Briefly, [^{18}F] fluoromethyl triflate was bubbled through a dimethylsulphoxide solution of L-tyrosine disodium salt at room temperature, and the reaction mixture was purified by high-performance liquid chromatography (HPLC). 2-Deoxy-D-[1-^{14}C]glucose ([^{14}C]DG, specific activity 2.07 GBq/mmol, radiochemical purity 98.3%, Amersham Biosciences UK Limited) as a substitute for [^{18}F]FDG and [6-^{3}H] thymidine ([^{3}H]Thd, specific activity 814 GBq/mmol, radiochemical purity 99.8%, Amersham Biosciences UK Limited) were obtained commercially.

Animals, tumours and chemotherapy

Five-week-old male Donryu rats were injected subcutaneously under their back skin with a 0.1 ml suspension containing 3.0×10^6 AH272 cells. Chemotherapy was performed with commercially available mitomycin C (MMC, Kyowa Hakko Kogyo Co. Ltd.) on the 10th day after the inoculation, when the tumours had grown to between 1.0 and 1.5 cm in diameter. Rats were anaesthetised with the inhalation of ether, and 2.5 mg/kg of MMC dissolved in 0.2–0.4 ml of physiological saline was administered to each rat via a lateral tail vein.

Tumour growth study

Six rats per group were used for control and chemotherapy groups. Tumours were measured (length and width) with sliding calipers by the same person every day after the start of the experiment. The tumour volume (V) in mm^3 was calculated from the linear measurements using the formula: tumour volume (mm^3)=4×pi×((length (mm) plus width (mm))/2)^3/3. The relative mean tumour volume (RV) was calculated as V_i/V_o, where V_i is the mean tumour volume of a group at any given time and V_o is the mean tumour volume at the initial treatment. Growth curves after treatment were generated from the calculated RV.

Triple-tracer tissue distribution study

The triple-tracer tissue distribution studies were performed on four groups of six rats at 1, 3, 5 and 7 days after chemotherapy, and also on a control group of seven rats. After 8 h of fasting, a mixture of three tracers, consisting of 1.85 MBq [^{18}F]FMT, 74 kBq [^{14}C]DG and 74 kBq [^{3}H]Thd, in 0.2 ml of physiological saline, was injected via a lateral tail vein. Rats anaesthetised with ether were killed by decapitation 60 min after the injection and the tumours were removed and blotted. A cross-section of each tumour at its maximum diameter was sampled and weighed, followed by ^{18}F measurement with an automated NaI well counter (United Technologies Packard Auto-Gamma 500/800). Seven days later, when ^{18}F had decayed, tissue samples were prepared for liquid scintillation counting of ^{14}C and ^{3}H. Each sample was digested and bleached with 2 ml tissue solvent, Soluene-350 (PerkinElmer Life Sciences), in a heater at 50°C for 4–5 h. The samples were then mixed with 10 ml scintillation cocktail and left at room temperature overnight. The contamination of ^{3}H with ^{14}C was 1.3%, and that of ^{14}C with ^{3}H was 0.35%. Tissue radioactivity data were expressed as the differential uptake ratio (DUR): DUR = (tissue counts/tissue weight) / (injected dose counts/animal body weight).

The relative tumour uptake ratio was also calculated as DUR_i/DUR_o, where DUR_i is the mean DUR of a group at any given time and DUR_o is the mean DUR at the initial treatment.

Dual-tracer macroautoradiography

A control rat and another rat at 3 days after chemotherapy were prepared for dual-tracer macroautoradiography. After fasting overnight, a mixture of 37 MBq [^{18}F]FMT and 185 kBq [^{14}C]DG in 0.2 ml of physiological saline was injected via a lateral vein, and the rats were anaesthetised with ether and killed by decapitation 60 min thereafter. The tumours were quickly dissected, embedded in O.C.T. compound (Sakura Finetek Japan Co. Ltd.) and deep frozen in a block of dry ice. Several 20-μm-thick sections were cut and mounted on clean glass slides in a cryostat (HM-500 Carl Zeiss) at −20°C, air-dried and placed in direct contact with macroautoradiography films for 1.5 h to produce [^{18}F]FMT images. One week later, following the decay of ^{18}F, the same sections were placed in contact with separate films for 10 days to produce [^{14}C]DG images.

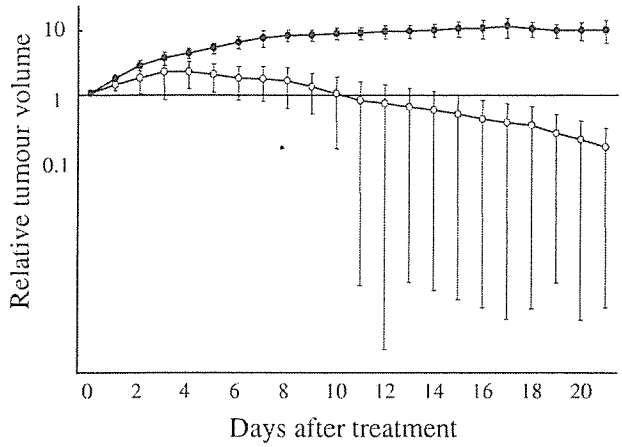

Fig. 1. The tumour growth curves for the chemotherapy (○) and control (●) groups. *Symbols* are mean values and *bars* are standard deviations. There were six rats in each group. In the control group, the tumours grew until day 18. In the chemotherapy group, tumour volumes begin to decrease on day 6, and the reductions were significant on day 11, as compared with the initial volume ($p<0.05$)

Microscopic examination

For comparison with the findings of dual-tracer macroautoradiography, a control rat and another rat at day 3 after chemotherapy were prepared for microscopic examination. Tumours were dissected, fixed with 10% neutral formalin and embedded in paraffin. Cross-sections with a thickness of 3 μm were produced with a microtome and stained with haematoxylin and eosin; the stained samples were examined with a BX51N microscope (Olympus) by the pathologist.

Statistical comparison

Student's *t* test was employed to determine the statistical significance of differences between tumour volumes and uptake data.

Ethics

The experimental protocol was approved by the Laboratory Animal Care Committee of Tohoku University.

Results

The growth curves for the chemotherapy and control groups are shown in Fig. 1. Each curve gives the mean and standard deviation for the relative tumour volume. In the control group, the volume increased until day 18. In the chemotherapy group, the relative tumour volumes slowed down from days 1 to 5, and began to decrease on day 6. The reduction became significant on day 11 (RV=0.75±0.76) as compared with the initial volume ($p<0.01$).

Data for the tumour uptake of [^{18}F]FMT, [^{14}C]DG and [^{3}H]Thd on the day before chemotherapy and on days 1, 3, 5 and 7 after chemotherapy are shown in Table 1, and the relative tumour uptake ratio curves of each tracer in Fig. 2. Decreases in uptake of all tracers were faster than those in the relative tumour volumes. The reduction in [^{18}F]FMT uptake compared with uptake on the day before chemotherapy was significant from day 1 ($p<0.01$), and [^{3}H]Thd uptake was also significantly reduced on day 1 ($p<0.05$) and days 3–7 ($p<0.01$). However, the decrease in [^{14}C]DG uptake occurred much later and was only significant from day 5 ($p<0.01$). The decreases in [^{14}C]DG and [^{3}H]Thd uptake after chemotherapy were larger than that in [^{18}F]FMT uptake. On the last day of the measurement, [^{14}C]DG and [^{3}H]Thd uptake had decreased to half of the untreated level, while [^{18}F]FMT uptake had decreased by one-third.

Macroautoradiographs of [^{18}F]FMT and [^{14}C]DG, and photographs of cross-sections of samples before and after chemotherapy are shown in Fig. 3. Tumour necrosis had been evident after chemotherapy, as shown in the photographs. Macroautoradiography of [^{18}F]FMT showed a diffuse pattern of uptake in tumour tissue, with gradual reduction of uptake in the necrotic area. On the other hand, [^{14}C]DG uptake was slightly spotty but dense close to the rim of the tumour, and there was strong contrast between the tumour and the necrotic area.

Microscopic examination was carried out separately from autoradiography at 3 days after chemotherapy, and the result is shown in Fig. 4. A large number of inflammatory cells appeared immediately adjacent to areas of necrosis.

Discussion

The present study provided clear evidence that uptake of [^{18}F]FMT and [^{3}H]Thd after MMC treatment of the AH272

Table 1. Tumour uptake of [^{18}F]FMT, [^{14}C]DG and [^{3}H]Thd after chemotherapy

	Pretreatment (control)	Day 1	Day 3	Day 5	Day 7
[^{18}F]FMT	4.68±0.72	3.34±0.66**	3.13±0.72**	3.42±0.45**	3.01±0.32**
[^{14}C]DG	3.26±0.40	3.09±0.55	3.01±0.97	2.28±0.35**	1.70±0.72**
[^{3}H]Thd	2.23±0.46	1.54±0.45*	1.28±0.37**	1.35±0.20**	0.94±0.12

Values are expressed as mean±standard deviation of DURs
The number of rats in each group was seven
DURs of each day after chemotherapy are compared with the DUR of the control
Statistical comparison: *$p<0.05$, **$p<0.01$ (Student's *t* test)

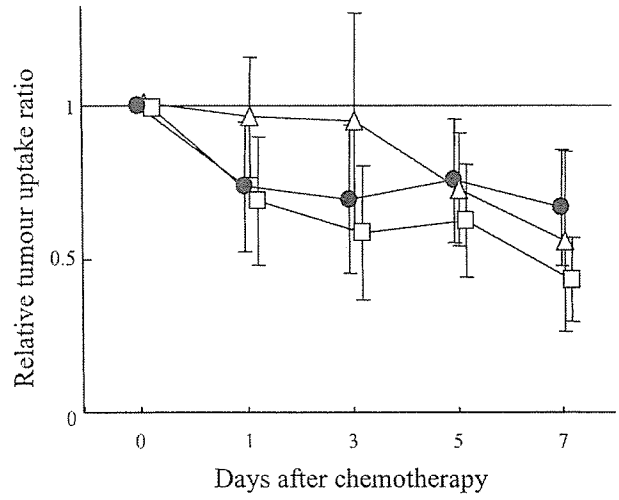

Fig. 2. Relative tumour uptake ratio curves of [^{18}F]FMT, [^{14}C]DG and [^{3}H]Thd after chemotherapy. *Symbols* are mean values and *bars* are standard deviations. ●, [^{18}F]FMT; △, [^{14}C]DG; □, [^{3}H]Thd

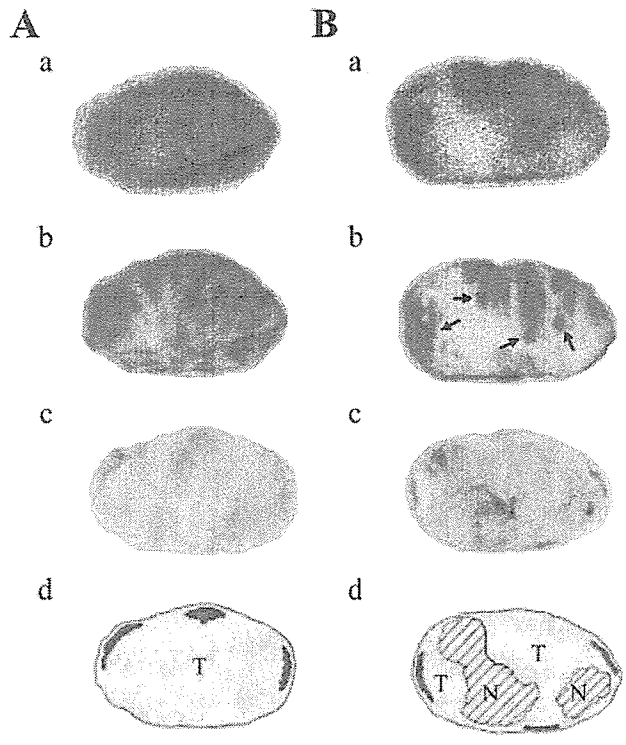

Fig. 3. Macroautoradiographs of control and MMC-treated tumours with [^{18}F]FMT and [^{14}C]DG, together with photographs and illustrations of their cross-sections. **A** is a control tumour and **B** is a tumour at 3 days after chemotherapy. **a** Macroautoradiographs of [^{18}F]FMT; **b** macroradioautographs of [^{14}C]DG; **c** photographs of the sample cross-sections; **d** illustrations of the photographs. In the illustrations, *T* indicates viable tumour and *N* indicates necrotic areas. The *arrow* in **B, b** indicates the rim of the tumour, where there is strong contrast between the tumour and the necrotic area on macroautoradiography with [^{14}C]DG

rat hepatoma transplanted into Donryu rats is more rapidly reduced than the uptake of [^{14}C]DG, providing an effective means for determination of early response to chemotherapy in vivo.

Amino acid transport tracers are amino acids that are only transported into the cells and do not actually participate in protein synthesis [10], examples being [^{18}F]FMT [23, 24], [^{18}F]FET [20] and [^{18}F]fluorine-α-methyl-tyrosine [21]. [^{11}C]Met is the most widely used amino acid tracer, and shows a well-documented rapid response to cancer radiotherapy [25]. However, clinical use is limited by high accumulation in the normal liver, pancreas and small intestine, and clinical interpretation of change in uptake of [^{11}C]Met during treatment is difficult because it participates in too many metabolic pathways [19]. The other amino acid tracers that enter protein synthesis, such as [^{18}F]TYR, are unsuitable for the evaluation of cell proliferation since protein synthesis is not always related to cell proliferation, in that the protein may be used for maintenance of cells [10]. Imaging with amino acid transport tracers shows the sum of protein synthesis and non-protein synthesis processes and the total amino acid signal is likely to correlate with the cell proliferation; thus these tracers are thought to be more appropriate for monitoring of chemotherapy. The fact that [^{18}F]FMT and [^{3}H]Thd, used as a nucleic acid tracer, showed similar changes in the time course after chemotherapy is of clear interest in this respect.

As already mentioned, in the present study, macro-autoradiography demonstrated [^{18}F]FMT to be diffusely distributed in tumour tissue with gradual reduction of uptake in the necrotic area, while [^{14}C]DG uptake was slightly spotty but dense close to the rim of the tumour, with marked contrast between the tumour and the necrotic area. Microscopy showed many inflammatory cells between the tumour and the necrotic area. Rau et al. [26]

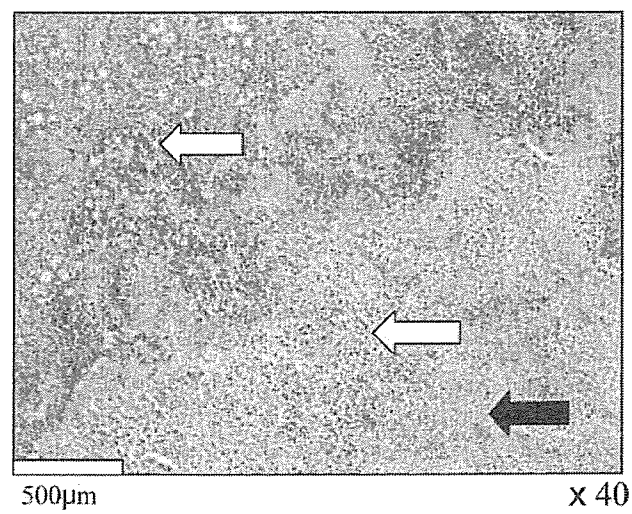

Fig. 4. Microscopic examination of tumour at day 3 after chemotherapy. The *white, yellow and black arrows* indicate the viable tumour, inflammatory cells and necrotic areas, respectively. A number of inflammatory cells appear around the necrotic area

and Suzuki et al. [27] respectively reported [^{18}F]FET and [^{18}F]FMT to show only limited accumulation in acute and chronic inflammatory lesions, whereas there is evidence that [^{18}F]FDG is readily taken up by inflammatory cells and granulation tissue [8]. However, it has also been reported that prenecrotic or hypoxic cells [28] and apoptosis [29] can be responsible for high accumulation of [^{18}F]FDG, and it might be difficult to explain the dense accumulation of [^{14}C]DG in the rim of the tumour only by reference to the presence of inflammatory cells. Nevertheless, at least in our study, it is possible that inflammatory cells affected the accumulation of [^{14}C]DG but not [^{18}F]FMT, and thus [^{18}F]FMT might be more appropriate than [^{18}F]FDG for the early detection of chemotherapeutic effects.

In our study, the final range of decrease in [^{18}F]FMT uptake after chemotherapy tended to be small, compared with the decrease in [^{14}C]DG uptake, in line with earlier findings for [^{14}C]Met after radiotherapy in comparison with [^{18}F]FDG [3]. Amino acids are also used for non-protein synthesis and this might be at least partially responsible for the small reduction in [^{18}F]FMT uptake after chemotherapy, but actual causes remain to be clarified. The small reduction in the amino acid transport tracers after chemotherapy or radiotherapy might be a disadvantage when using them to evaluate the effect of treatment.

We employed [^{18}F]FMT as an amino acid transport tracer in this study since it was recently developed in our institute using [^{18}F]fluoromethyl triflate as a novel [^{18}F] fluoromethylating agent [30]. The preparation method is simpler and more efficient than that for [^{18}F]FET and [^{18}F] fluorine-α-methyl-tyrosine, and is suitable for routine production [23] of sufficient amounts for a number of patients and distribution to satellite PET centres.

Recent development of 3′-deoxy-3′-[^{18}F]fluoro-thymidine ([^{18}F]FLT) has made it possible to obtain DNA synthesis-specific proliferative images of tumours [31, 32], and [^{18}F]FLT has been expected to be applicable for the monitoring of antiproliferative therapy [33]. In our study, the reduction in the uptake of [^{3}H]Thd was similar to that in [^{18}F]FMT, suggesting that in this model [^{18}F]FLT would perform as well as [^{18}F]FMT. Because [^{18}F]FLT reflects DNA synthesis, it might be more specific for monitoring antiproliferative therapy than amino acid transport tracers. However, because amino acid transport tracers reflect the sum of protein synthesis and non-protein synthesis processes, they could yield some information beyond that on DNA synthesis. Further comparative studies on the particular advantages and disadvantages of [^{18}F]FMT and amino acid transport tracers will be needed.

To our knowledge, this study is the first experimental in vivo study to compare the utility of an amino acid transport tracer and a glucose analogue for monitoring of chemotherapeutic effects. Recently, Pauleit et al. reported [^{18}F]FET to be inferior to [^{18}F]FDG as a PET tracer for general tumour diagnosis [34], but our data suggest that amino acid transport tracers may be superior for assessing treatment efficacy.

In conclusion, [^{18}F]FMT showed as rapid and sensitive a response to chemotherapy as [^{3}H]Thd, with advantages over [^{14}C]DG. The data suggest that [^{18}F]FMT is a promising PET tracer for monitoring proliferative activity after cancer chemotherapy and that clinical trials should now be considered.

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Clinical Studies

Clinical outcome and risk factors for recurrence in borderline ovarian tumours

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We investigated the long-term prognosis of borderline ovarian tumours and determined risk factors for recurrence. One hundred and twenty-one borderline ovarian tumours treated between 1994 and 2003 at the participating institutions in the Tohoku Gynecologic Cancer Unit were retrospectively investigated for clinical stage, histopathological subtype, surgical technique, postoperative chemotherapy, the presence or absence of recurrence, and prognosis. The median follow-up period was 57 months (1–126 months). One hundred and nine cases (90.6%) were at clinical stage I. The histopathological subtypes consisted of 91 cases of mucinous tumour (75.2%), 27 cases of serous tumour (22.3%), and three cases of endometrioid tumour. Conservative surgery was used in 53 cases (43.8%), radical surgery in 68 cases (56.2%), a staging laparotomy in 43 cases (35.5%), and postoperative adjuvant therapy in 30 cases (24.8%). Recurrence was found in eight cases, but no tumour-related deaths were reported. Although no significant difference in disease-free survival rate was seen between different clinical stages, the difference in disease-free survival rate between serous and nonserous (mucinous and endometrioid) types was significant ($P<0.05$). The 10-year disease-free survival rate was 89.1% for the radical surgery group and 57.4% for the conservative surgery group – this difference was significant ($P<0.05$). In the conservative surgery group, cystectomy and serous tumour were independent risk factors for recurrence. Although recurrence was observed, the long-term prognosis of borderline ovarian tumour was favourable, without tumour-related deaths. Considering the favourable prognosis, conservative surgery can be chosen as far as the patient has a nonserous tumour and receive adnexectomy. However, in cases of serous type and/or receiving cystectomy special care should be given as relative risk rates of recurrence elevate by 2–4-folds.

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Taylor (1929) found that some epithelial ovarian tumours showed clinically intermediate behaviour between benign and malignant, and called them 'semimalignant'. The International Federation of Gynecology and Obstetrics (FIGO) has formally introduced this concept as 'carcinoma of low malignant potential' in 1971, and the World Health Organization (WHO) as 'borderline tumour' in 1973, when the histological diagnostic criteria was proposed. The concept of borderline ovarian tumours was histologically defined as a disease entity that had been proposed clinically, and the adequacy of this histological definition has been repeatedly verified clinically.

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With an accumulated experience and knowledge regarding the characteristics and management of borderline ovarian tumours, reclassification and redefinition have been attempted (Seidman and Kurman, 1996), and new prognostic factors have been proposed (de Nictolis *et al*, 1992; Gershenson *et al*, 1999). At present, many conflicting reports are causing confusion. As many of the patients are relatively young (Harris *et al*, 1992), preservation of fertility has been attempted with favourable results (Morice *et al*, 2001). However, there are also reports of recurrence or poor prognosis (Kaern *et al*, 1993; Gilks, 2003), and more precise prognostic factors are required. We believe that it is important to get a clear picture of the present status of borderline ovarian tumours, as it has been more than 30 years since the introduction of the concept of these tumours. Our retrospective multicentre study conducted an overall clinical analysis of borderline ovarian tumours. Our ultimate aim is to investigate the long-term prognosis of borderline ovarian tumours, and to determine the risk factors for recurrence.

MATERIALS AND METHODS

Information on 124 patients with a diagnosis of epithelial borderline ovarian tumour who were treated at the Tohoku Gynecologic Cancer Unit consisting of eight institutes from 1994 to 2003 was collected using each institutional databases.

Central pathological review was adopted in this study. One of the authors reviewed 124 cases diagnosed by the gynaecological pathologists of each institutes concerning histologic typing and grading of the primary lesion and 121 cases of those were determined as an epithelial borderline tumour. The histopathologic criteria embodied in a recent conference with published commentaries (Bell *et al*, 2004; Ronnett *et al*, 2004), some of which are included in the current WHO classification of ovarian tumours (Tavassoli and Devilee, 2003) were used for the diagnosis of borderline ovarian tumours in this study. These tumours were staged according to FIGO criteria (1987).

Radical surgery was defined as hysterectomy with bilateral salpingo-oophorectomy. Conservative surgery was defined as any surgery that preserved the uterus and one or both ovaries. Conservative surgical procedure was performed as cystectomy or adnexectomy. Peritoneal cytology was performed systematically in both surgical procedures. Surgical staging in the present study was defined as including peritoneal cytology, omentectomy, and pelvic lymphadenectomy with or without paraaortic exploration (lymphadenectomy or biopsy or palpation), and peritoneal biopsy in radical or conservative surgery on occasion. These surgical procedures were performed depending on the surgical teams who provided the treatment and whether borderline tumour was diagnosed during or after the surgical procedure.

With regard to adjuvant chemotherapy, all women with advanced disease, those with stage Ic, and those with a likely persistence of residual tumour after cystectomy received platinum-based treatment in the early years of this study. Thereafter, chemotherapy was usually confined to women with advanced disease.

Comparisons of categorical variables were conduced by two-tailed χ^2 and Fisher's exact tests where appropriate. Evaluation of independent factors predicting disease-specific recurrence was conducted by nominal logistic regression analysis. Survival estimates were calculated using the Kaplan–Meier product limit method. Comparison between survival curves was made using the generalised Wilcoxon's test. Statistical significance was set at $P<0.05$. The patients who lost to follow-up were censored from the survival data.

Detailed information regarding patient's characteristics, treatment method, recurrence, and prognosis of the disease was abstracted from the medical record. We did not request institutional review board approval for this study because of its retrospective nature.

RESULTS

The median age of patients was 43 years old (range 15–76 years). Fifty-one patients (42.1%) were below 40 years of age, and 29

Table 1 Clinical and histopathologic characteristics of patients with borderline tumours

	Alive with NED	Recurrence	Died of disease	Died of ICD	Lost	Total
Number of patients	94	8	0	2	17	121
Age (years)						
<20	7	0	0	0	0	7
20–30	14	1	0	0	4	19
31–40	19	4	0	0	2	25
41–50	14	1	0	0	2	17
51–60	17	1	0	1	5	24
> 60	23	1	0	1	4	29
Histological type						
Mucinous	73	4	0	2	12	91
Serous	19	4	0	0	4	27
Endometriod	2	0	0	0	1	3
Stage						
Ia	62	4	0	2	10	78
Ib	1	0	0	0	0	1
Ic	24	3	0	0	3	30
II	2	0	0	0	0	2
IIIa	0	1	0	0	1	2
IIIb	1	0	0	0	1	2
IIIc	3	0	0	0	1	4
IV	1	0	0	0	0	1
Unknown	0	0	0	0	1	1
Surgical procedure						
Radical	57	2	0	2	7	68
Conservative	37	6	0	0	10	53
Staging laparotomy						
Staged	36	3	0	1	3	43
Unstaged	58	5	0	1	14	78
Adjuvant chemotherapy						
Yes	22	4	0	1	3	30
No	72	4	0	1	14	91

NED = no evidence of disease; ICD = intercurrent disease.

patients (24.0%) were above 60 years of age (Table 1). The follow-up period varied from 1 to 126 months, with a median of 57 months. One hundred and nine patients (90.6%) had stage I disease, two had stage II disease, and nine had stage III and IV disease (Table 1). The dominant histopathological subtypes were mucinous (91 cases; 75.2%) and serous (27 cases; 22.3%) (Table 1). Only three tumours (2.5%) were of endometrioid type. Seventy-five (82.4%) and 16 of the 91 mucinous borderline tumours were intestinal and endocervical types, respectively. Radical treatment was performed in 68 (56.2%) patients, and 53 (43.8%) patients underwent conservative management (Table 1). Complete surgical staging was performed in 43 (35.5%) patients (Table 1). Adjuvant chemotherapy was given to 30 (24.8%) patients (Table 1). Seventeen patients were lost to follow-up, and two patients died of the other diseases (Table 1). Four patients had a mucinous tumour with pseudomyxoma peritonei and were excluded from the present study because presence of pseudomyxoma peritonei changes the scope of management and the category of pseudomyxoma peritonei is recognised as tumour that can simulate primary mucinous borderline ovarian tumour (Ronnett *et al*, 2004).

Among 102 patients who were finally evaluated for clinical outcome and prognostic factors, eight had tumour recurrence but none of them died of the disease (Table 1). The median time to recurrence was 46 ± 33 months (range 14–107 months). The 5- and 10-year disease-free survival rates were 91.7 and 69.2% for stage I diseases, respectively, and the 5- and 7-year disease-free survival rates were 100 and 66.7% for stage II–IV diseases, respectively (Figure 1A). The 10-year disease-free survival rate was 91.5 and 36.0% for mucinous and serous tumours, respectively (Figure 1B). Although no significant differences in disease-free survival rate were seen between different clinical stages, the difference between serous and nonserous (mucinous and endometrioid) types was significant. On the other hand, the 10-year disease-free survival rate was 89.1% for the radical surgery group and 57.4% for the conservative surgery group (Figure 1C). This difference was significant ($P<0.05$). In univariate analysis, serous type and conservative surgery were found to be important variables affecting recurrence of disease (Table 2). Frequency of recurrence was not influenced by clinical stage, staging laparotomy, and postoperative adjuvant chemotherapy (Table 2). Multivariate analysis showed that only conservative surgery had independent prognostic value regarding recurrence of disease (Hazard ratio 2.2, 95% confidence interval, 0.02–0.52) (Table 2). Subsequently, risk factors for recurrence were evaluated among 43 patients who underwent conservative surgery (Table 3). Of these patients, six had tumour recurrence (Table 3). Three of eight patients who had cystectomy and three of 35 patients who had adnexectomy experienced tumour recurrence (Table 3, $P<0.03$). Recurrence occurred more frequently in patients with serous tumour than with nonserous tumour (Table 3). No correlation was found between recurrence and the factors such as clinical stage, staging laparotomy, or postoperative adjuvant chemotherapy among conservative surgery group (Table 3). Multivariate analysis confirmed cystectomy and serous type as an independent risk factor for recurrence of disease among the patients who underwent conservative surgery (Table 3). Table 4 shows estimated relative risk of having recurrence of disease for different combination of procedure of conservative surgery and histopathological subtype. For example, the relative risk for a patient receiving cystectomy for her serous tumour is 4.33 times greater than the risk for a patient receiving adnectomy for her nonserous tumour.

The clinical and pathological features of the eight patients who developed recurrence were demonstrated in Table 5. None of these eight patients died of progression of their disease. Three of the four serous tumours with recurrence were a noninvasive peritoneal implant, one of which was diagnosed as a serous adenocarcinoma at recurrence. The case developed adenocarcinoma in contralateral ovary 107 months after cystectomy. All mucinous tumours with recurrence were of intestinal subtype. All patients with recurrence who were initially treated conservatively are free of disease after secondary surgical treatment.

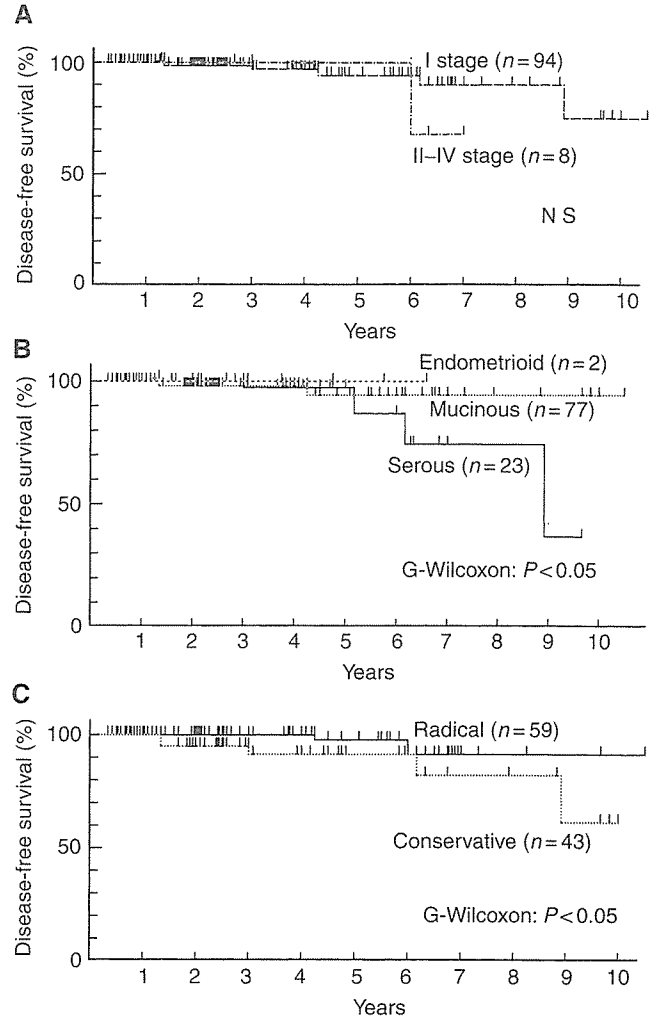

Figure 1 (**A**) Clinical stages and disease-free survival in patients with borderline ovarian tumour. There is no significant difference between two curves. (**B**) Histological type and disease-free survival in patients with borderline ovarian tumour. There is significant difference in disease-free survival between serous and nonserous (mucinous and endometrioid) type ($P<0.05$). (**C**) Surgical procedure and disease-free survival in patients with borderline ovarian tumour. There is significant difference between two curves ($P<0.05$).

DISCUSSION

It has been shown that the 5-year survival rate was 95–97% for stage I and 65–87% for stages II and III (Trope *et al*, 2000; Trimble *et al*, 2002; Sherman *et al*, 2004) suggesting that the prognosis for borderline ovarian tumours depends on extraovarian extension of the tumour. In addition, prognostic factors included clinical stage, histopathological subtype, and residual tumour, but the surgical method was not regarded as a prognostic factor (Trope *et al*, 2000; Gilks, 2003). The results of the present study, however, showed neither the stage nor the histopathological subtype of the disease was related with long-term prognosis, but showed that disease-free survival rates were significantly lower in cases managed by conservative surgery (Figure 1).

Table 2 Risk factors for recurrence in borderline tumours

Factors	Recurrence ($n=8$)	No recurrence ($n=94$)	Univariate *P*	Multivariate *P*
Mean age (years)	42.2±13.7	43.5±16.2		
Histology, n (%)				
Serous	4 (50)	19 (20.2)		
Nonserous	4 (50)	75 (79.8)	0.053	0.09
Surgical procedure, n (%)				
Radical	2 (33.3)	57 (60.6)		
Conservative	6 (66.7)	37 (39.4)	0.05	0.031
Staging laparotomy, n (%)				
Staged	3 (37.5)	36 (38.3)		
Unstaged	5 (62.5)	58 (61.7)	0.96	0.58
Stage, n (%)				
I	7 (87.5)	87 (92.6)		
II–IV	1 (12.5)	7 (7.4)	0.61	0.79
Adjuvant chemotherapy, n (%)				
Yes	4 (50)	22 (23.4)		
No	4 (50)	72 (76.6)	0.098	0.33

Table 3 Risk factors for recurrence in the patients who underwent conservative surgery for borderline tumours

Factors	Recurrence ($n=6$)	No recurrence ($n=37$)	Univariate *P*	Multivariate *P*
Surgical procedure, n (%)				
Cystectomy	3 (50)	5 (13.5)		
Adnexectomy	3 (50)	32 (86.5)	0.03	0.047
Staging laparotomy, n (%)				
Staged	1 (16.7)	3 (8.1)		
Unstaged	5 (83.3)	34 (91.9)	0.51	0.137
Adjuvant chemotherapy, n (%)				
Yes	3 (50)	7 (18.9)		
No	3 (50)	30 (81.1)	0.095	0.593
Stage, n (%)				
Ia	3 (50)	23 (62.2)		
Ic	3 (50)	14 (37.8)	0.57	0.198
Histology, n (%)				
Serous	3 (50)	6 (16.2)		
Non-serous	3 (50)	31 (83.8)	0.059	0.041

Table 4 Relative risk of recurrence in borderline tumours

	Histological type	
Conservative surgery	**Nonserous**	**Serous**
Adnexectomy	1	2.11
Cystectomy	2.05	4.33

In our study, surgical procedure was found to be an independent risk factor for recurrence and the risk could be reduced by radical surgery (Table 2). Because borderline tumours are seen more frequently in younger females than definitive carcinomas (Harris *et al*, 1992), whether conservative surgery is appropriate for

Table 5 Eight patients with borderline tumour who developed recurrence

Age	Histological type	Stage	Initial surgery	Staging procedure	Adjuvant chemotherapy	Time to recurrence	Site of recurrence	Treatment after recurrence	Histology of recurrence site	Status
33	SBT, noninvasive	Ia	Conservative (adnexectomy)	(−)	(+)	74	Intrapelvis	Surgery alone	SBT	NED
35	SBT, noninvasive	Ia	Conservative (adnexectomy)	(−)	(−)	36	Contralateral ovary	Surgery alone	SBT	NED
36	SBT, noninvasive	Ia	Conservative (cystectomy)	(−)	(+)	107	Contralateral ovary	Surgery+chemotherapy	Serous adenocarcinoma	NED
46	SBT, invasive	IIIa	Radical	(+)	(+)	62	Perihepatic	Chemotherapy alone	Unknown	AWD
28	MBT, intestinal	Ic	Conservative (cystectomy)	(−)	(−)	14	Ipsilateral ovary	Surgery alone	MBT, intestinal	NED
35	MBT, intestinal	Ic	Conservative (cystectomy)	(+)	(+)	16	Intrapelvis	Surgery alone	MBT, intestinal	NED
58	MBT, intestinal	Ic	Conservative (adnexectomy)	(−)	(−)	20	Intrapelvis	Surgery alone	MBT, intestinal	NED
67	MBT, intestinal	Ia	Radical	(+)	(−)	35	Lung	None	Unknown	AWD

SBT = serous borderline tumour; MBT = mucinous borderline tumour; NED = no evidence of disease; AWD = alive with disease.

borderline ovarian tumours is an important matter to be resolved. Zanetta *et al* (2001) reported that only three of 119 stage I (2.5%) cases that underwent radical surgery recurred, whereas 20 out of 164 stage I (12.1%) cases that underwent conservative surgery recurred, with one case resulted in death from the disease. Morice *et al* (2001) demonstrated that the majority of recurrent cases, including stages II and III, were cured completely by subsequent surgery, and few cases resulted in death. More over, Donnez *et al* (2003) reported that although recurrence was commoner in cases treated by conservative surgery (3 out of 16, 18.7%) than by radical surgery (0 out of 59, 0%), subsequent treatment resulted in no tumour-related deaths, and 63.6% of conservative surgery cases subsequently became pregnant, suggesting that conservative surgery can be an option for management of borderline malignant ovarian tumours in young subjects who need to reserve fertility. However, it is also reported that all of deaths as a result from recurrence were seen in cases treated by conservative surgery (Morris *et al*, 2000; Zanetta *et al*, 2001). Therefore, it is of quite importance to investigate underlying risk factors for recurrence after conservative surgery. As shown in Table 3, we found that cystectomy and serous tumours were independent risk factors for recurrence in patients who received conservative surgery. Previous reports have shown that recurrence after cystectomy did not necessarily occur ipsilaterally (Morris *et al*, 2000, 2001; Zanetta *et al*, 2001). So it seems that the residual tumour during cystectomy is solely responsible for recurrence. Then, it may be rational for young women who wish pregnancy to select cystectomy as an option if the surgical margin is free of tumour. However, results by Morice *et al* (2001) did not support this as they found that the recurrence rate was high after cystectomy compared with adnexectomy. Morris *et al* (2000) also demonstrated that recurrence was higher in cases treated by cystectomy rather than by adnexectomy. The present study confirmed this and further demonstrated for the first time that a difference in pathohistology affects the recurrence rate. As shown in Table 3, it was revealed that serous tumour is a significant risk factor for recurrence in cases managed by conservative surgery. Morris *et al* (2000) also showed similar tendency, but they regrettably missed statistical analysis. As shown in Table 4, the present study clearly demonstrated that the risk of recurrence when serous tumours were treated by cystectomy was approximately four times higher than for adnexectomy of nonserous tumours.

As a prognostic factor for borderline ovarian serous tumours, the concept of peritoneal implant is attracting attention (Bell *et al*, 1988). When estimating the prognosis of borderline ovarian serous tumours, peritoneal lesions should be explored and biopsied at the time of the surgery – in other words, accurate surgical staging is required. Clinical stage is one of the most important prognostic factors in borderline ovarian tumours (Kliman *et al*, 1986), and an accurate surgical staging is indispensable for follow-up after conservative surgery, as well as selecting postoperative therapy. Winter *et al* (2002) compared 48 cases that underwent complete surgical staging, and 45 cases without surgical staging – a higher stage was found in 17% (8 out of 48) of those assessed by surgical staging, but there was no difference in recurrence and survival rates between the groups. Camatte *et al* (2002) found metastasis to lymph nodes in 19% (8 out of 42) of cases. All cases with metastases were seen with borderline ovarian serous tumours associated with peritoneal dissemination, but no cases resulted in death – there was no difference in prognosis when compared with cases without metastases. The presence or absence of a peritoneal lesion is an important predictive factor of recurrence as well as an important prognostic factor, and we do not deny the importance of surgical examination of the abdominal cavity where possible. However, many reports have indicated that the presence or absence of lymph node metastasis is not related to the prognosis for borderline ovarian tumours (Camatte *et al*, 2002; Winter *et al*, 2002), and it is still debatable whether or not to perform a biopsy or dissection of the lymph nodes. As shown in Table 3, the present study could not show a significant relevance to risk of recurrence. The limitation of the present study is that surgical staging was not considered beforehand in all cases so that our data may be biased in this respect. Further studies using a prospective design with emphasis on surgical staging are required to investigate the risk of recurrence in borderline ovarian serous tumours after conservative surgery. Therefore, it is important that conservative surgery should only be performed in cases that truly require conservative surgery, after giving a full explanation of the risk of recurrence.

Barakat *et al* (1995) reported that cisplatin-based chemotherapy induced complete remission in six of 23 (26%) advanced cases with macroscopic diseases, and in 17 of 25 (68%) cases with microscopic disease, and proposed that adjuvant chemotherapy could be considered as a therapeutic option although a life-extension effect of chemotherapy was not clear. In the present study, the regimen or frequency of chemotherapy used was not uniform, and differed among institutions, and no relationship was found between the presence or absence of postoperative adjuvant chemotherapy and recurrence (Table 2). Kaern *et al* (1993) showed that adjuvant chemotherapy did not improve neither recurrence free survival nor overall survival rate in 364 cases without residual tumour. Morice *et al* (2001) demonstrated that postoperative chemotherapy did not improve the survival rate in 80 cases of advanced borderline ovarian serous tumour in stages II and III with extraovarian extension, and that deaths were more closely related to the treatment than to the tumour. Thus, the efficacy of chemotherapy for borderline ovarian tumours is not yet established.

In conclusion, although recurrence was detected in eight out of 102 cases with borderline ovarian tumour that were available for follow-up, no tumour-related deaths were found, and there was a favourable long-term prognosis. Although the relative risk of recurrence is high, conservative surgery appears to be worth trying to preserve fertility, considering the favourable prognosis. When considering conservative surgery, special care should be given when cystectomy is chosen as a surgical procedure or the histological subtype is borderline serous ovarian tumour. Consensus has not been reached on such issues as to the significance of surgical staging, the indication for postoperative adjuvant chemotherapy, or the indications for conservative surgery. To reinforce the present study results, we expect that a large scaled prospective clinical study involving many institutions will be designed to obtain more evidence.

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Clear cell carcinoma of the ovary: a retrospective multicentre experience of 254 patients with complete surgical staging

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A retrospective analysis was performed to evaluate the clinical characteristics and prognostic factors in the patients with clear cell carcinoma (CCC) of the ovary. After central pathological review and scanning of the medical records of nine Japanese institutions between 1992 and 2003, a total of 254 patients with CCC of the ovary were enrolled in the present study. Mean age was 52.4 years (range 23–73 years). Tumours were 13% (33/254) stage Ia, 36% (92/254) stage Ic, 13% (33/254) stage II, 30% (80/254) stage III, and 6% (16/254) stage IV. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively. Retroperitoneal lymph node metastasis was observed in 9% in pT1a tumours, 7% in pT1c tumours, 13% in pT2 tumours, and 58% in pT3 tumours, respectively. There was no survival benefit according to chemotherapeutic differences in the patients who received complete surgical staging procedures and conventional chemotherapy. Peritoneal cytological status was an independent prognostic factor in stage Ic patients ($P = 0.03$) and only residual tumour diameter was an independent prognostic factor in stage III, IV patients ($P = 0.02$). Our results suggest that cytoreductive surgery resulting in no residual tumour only could improve the prognosis of advanced CCC patients.
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Cancer of the ovary has the worst prognosis of all gynaecological malignancies in the United States (Edwards *et al*, 2005) and Europe (Bray *et al*, 2005). Survival rate of patients with ovarian cancer has dramatically improved after introduction of platinum-based chemotherapy, but there still exist a large number of patients showing no response to the treatments. Although response to anticancer drugs is not easy to predict, *in vitro* studies suggested that acquired resistance to cisplatin has been associated with increased levels of glutathione and glutathione-S-transferase activity, increased metallothionein and decreased accumulation of cisplatin (Kikuchi *et al*, 1998). Histological subtypes such as clear cell carcinoma (CCC) and mucinous adenocarcinoma had been suggested as one of the most reliable criteria predicting the ineffectiveness of chemotherapy.

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Clear cell carcinoma (CCC) was initially termed as mesonerhroid in 1939 (Schiller, 1939), and since 1973 it was strictly defined by World Health Organization as lesions characterised by clear cells growing in solid/tubular or glandular patterns as well as hobnail cells (Serov *et al*, 1973). Since then, many literatures have identified the distinctive behaviour of the tumors as compared with other histological subtypes of ovarian neoplasms. The most distinctive difference is that patients with CCC of the ovary have lower response rate to anticancer drugs. To our knowledge, only a few clinical studies have evaluated the response rates for CCC patients with measurable disease. The response rate of chemotherapy for CCC was 11.1% with platinum-based regimens (Sugiyama *et al*, 2000) and 22–56% with paclitaxel plus carboplatin. (Enomoto *et al*, 2003; Ho *et al*, 2004).

Another factor that might contribute to prognosis of ovarian cancer is the degree of cytoreductive surgery including lymphadenectomy. Complete surgical staging including para-aortic lymphadenectomy might influence the prognosis in early-stage CCC cases (Ho *et al*, 2003). Furthermore, the patients with pure-type CCC had worse overall survival than those with mixed-type CCC (Ho *et al*, 2004).

To evaluate the clinical characteristics of the patients with CCC of the ovary and to determine the impact of surgery and

chemotherapy on prognosis of those patients, we conducted a retrospective study over 11-year period of a sample of 254 patients diagnosed with pure-type CCC in the departments of nine Japanese institutions.

MATERIALS AND METHODS

Patients and tumours

Between 1992 and 2002, 254 patients with CCC of the ovary were identified by scanning the medical records of the collaborating institutions and central pathological review. Patients received initial treatment and follow-up at nine institutions belonging to Japan Clear Cell Carcinoma Study Group; National Defence Medical College Hospital, Tohoku University Hospital, Aichi Cancer Center Hospital, Osaka Medical College Hospital, Osaka City General Hospital, Jichi Medical College Hospital, Tottori University Hospital, Kobe National Hospital, Iwate Medical College Hospital.

Initially, 337 patients were accrued from medical records of each institution. All pathological specimens from primary surgery were reviewed under central pathological review by two independent pathologists with no knowledge of patients' clinical data. Tumours were diagnosed as CCC if typical clear or hobnail cells growing in a papillary, solid, or tubulocystic pattern appeared in >90% of all pathological specimens. After pathological review, three cases were excluded; two diagnosed as mixed epithelial ovarian cancers and the other diagnosed as CCC derived from mature cystic teratoma, and 334 cases were identified as the patients with pure-type CCC of ovary. In those patients, 80 patients were excluded owing to insufficient surgery lacking complete surgical staging procedures: 13 cases in pT1a tumours, 51 cases in pT1c tumours, 16 cases in pT2 tumours, respectively. The rest 254 patients were enrolled on the present study. Patients of FIGO stage Ic were classified into three subtypes according to pathological characteristics; Ic (capsule ruptured) for the patients with ruptured capsule at laparotomy, Ic (ovarian surface) for those with tumour on ovarian surface, and Ic (ascites/malignant washing) for those with positive malignant cells in the ascites or positive peritoneal washing.

All 254 patients underwent complete surgical staging procedures including hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing, omentectomy, pelvic lymphadenectomy and paraaortic lymphadenenctomy. Staging was based on the FIGO classification. The resected lymph node counts were not considered for the completion of the lymphadenectomy. A pN1 case was determined as having one or more lymph node metastasis in pelvic or paraaortic lymph nodes.

Chemotherapy

Two hundred and forty-two (95.3%) patients received postoperative chemotherapy after initial surgery. Second look operation or second reductive surgery was done by surgeon's preference. Combination therapy of cyclophosphamide and doxorubicin and cisplatin (CAP) was as follows: one cycle consisted of a drip infusion of 50–75 mg m^{-2} cisplatin for 3 h accompanied by an i.v. injection of 50 mg m^{-2} doxorubicin and 500 mg m^{-2} cyclophosphamide and six cycles were given every 4 weeks. Paclitaxel and platinum regimen consisted of an infusion of 175–180 mg m^{-2} of paclitaxel and 50–75 mg m^{-2} of cisplatin or carboplatin (AUC = 5–6). Other regimens included the combination chemotherapy irinotecan hydrochloride and cisplatin (40 cases) and irinotecan hydrochloride and mitomycin C (20 cases) and irinotecan hydrochloride and etoposide (3 cases). One cycle of irinotecan hydrochloride and platinum regimen consisted of a drip infusion of 50–60 mg m^{-2} of cisplatin on day 1 and 50–60 mg m^{-2} of CPT-11 on day 1, 8, 15 and 1 week off and it was repeated every 4 weeks.

Response was evaluated with CT or MR images for patients with measurable disease. A complete response (CR) was defined as the complete disappearance of all detectable disease for at least 4 weeks. A partial response (PR) was defined as a >50% decrease in tumour size for at least 4 weeks. Stable disease (SD) was defined as the absence of any significant change in measurable lesions for at least 4 weeks. Progressive disease (PD) was defined as the appearance of a new lesion or a >25% increase in tumour size. Serum levels of tumour markers including CA125 were not used for response evaluation of chemotherapy in the present study.

The time to progression was defined as the interval from the date of primary surgery until the date of recurrence or tumour progression (PD). Survival duration was determined as the time from the date of primary surgery until death or the date of last follow-up contact.

Statistical methods

Kaplan–Meier method was used for calculation of patient survival distribution. The significance of the survival distribution in each group was tested by a generalized Wilcoxon test and the log-rank test. The χ^2-test and Student's t-test for unpaired data were used for statistical analysis. A P-value of <0.05 was considered statistically significant. The Stat View software ver.5.0 (SAS Institution Inc., Cary, NC, USA) was used to analyse the data.

RESULTS

Patients and tumours

The characteristics of the study population are summarized in Table 1. Mean age was 52.4 years (range 23–73 years). Tumours were 13% (33/254) stage Ia, 36% (92/254) stage Ic, 13% (33/254) stage II, 31% (80/254) stage III, and 6% (16/254) stage IV, respectively. There is no case with stage Ib tumours. Among 92 cases of stage Ic, there were 45 cases (49%) of Ic (capsule

Table 1 Characteristics of the patients

Characteristics	No. of patients (%)
All cases	254
Age (years)	
<55	147 (57.9)
>55	107 (42.1)
FIGO Stage	
Ia	33 (13.0)
Ic (ovarian surface)	3 (1.2)
Ic (capsule ruptured)	45 (17.7)
Ic (ascites/malignant washing)	44 (17.3)
II	33 (13.0)
IIIa,b	5 (2.0)
IIIc	75 (29.5)
IV	16 (6.3)
Residual tumour diameter	
0 cm	176 (69.3)
<1 cm	18 (7.1)
>1 cm	60 (23.6)
Postoperative chemotherapy	
CAP[a]	76 (29.9)
Paclitaxel+platinum	103 (40.6)
Others	63 (24.8)
None	12 (4.7)

[a]CAP, cyclophosphamide+doxorubicin+cisplatin.

ruptured), 3 cases (3%) of Ic (ovarian surface) and 44 cases (48%) of Ic (ascites/malignant washing), respectively. In 75 stage IIIc tumours, 15 cases (20%) were upstaged to stage IIIc because of retroperitoneal lymph node metastasis and 20 patients (27%) had both retroperitoneal lymph node metastasis and intra-peritoneal disease. Residual tumour diameter after primary debulking surgery was 0 cm in 176 cases (69%), less than 1 cm in 18 cases (7%), and more than 1 cm in 60 cases (24%), respectively.

Postoperative chemotherapy was offered for all patients, and 242 patients (95%) received anticancer drugs. Eight patients in stage Ia and four patients with stage Ic (capsule ruptured) refused postoperative chemotherapy.

Precise lymph node status according to pT distribution was documented in Table 2. Lymph node metastasis was documented in 3 of 36 patients (9%) in pT1a tumours, 7.1% in pT1c tumours, 13% in pT2, and 58% in pT3 tumours, respectively. Retroperitoneal lymph node metastasis in pT3 tumours was observed significantly more frequent than in pT1, 2 tumours (58.0 *vs* 8.7%, $P<0.001$, χ^2-test).

Response of chemotherapy

Response judged with CT or MRI images was assessable in 73 cases (29%) in 242 patients who received postoperative chemotherapy. Only 5 of 30 cases (16%) responded to CAP regimen. Progressive disease was documented in 23 patients (77%) and SD was observed in 2 patients (7%). In 28 patients treated with paclitaxel and platinum, response was observed in nine cases (32%) including one case with CR. In the patients treated with other regimens, response was observed in 3 of 10 patients (30%) treated with irinotecan hydrochloride and cisplatin. There is no responder in seven assessable patients who received combination with irinotecan hydrochloride and mitomycin C.

The median duration of progression-free survival for the patients with measurable disease was 4 months (range, 1–20 months) in CAP regimen, 5 months (range, 1–21 months) in paclitaxel and platinum, and 3 months (range, 2–20 months) in irinotecan hydrochloride and cisplatin, respectively.

Table 2 Rates of lymph node metastasis according to pT status

pT status	pN1	pN0	Rate of Lymph Node metastasis (%)
pT1a ($n=36$)	3	33	9.1
pT1c ($n=99$)	7	92	7.1
pT2 ($n=38$)	5	33	13.1
pT3 ($n=81$)	47	34	58.0
Total ($n=254$)	62	192	24.4

Clinical course

Average follow-up for all CCC patients in the present study is 47.4 months. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively (Figure 1). Although there is no statistically significant difference in progression-free survival between patients with stage Ic (capsule ruptured) and those with stage Ia ($P=0.11$), progression-free survival of the patients with stage Ic (ascites/malignant washing) and Ic (ovarian surface) was significantly worse than that of stage Ic (capsule ruptured) ($P=0.04$)(Figure 2). Multiple regression survival analysis for stage Ic patients with CCC revealed that positive peritoneal cytology was the only independent prognostic factor ($P=0.03$; Relative risk, 3.40; 95% CI, 1.14–10.18). Cumulative progression-free survival of pT1M0 patients with positive node was significantly lower than those with negative node ($P<0.01$). Five-year progression-free survival was 84% in pT1N0 patients and 56% in pT1N1 patients, respectively.

Progression-free survival curves of stage III, IV patients according to the residual tumour diameter were shown in Figure 3. Median progression-free survival duration was 39 months in the

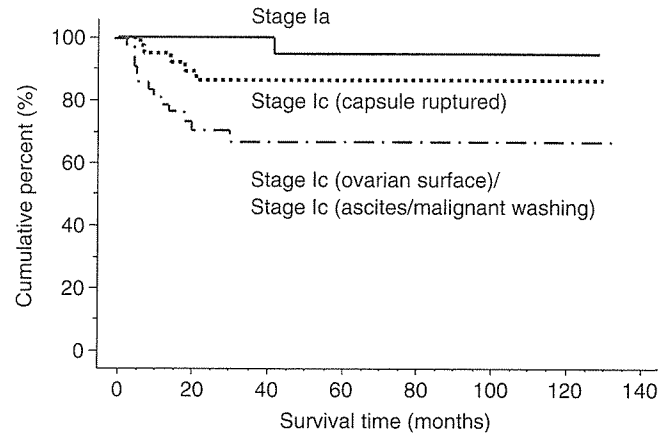

Figure 2 Progression-free survival of patients with FIGO stage I patients. There is no significant difference between patients with stage Ic (capsule ruptured) and those with stage Ia ($P=0.11$). Survival of the patients with stage Ic (ascites/malignant washing) and Ic (ovarian surface) was significantly worse than that of stage Ic (capsule ruptured) ($P=0.04$).

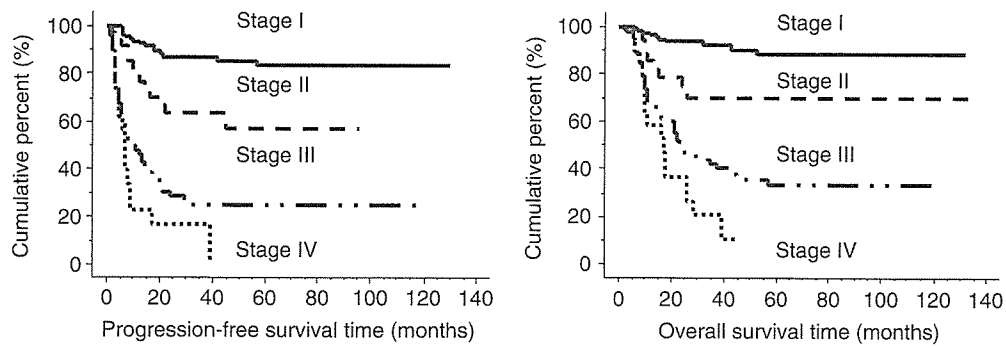

Figure 1 Progression-free survival and overall survival of patients depending on their FIGO stage. Five-year progression-free survival and overall survival was 84 and 88% in stage I, 57 and 70% in stage II, 25 and 33% in stage III and 0 and 0% in stage IV, respectively. *P*-values in progression-free survival were as follows: Stage I *vs* stage II, $P<0.01$; stage II *vs* stage III, $P<0.01$; stage III *vs* stage IV, $P=0.35$. *P*-values in overall survival were as follows: Stage I *vs* stage II, $P<0.01$; stage II *vs* stage III, $P<0.01$; stage III *vs* stage IV, $P=0.17$.

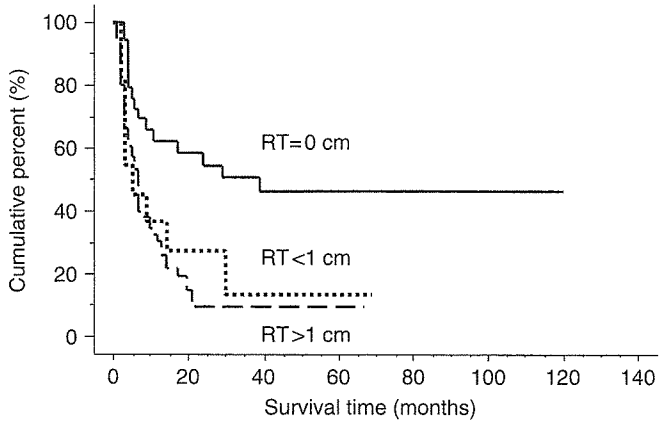

Figure 3 Progression-free survival of stage III, IV patients according to the residual tumour (RT) diameter. There is no significant prognostic difference between the patients with the tumour diameter less than 1 cm and those with the tumour diameter more than 1 cm ($P=0.40$). The patients with no residual tumour had significantly better progression-free survival than those with the tumour less than 1 cm ($P=0.04$) or those with tumour diameter more than 1 cm ($P<0.01$), respectively. Median progression-free survival duration was 39 months in the patients with no residual tumour, 7 months in those with the tumour diameter less than 1 cm, and 5 months in those with residual tumour diameter more than 1 cm, respectively.

Table 3 Multiple regression survival analysis for stage III, IV patients with CCC

Variables	Hazard ratio	95% confidence interval	P
Age (years)			0.96
<54	1		
>55	0.99	0.60; 1.61	
PS			0.67
0	1		
1,2	1.06	0.79; 1.43	
FIGO stage			0.22
III	1		
IV	1.47	0.80; 2.70	
Residual tumour			0.02
None	1		
<1 cm	2.23	0.89; 5.54	
>1 cm	3.17	1.68; 6.00	
Chemotherapy			0.24
CAP[a]	1		
Paclitaxel+platinum	0.56	0.48; 1.88	
Others	0.95	0.32; 1.22	

[a]CAP, cyclophosphamide+doxorubicin+cisplatin.

patients with no residual tumour, 7 months in those with the tumor diameter less than 1 cm, and 5 months in those with residual tumour diameter more than 1 cm, respectively. There is no significant prognostic difference between the patients with the tumour diameter less than 1 cm and those with the tumour diameter more than 1 cm ($P=0.40$). The patients with no residual tumour had significantly better progression-free survival than those with the tumour less than 1 cm ($P=0.04$) or those with tumour diameter more than 1 cm ($P<0.01$), respectively.

Multiple regression analysis in stage III and IV patients revealed that chemotherapeutic regimen was not an independent prognostic factor ($P=0.24$) and only residual tumour diameter was an independent prognostic factor in stage III and IV patients ($P=0.02$) (Table 3).

DISCUSSION

The present study and previous studies support that CCC of the ovary tended to present at earlier stages. Proportion of stage I/II tumours ranged from 59 to 71% (Yoonessi *et al*, 1984; Crozier *et al*, 1989; Jenison *et al*, 1989; Kennedy *et al*, 1989; O'Brien *et al*, 1993; Behbakht *et al*, 1998; Sugiyama *et al*, 2000). One of the reasons for the early detection was explained by the slow growing tumour behaviour (Itamochi *et al*, 2002a) and frequent presentation of the tumours as relatively large pelvic masses (Kennedy *et al*, 1989; Behbakht *et al*, 1998). In the present study, the status of peritoneal cytology was identified as an independent prognostic factor in FIGO stage Ic patients. Although tumour progression was observed in 5 (11%) of 45 stage Ic (capsule ruptured) tumours and one (3%) of 33 stage Ia tumours, there is no significant survival difference between two groups. Recent report analysing prognosis of early-staged ovarian cancer including only 25 CCC cases (26.6%) in 94 carcinomas showed no statistical significant difference between stages Ic preoperative *vs* intraoperative rupture (Leitao *et al*, 2004). Another report including higher ratio of CCC patients identified that stage Ic (capsule ruptured) patients showed significantly poorer survival than stage Ia patients (Mizuno *et al*, 2003). The present study implied the importance to remove the tumour mass without intraoperative rupture, especially in CCC patients.

Even in stage I ovarian cancer including all histological subtypes, the incidence of positive lymph nodes was not low, ranging from 5.1 to 20% (Sakuragi *et al*, 2000; Cass *et al*, 2001; Morice *et al*, 2003). It was reported that serous tumour had a higher incidence of lymph node involvement than non-serous tumors (Takeshima *et al*, 2005). Although the true incidence of lymph node metastasis in CCC tumour had not been clear, the present study revealed the frequency of metastasis in a large number of the CCC patients. Lymph node metastasis was observed in 3 of 36 patients (9.1%) in pT1a tumours, 7.1% in pT1c tumours, 10.8% in pT2 tumours, respectively. Fifteen (8.7%) of 173 patients who had pT1 or pT2 tumors were upstaged as stage IIIc tumours based on lymph node status. In general, prognostic significance of retroperitoneal lymph node metastasis in early-staged ovarian cancer patients was controversial. Survival rates with node-positive disease were significantly lower in clinical stage I and II disease (Kanazawa *et al*, 1999; Sakuragi *et al*, 2000; Negishi *et al*, 2004). In contrast, another report showed that the prognoses for clinical stage I/II patients with or without lymph node metastasis were similar (Onda *et al*, 1998). In pT1 CCC patients of the present study, lymph node status was identified as a strong prognostic factor and it is essential to accurately evaluate the lymph node status through complete surgical staging procedures. The study, called Adjuvant ChemoTherapy in Ovarian Neolasm (ACTION), revealed that no benefit of adjuvant chemotherapy was observed in early-stage ovarian cancer with optimal surgical procedures (Trimbos *et al*, 2003). In the present study, 12 patients with stage Ia or stage Ic (capsule ruptured) refused to receive chemotherapy, but there was no evidence of recurrence in median follow-up period of 44 months (range: 6–63 months), which might support the results of ACTION study.

Previous Japanese report have shown that the chemotherapeutic effect was assessable in only 27 patients (26.7%) in 101 CCC cases, in contrast it was assessable in 47% of serous adenocarcinoma (Sugiyama *et al*, 2000). In our series of CCC patients, patients with residual tumour diameter more than 1 cm were documented in only 60 (18%) of 254 cases, and the chemotherapeutic effect was assessable in only in 73 cases (29%) in 242 patients who received adjuvant chemotherapy. As the residual tumour after debulking surgery often lacked measurable tumour diameter to evaluate the

effects of adjuvant chemotherapy in CCC patients, it has been quite difficult to select superior regimen.

There have been only a few reports to document the response of anticancer agents for CCC patients, but each of them included relatively small number of cases. The present study confirmed that CAP regimen showed a low response rate and quite a high incidence of PD in CCC patients as described previously (Sugiyama *et al*, 2000). The combination chemotherapy consisting of paclitaxel and platinum has been established as standard therapy for ovarian cancer. One report of paclitaxel and platinum regimen for CCC patients revealed that the response was observed in two of nine cases (22%) (Enomoto *et al*, 2003), and the other report of paclitaxel plus platinum chemotherapy showed the response was observed in 9 of 15 cases (56%) (Ho *et al*, 2004). These two studies including the present study suggested that paclitaxel plus platinum regimen had higher response rate compared to platinum-based chemotherapy. One report showed survival benefit of conventional chemotherapy with paclitaxel and platinum after complete surgery in CCC patients (Ho *et al*, 2003). However, the results from our series of CCC patients showed that there was no survival benefit with chemotherapy with paclitaxel and platinum compared with CAP regimen in both early and advanced cases. Irinotecan hydrochloride was preliminary introduced for CCC patients in clinical settings (Shimizu *et al*, 1998; Adachi *et al*, 1999; Kita *et al*, 2000), but there is no large clinical trial for the treatment of CCC patients of the ovary. Further studies are needed to establish the candidate regimen for CCC of the ovary.

Recent studies have suggested that CCC tumour showed a distinctive molecular behaviour from other histological subtypes. *In vitro* study suggested that paclitaxel and irinotecan hydrochloride were the candidates for anti-neoplastic agents for CCC (Itamochi *et al*, 2002b), but the present study has failed to prove the survival benefit of these two drugs in CCC patients. Another strategy for CCC tumours might be the additive use of molecular targeting agents. It was reported that hepatocyte nuclear factor-1 beta (HNF-1 β) was a CCC-specific marker and had anti-apoptotic effects in CCC cell lines (Tsuchiya *et al*, 2003). Another candidate marker could be ABCF2, which belongs to the ATP-binding cassette gene superfamily and is highly expressed in CCC and non-responders for chemotherapy (Tsuda *et al*, 2005). Suppression of CCC-specific molecular markers such as HNF-1 β or ABF2 may be another strategy for the treatment of CCC of the ovary. The present study clarified the significant prognostic importance of positive peritoneal cytology in early-stage CCC disease, and no macroscopic residual tumour in advanced CCC tumours, respectively. However, there was a little impact of chemotherapeutic effects on both early and advanced diseases. Although further studies are needed to identify effective agents in both anti-neoplastic agents and molecular targeting agents, our study provides the fundamental characteristics of CCC of the ovary.

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Paclitaxel–platinum combination chemotherapy for advanced or recurrent ovarian clear cell adenocarcinoma: a multicenter trial

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Abstract. Utsunomiya H, Akahira J, Tanno S, Moriya T, Toyoshima M, Niikura H, Ito K, Morimura Y, Watanabe Y, Yaegashi N. Paclitaxel–platinum combination chemotherapy for advanced or recurrent ovarian clear cell adenocarcinoma: a multicenter trial. *Int J Gynecol Cancer* 2006;**16**:52–56.

The therapeutic effect of a combination of paclitaxel (PTX) and platinum (PLT) in ovarian clear cell adenocarcinoma (CC) patients with measurable disease has yet to be elucidated. In this study, we used retrospective review to evaluate the results of treatment with a combination of PTX and PLT in CC patients with measurable disease. A total of 28 patients with measurable residual CC (15 cases with primary disease, 13 cases with recurrent disease) treated with combination PTX-PLT chemotherapy was identified through medical records from ten institutions. Clinical response to chemotherapy was evaluated using Response Evaluation Criteria in Solid Tumors criteria. Of the 28 cases, 8 of 15 patients with primary disease (53.3%) and 3 of 13 patients with recurrent disease (23.1%) responded to PTX-PLT chemotherapy. The response rate for cases with late recurrent disease (>12 months) was 20% (1/5), whereas the rate was 25% (2/8) for cases with early recurrent (<12 months) or refractory disease. Our results indicate that the combination of PTX and PLT may have greater efficacy against CC than conventional PLT-based chemotherapy that does not include PTX.

KEYWORDS: chemotherapy, clear cell adenocarcinoma, ovary, paclitaxel, platinum.

Epithelial ovarian cancer is the leading cause of death among gynecological malignancies in the great majority of developed countries[1]. Treatment for this disease has improved over the past 30 years with advances in surgery and in platinum (PLT)-based chemotherapy. However, most women with ovarian cancer still develop recurrent disease and die within 5 years. This high mortality is considered to be, in large part, due to the high frequency of advanced-stage disease at time of diagnosis. However, many clinical studies have reported that there are other prognostic factors such as histologic type, degree of primary surgical cytoreduction, and response to chemotherapy[1–3].

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Standard chemotherapy for ovarian cancer has been a combination of cyclophosphamide and a PLT agent, with or without doxorubicin[4]. Recently, the therapeutic effect of a combination of cisplatin (CDDP) and paclitaxel (PTX) was shown to be superior to that of a combination of cyclophosphamide and CDDP, with a clinical response rate (RR) for the PTX-CDDP combination of roughly 70%[5]. An increasing amount of evidence shows that this general combination of PTX-PLT chemotherapy seems to improve overall and disease-free survival not only for primary ovarian cancer patients but also for patients with relapsed disease[6–8].

Ovarian clear cell adenocarcinoma (CC) has been recognized as a distinct histologic entity in the World Health Organization classification of ovarian tumors since 1973[9]. The incidence of CC among epithelial ovarian carcinomas is cited as 3.7–12.1%, with approximately 60% of CC patients presenting with

early-stage disease[3,10–14]. Recently, CC has increased in prevalence. It now accounts for 18.5–20% of all epithelial ovarian cancers in Japan[15,16]. It has become obvious that chemosensitivity of ovarian cancers is closely related with histologic type. Several reports indicate that CC has a poor response to conventional therapies such as chemotherapy and irradiation and has a significantly worse prognosis than other histologic types of ovarian carcinoma[17–21]. However, those previous studies were conducted mainly for general ovarian cancer patients and with combination chemotherapy that did not include PTX. Moreover, prognosis for CC patients is considered to be influenced not only by chemotherapeutic factors but also by operative procedure[22]. In this study, we conducted a multicenter, retrospective analysis to evaluate true chemosensitivity for PTX-PLT (with either CDDP or carboplatin [CBDCA]) combination chemotherapy for CC patients who had measurable target lesions.

Materials and methods

In April 2003, ten Japanese institutions received questionnaires regarding CC cases treated with chemotherapy between 1998 and 2003: Tohoku University Hospital, Kinki University Hospital, Tsuboi Hospital, Miyagi Municipal Cancer Center, National Sendai Hospital, Takeda Hospital, Yamagata Municipal Central Hospital, Yuri-kumiai Hospital, Ichinoseki Hospital, and Hachinohe Municipal Hospital. All the selected institutions from which patients were enrolled in this study are considered highly specialized in gynecologic oncology. Patient data were collected from patient chart review by a responsible person at each institution.

Eligible patients included those with primary advanced disease (eg, stage II/III/IV) and those with recurrent or persistent CC disease. Both patient groups had to have measurable disease before chemotherapy. Tumors were diagnosed as CC if the following appeared in >50% of all histologic specimens: small to large sheets of polyhedral clear cells with delicate fibrovascular septa; tubules and papillae; clear, hobnail, or eosinophilic cells of organoid appearance; or clear cells with coalescent vacuoles containing "targetoid" eosinophilic, periodic acid–Schiff stain–positive globules. Histologic evaluation was performed under central pathologic review by one of the authors (T.M. or J.A.). All patients underwent complete surgical staging including intraperitoneal cytology, bilateral salpingo-oophorectomy, hysterectomy, omentectomy, pelvic and/or para-aortic lymphadenectomy, and aggressive cytoreductive surgery as initial treatment. Each chemotherapy cycle consisted of PTX 175 mg/m^2 and CBDCA with an area under the curve equal to five or PTX 175 mg/m^2 and CDDP 50 mg/m^2 after initial suboptimal surgery (residual tumor >1 cm) in cases of primary CC disease. The same therapy was administered for patients with persistent or recurrent measurable disease. For patients with recurrent or persistent CC disease, the number and regimens of previous chemotherapy were not used as exclusion criteria. Patients received more than three cycles of PTX-PLT combination chemotherapy every 3 weeks. In addition, to evaluate the specific response to PTX-PLT, patients who received only this regimen were enrolled in the study.

Clinical response to chemotherapy was evaluated by two of the authors (K.I. and H.N.) according to Response Evaluation Criteria in Solid Tumors[23] with use of computed tomography after three cycles of planned chemotherapy and/or when all planned chemotherapy was finished. Toxicity of chemotherapy was not evaluated in this study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan. Each survival curve was obtained by the Kaplan–Meier method.

Results

A total of 28 patients were identified who met all the study eligibility criteria described above. Patient characteristics are summarized in Table 1. Of the 28 patients included in the study, 15 women with primary disease (53.6%) received PTX-PLT as first-line chemotherapy and 13 women with recurrent or refractory disease (46.4%) received PTX-PLT as second- or third-line chemotherapy. The details of previous

Table 1. Patient characteristics

	Clear cell	
	Primary (n = 15)	Recurrence (n = 13)
Age (years)		
Median (range)	52 (43–74)	54 (48–68)
Performance status		
0	12	11
1	3	1
2	0	1
Stage at primary treatment		
1	0	7
2	1	4
3	12	1
4	2	1