repression occurred in primary cervical cancers with comparable frequency, we analyzed *RAR*β2 expression in clinical cancer tissues (Fig. 1E). *RAR*β2 was completely repressed in 13 cancers (76.4%), highly repressed in two cancers, and moderately down-regulated in two cancers. The frequency of complete repression is similar to that of cancer cell lines (67%).

3.2. The frequent methylation of RARβ2 promoter CpG islands is correlated with RARβ2 repression

To determine if the repression is associated with promoter hypermethylation, COBRA was performed on nine cancer cell lines to analyze the DNA methylation status of the *Acc*II site in CpG island 2 (Fig. 1A and C). The results showed that four of eight *RAR*β2 repressed cell lines had methylated promoters, and the remainder had unmethylated promoters. Promoter methylation was also examined in the primary cancer tissues (Fig. 1F). Nine of the 13 *RAR*β2 repressed samples (69%) had methylated promoter, and the remaining four were unmethylated. Promoter methylation was not seen in any of the *RAR*β2-positive cells and tissues, including all of the six normal cervixes, one cancer cell line (HT-3), and four cancer tissues. Detailed methylation analyses were then carried out on the cancer cell lines by bisulfite sequencing at 32 CpG sites encompassing the two CpG islands, CGI1 and CGI2, in the promoter (Fig. 1D). These analyses confirmed the results of the COBRA and indicated that the two CpG islands showed essentially the same methylation statuses in all the samples examined. Importantly, all the samples with methylated promoters were *RAR*β2-negative, indicating that promoter DNA methylation is tightly correlated with and may possibly be responsible for *RAR*β2 repression. On the other hand, it is noteworthy that some *RAR*β2-negative cell lines and cancer tissues have unmethylated or hypomethylated promoters, implying that a mechanism other than DNA methylation is at work in *RAR*β2 epigenetic silencing.

3.3. Profiles of histone modification at the RARβ2 promoter region

To assess the role of histone modification in the repression of *RAR*β2 as well as its relationship with DNA methylation, we then used ChIP assay to investigate the enrichments of four kinds of modified histones, acetylated histones H3 and H4 (H3Ac and H4Ac), di-methylated histone H3K4 (H3me2K4), and di-methylated histone H3K9 (H3me2K9) at the two CpG islands in four representative cell lines (Fig. 2). In HT-3, which is the *RAR*β2-positive cell line with an unmethylated promoter, levels of H3Ac, H4Ac, and H3me2K4 are high and that of H3me2K9 is very low. This pattern of histone modification is known to mark an open chromatin structure associated with active gene expression. HeLa, which represented the *RAR*β2-negative cell lines with a hypermethylated promoter, showed a contrary pattern (low levels of H3Ac, H4Ac, and H3me2K4, and a high level of H3me2K9), known as repressive histone modifications [10,11]. SKG-IIIa and SiHa, which are the *RAR*β2-negative cell lines with an unmethylated promoter, also showed repressive histone modifications similar to those of HeLa cells. These identical results were obtained from three independent ChIP assays. The results for each of the two CpG islands were essentially the same in all the samples. These results indicated that the histone modifications at the *RAR*β2 promoter region were strongly correlated with the *RAR*β2 expression, because the repressive pattern emerges in all the *RAR*β2-negative cells regardless of the DNA methylation status (Figs. 1 and 2).

3.4. Re-expression of RARβ2 in the RARβ2-repressed cells after treatment with DAC or TSA

To find out whether these two epigenetic factors cause of *RAR*β2 repression and whether these factors are interdependent, we then examined the changes of *RAR*β2 expression and DNA methylation and histone modification in the promoter region after treatments of cells with 5-aza-2′-deoxycytidine (DAC), an inhibitor of DNA methyltransferases (DNMTs) or trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor.

As shown in Fig. 3A, treatment with DAC restored *RAR*β2 expression only in the HeLa cell, a *RAR*β2-negative cell with a methylated promoter. DAC treatment caused a faint elevation of expression in the *RAR*β2 positive cell line, HT-3. The HeLa cell showed no reactivation of *RAR*β2 when the cell was treated with TSA. Consistent with this result, TSA had no additional effect on *RAR*β2 reactivation either when it was added to the cell treated with DAC, in comparison with the induction by DAC alone. Similar results were also observed in the cells with a methylated promoter, HeLa-TG, JSK-1, and TMCC-1 (data not shown). COBRA was performed on *RAR*β2 promoter in the HeLa cell to see if the reactivation induced by DAC is in fact accompanied by demethylation of the promoter. As expected, almost complete demethylation of the promoter occurred in the cells treated with DAC alone or DAC/TSA, but not in the cell treated with TSA alone (Fig. 3B, HeLa). SKG-IIIa and SiHa cells do not express *RAR*β2 even though their promoters are hypomethylated. These cells have histone modifications of repressive pattern in the promoter. Treatment with an HDAC inhibitor, TSA, reactivated *RAR*β2 expression in these cells (Fig. 3A). DAC treatment showed no effect on *RAR*β2 expression, being consistent with their hypomethylated promoters. Similar results were obtained with OMC-4 and C33A, the cells with decreased expression and the unmethylated promoter (data not shown).

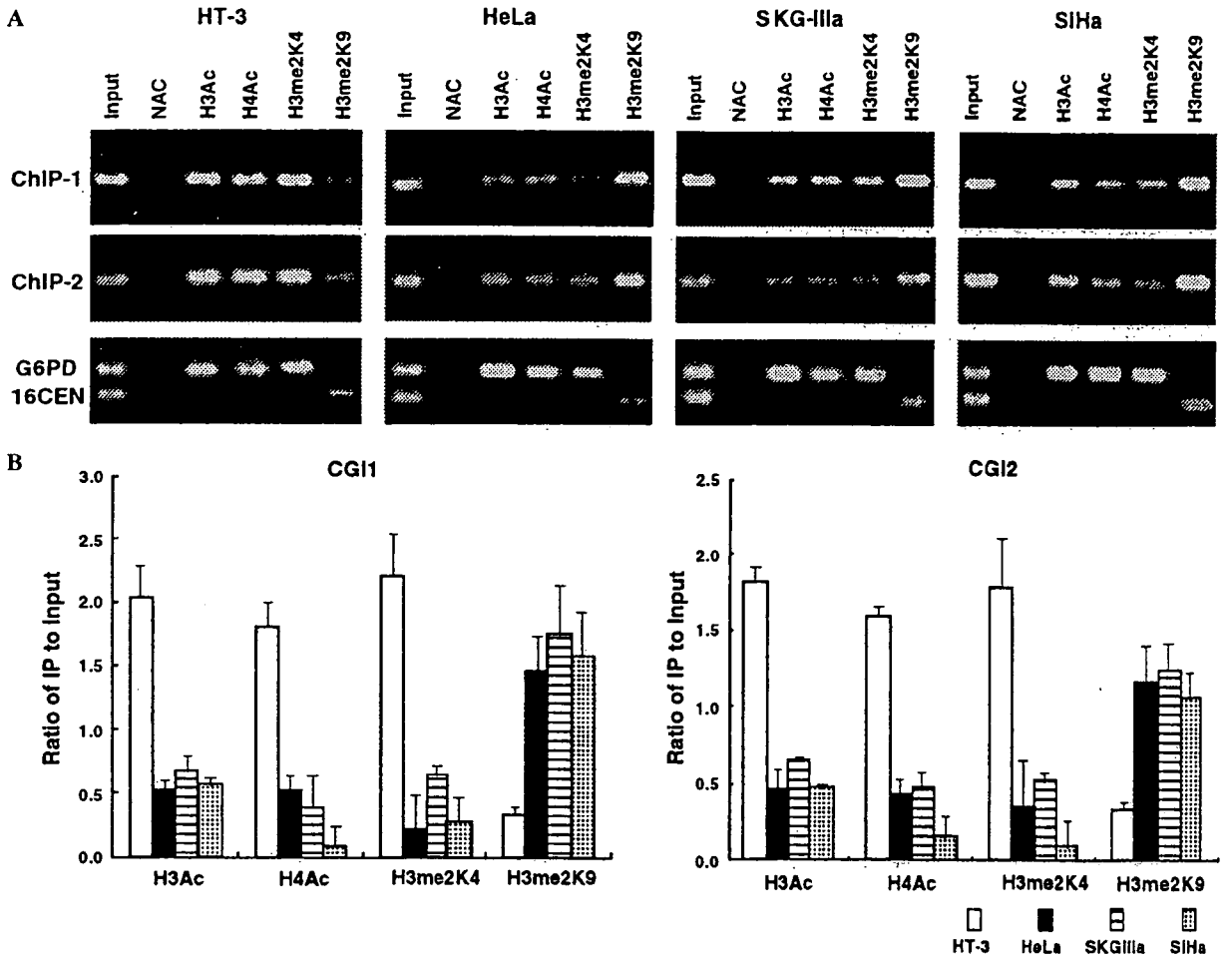

Fig. 2. Histone modification analyses at two CpG islands of *RARβ2* promoter region by ChIP assay. (A) Representatives of ChIP-PCR. Input, DNA from cell lysate before immunoprecipitation; NAC, no antibody control; G6PD, a positive control for H3Ac, H4Ac, and H3me2K4, and negative control for H3me2K9. 16CEN, an inverse control for those of G6PD. (B) Quantification of the ChIP-PCR. Vertical bars represent the ratios of PCR products with immunoprecipitated DNA (IP) versus input DNA. Error bars denote standard deviation. H3Ac, H4Ac, and H3me2K4 are higher, and H3me2K9 is lower in the *RARβ2*-positive cell (HT-3). *RARβ2*-negative cells (HeLa, SKG-IIIa, and SiHa) have a reversed histone modification pattern.

We then asked whether histone modification in the promoter also changed during the re-expression induced by the drugs. Upon treatment of HeLa with DAC or DAC/TSA, there were substantial increases of H3Ac, H4Ac, and H3me2K4 and a simultaneous decrease of H3me2K9 in CpG island 1, that is, changes into the active pattern of histone modifications (Fig. 3 C and D). The observed changes are thus completely consistent with activation of the gene. Treatment with TSA alone caused the same but only moderate change in histone modifications. In SKG-IIIa and SiHa cells, TSA induced a drastic change of histone modifications into the active pattern. The change included not only increases of H3Ac and H4Ac but also an increase of H3me2K4 and a decrease of H3me2K9. DAC induced almost no change of histone modifications (Fig. 3 C and D). Essentially the same results were obtained in CpG island 2 (data not shown).

4. Discussion

In the present study, it was shown that *RARβ2* was repressed in most of the cancer cell lines and primary cancer tissues examined. The repression was frequently associated with promoter hypermethylation. There was no cell expressing the gene with a methylated promoter. These results strongly suggest that promoter methylation was the epigenetic cause of *RARβ2* repression in cervical cancers harboring a methylated promoter. DAC, a DNA

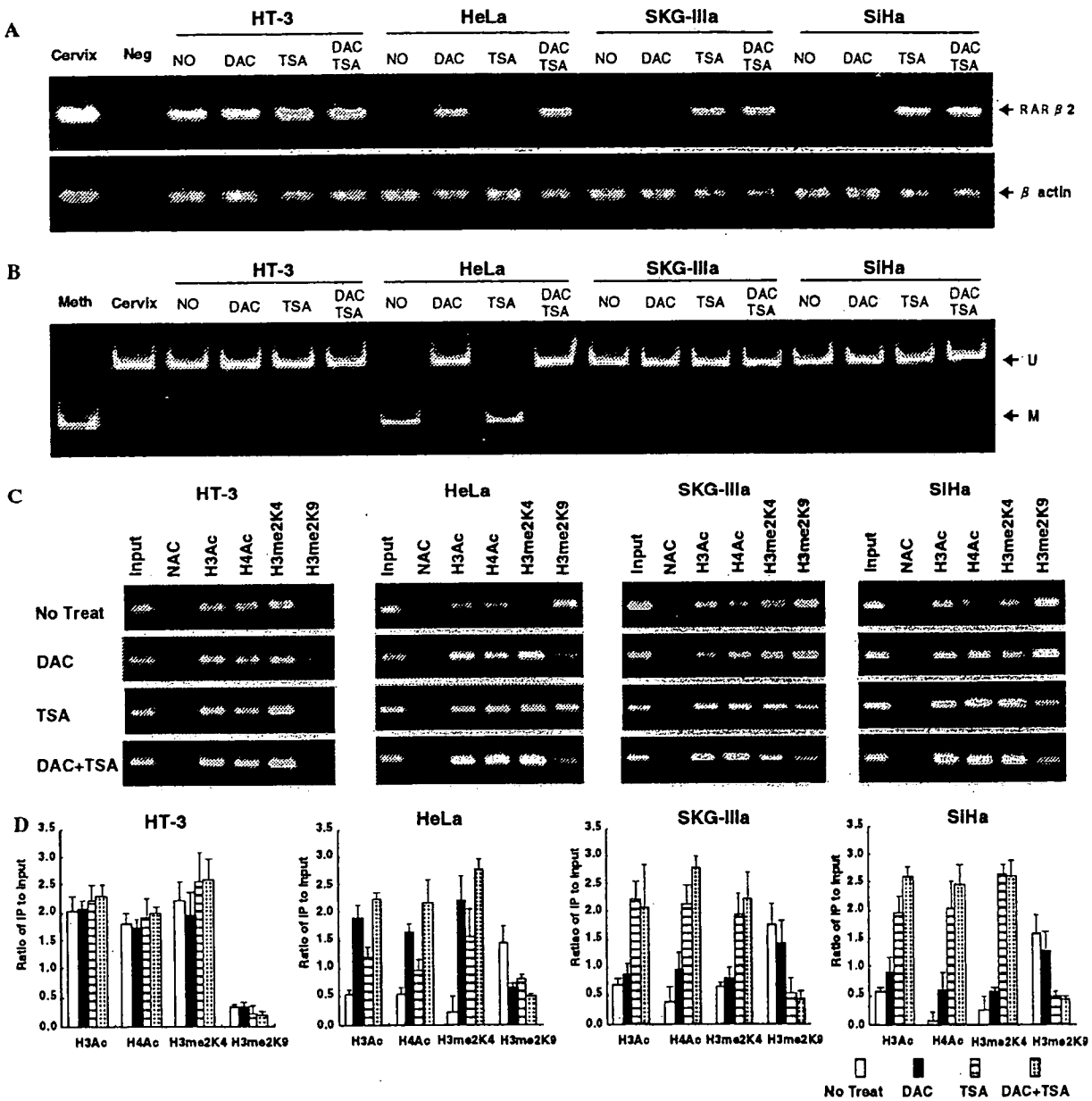

Fig. 3. Analyses of *RARβ2* expression, DNA methylation, and histone modifications at the promoter region after drug treatments. (A) RT-PCR analyses of *RARβ2* expression in cells treated with DAC, TSA, and DAC + TSA. NO, no treatment. The *RARβ2* suppressed cell line with hypermethylated promoter (HeLa) restored the expression by DAC treatment. The cell lines with unmethylated promoters (SKG-IIIa and SiHa) restored the expression by TSA treatment. (B) DNA methylation analyses of CGI2 by COBRA. DAC treatment induced demethylation of the promoter in HeLa cell. (C) Analyses of histone modifications by ChIP assay. Shown are the representatives of ChIP-PCR for CGI1. (D) Quantification of the ChIP-PCR.

demethylating reagent, could reactivate the expression of the gene with simultaneous induction of drastic demethylation of the promoter in the repressed cells carrying a methylated promoter. This consistency between the promoter demethylation and the re-expression strongly suggested that the primary cause of *RARβ2* repression was indeed promoter hypermethylation in these cells. Several hypotheses have been proposed with regard to the mechanisms of DNA methylation leading to silencing of genes [2,37]. In these hypotheses, once DNA methylation occurs in an originally active chroma-

tin, MDB proteins bind to the methylated promoter and then recruit histone deacetylases and histone methyltransferases. These enzymes form repressive histone modifications leading to heterochromatin formation, and cause transcriptional silencing. Treatment with DAC also changed histone modifications to an active pattern in the promoter. This indicated that DNA methylation was required for not only formation but also maintenance of the repressive histone modifications. TSA did not induce acetylation of histone H3 and H4 so much in methylated promoters as it did in the unmethylated promoters. TSA may require an unmethylated status of the promoter DNA to fully induce histone acetylation. Because TSA did not reactivate the expression of *RAR*β2, this moderate change to an active pattern seems not to be sufficient for the expression of the gene. Alternatively, DNA methylation in the promoter may inhibit active transcription even with the active modifications of histones.

In some cancer cells and tissues examined, *RAR*β2 was repressed without promoter hypermethylation. Some TSGs were known to be repressed with hypomethylated promoters [4–6]. These facts indicated that although DNA methylation was the major epigenetic mechanism for gene silencing, there were other epigenetic silencing pathways independent of DNA methylation. Histone modifications were found to be the repressive form in such cells, SKG-IIIa and SiHa. In these cells, an HDAC inhibitor, TSA, simultaneously induced the expression of *RAR*β2 and the change of histone modifications into the active form. This result suggested that repressive histone modifications were the direct cause of *RAR*β2 silencing in these cells. TSA induced not only acetylation of histones H3 and H4 but also changes in H3me2K4 and H3me2K9. Although we do not know the exact mechanisms linking the acetylation and methylation of histones, increasing evidence indicate an interplay between different post-translational modifications occurring on histones [38,39]. OMC-4 and C33A expressed *RAR*β2 at a very low level but have a hypomethylated promoter indistinguishable from those of HT-3, SKG-IIIa, and SiHa cells (Fig. 1B, C, and D). We did not analyze the histone modification statuses in these cells, but they may have repressive modifications in a lesser extent than those of SKG-IIIa and SiHa.

In SKG-IIIa and SiHa, DAC has almost no effect on the expression of *RAR*β2 or on the histone modifications in the promoter. DAC can induce reactivation of some genes repressed without promoter hypermethylation [4,36,40]. For example, a DNA repair gene, *MGMT*, is repressed without DNA hypermethylation in some cancer cells. DAC reactivated the repressed *MGMT* by inducing the dissociation of bound MeCP2 and a decrease of H3me2K9 at the promoter, independent of DNA demethylation [36]. Moreover, *MGMT* could not be reactivated by treatment with TSA. The difference in the effectiveness of TSA treatment suggests that there are different molecular mechanisms of gene silencing without promoter hypermethylation.

In conclusion, *RAR*β2 is frequently silenced in cervical cancers by one of two epigenetic mechanisms. One is DNA methylation, a well-known epigenetic factor leading to transcriptional silencing of genes. The present study reveals another pathway, formation of repressive histone modifications at the promoter, by unknown mechanisms independent of DNA methylation. At present, we do not know the initial causes of these epigenetic changes during carcinogenesis. Silenced *RAR*β2 with promoter methylation can be reactivated by DAC but not by TSA, and the silenced gene with promoter hypomethylation is reactivated by the reverse process. This result suggests the importance of examining promoter methylation if these epigenetic modulating drugs are used for chemotherapy of cervical cancers.

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A validation study of a scoring system to estimate the risk of lymph node metastasis for patients with endometrial cancer for tailoring the indication of lymphadenectomy☆

Yukiharu Todo [a,*], Kazuhira Okamoto [b], Masaru Hayashi [c], Shinichiro Minobe [d], Eiji Nomura [e], Hitoshi Hareyama [f], Mahito Takeda [a], Yasuhiko Ebina [a], Hidemichi Watari [a], Noriaki Sakuragi [a]

[a] *Department of Obstetrics and Gynecology, Hokkaido University School of Medicine, Kita-15, Nishi-7, Kita-Ku, Sapporo 060-8638, Japan*
[b] *Division of Obstetrics and Gynecology, Asahikawa Kosei General Hospital, Asahikawa, Japan*
[c] *Division of Obstetrics and Gynecology, Obihiro Kosei General Hospital, Obihiro, Japan*
[d] *Division of Obstetrics and Gynecology, Kushiro Red Cross Hospital, Kushiro, Japan*
[e] *Division of Obstetrics and Gynecology, Oji General Hospital, Tomakomai, Japan*
[f] *Division of Obstetrics and Gynecology, Sapporo City General Hospital, Sapporo, Japan*

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Abstract

Objective. The aim of this study was to verify whether a preoperative scoring system to estimate the risk of lymph node metastasis (LNM) in endometrial carcinoma is clinically useful for tailoring the indication of lymphadenectomy.

Study design. LNM score was set up using volume index, serum CA125 level, and tumor grade/histology, which were found to be independent risk factors for LNM in a pilot study. Based on the LNM score before a validation study was started, the estimated rates of LNM (para-aortic LNM) were 3.4% (0.0%) in a low risk group, 7.7% (5.8%) in an intermediate group, 44.4% (30.6%) in a high risk group and 70.0% (50.0%) in an extremely high risk group. The validation study was carried out using data for 211 patients with endometrial carcinoma for whom three risk factors were preoperatively confirmed. Logistic regression analysis was used to determine whether these factors remain valid. The actual rate of LNM was investigated according to the LNM score.

Results. Volume index, serum CA125 level, and tumor grade/histology were found to be independent risk factors for LNM in the cohort of this study. The actual rates of LNM (para-aortic LNM) were 3.2% (1.0%) in the low risk group, 15.3% (11.9%) in the intermediate group, 30.2% (23.8%) in the high risk group and 78.6% (57.1%) in the extremely high risk group.

Conclusion. The actual rate of LNM for each score was fairly consistent with the estimated rate of LNM. Para-aortic lymphadenectomy may not be necessary in cases of a low risk group. A large prospective multicenter clinical trial needs to be conducted to establish the clinical usefulness of our preoperative scoring system.

Keywords: Endometrial carcinoma; Lymph node metastasis; MRI; CA125

Introduction

Retroperitoneal lymph node metastasis (LNM) is a critical prognostic factor for patients with endometrial carcinoma [1]. Lymphadenectomy has become accepted as the standard treatment for women with endometrial cancer but still has some issues of debate. Many gynecologists would agree that patients with endometrial carcinoma who have grade 1 tumor with no myometrial invasion do not need lymphadenectomy [2]. However, there has not been a gold-standard method for selecting patients who do not need lymphadenectomy in a preoperative setting. It has been suggested that para-aortic lymphadenectomy has a potential therapeutic role in node-positive endometrial cancer [3]. However, a consensus has not been reached regarding the issue of whether to extend the

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* Corresponding author. Fax: +81 11 706 7711.
E-mail address: yukiharu@med.hokudai.ac.jp (Y. Todo).

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application field of lymphadenectomy to the para-aortic area. We previously reported that volume index, which is a substitute for tumor volume, preoperative serum CA125 level, and histologic grade 3 tumor or serous adenocarcinoma determined by preoperative endometrial biopsy were independent risk factors for LNM [4]. Using these indexes, we formulated a scoring system to estimate the risk of LNM in endometrial cancer. The aim of this study was to determine whether this scoring system is valid in a different cohort of patients with endometrial cancer. Indication for lymphadenectomy is also discussed in this report.

Materials and methods

Study design

We designed a series of clinical studies to establish a scoring system that can determine the risk of LNM in an individual patient with endometrial cancer. These studies comprise of as follows: 1) a pilot study to define the independent risk factor of LNM that will be incorporated in the scoring system, 2) a validation study in which the scoring system will be applied to a different cohort of patients, and 3) a large multi-institutional observational study to verify the generalizability and applicability of the scoring system. This is a report of the validation study.

A pilot study for formulating lymph node metastasis score (LNM score)

The lymph node metastasis score (LNM score) was formulated based upon the data of 214 patients with endometrial cancer who underwent extensive surgical staging including systematic pelvic and para-aortic lymphadenectomy during the period between January 1993 and March 2000, which was reported in a previous paper [4]. In a pilot study, all patients with endometrial cancer underwent systematic lymphadenectomy. The pelvic lymph node groups that were dissected included the common iliac, external iliac, internal iliac, obturator, medial deep inguinal, lateral deep inguinal, parametrial, and sacral node group in the pelvic area. Para-aortic lymph nodes that were inferior to the level of the inferior mesenteric artery and para-aortic lymph nodes that were superior to the inferior mesenteric artery up to the level of the renal vessels were dissected. The clinicopathologic characteristics of the patients are shown in Table 1. Among the 214 patients, 31 (14.5%) had LNM and 19 (8.9%) had para-aortic LNM.

The scoring system includes volume index, serum CA125 level, and tumor grade/histology. Volume index was defined as the product of the maximum longitudinal diameter along the uterine axis, the maximum anteroposterior diameter (thickness) in a sagittal section image, and the maximum horizontal diameter in a horizontal section image. The results of measurements were used to obtain receiver operating characteristic (ROC) curves for LNM. Although we separately showed the ROC curve for pelvic LNM and that for para-aortic LNM in a previous report, the revised ROC curve for LNM that includes both pelvic LNM and para-aortic LNM is shown in Fig. 1. When determined on the curve, cut-off value for LNM was 36. The serum CA125 level was determined using a RIA kit (Fujirebio Diagnostics, Malvern, PA). The patient population was divided into two groups by age. Although we separately showed the ROC curve for pelvic LNM and that for para-aortic LNM in a previous report, the revised ROC curves for LNM that includes both pelvic LNM and para-aortic LNM are shown in Fig. 1. Using these curves, two cut-off values (70 U/ml for patients aged less than 50 years and 28 U/ml for patients aged 50 years or over) divided patients into low and high CA125 groups for LNM. Preoperative endometrial biopsy specimens were evaluated for tumor grade and histologic variant (three grades according to the 1988 FIGO criteria). Tumor grade/histology, which put two factors of tumor grade and histologic type together, was used as an independent variable in the revised analysis. In a previous report, we separately showed the results of logistic regression analysis in which pelvic LNM was used as a dependent variable and that in which para-aortic LNM was used as a dependent variable. Table 2 shows the results of revised logistic regression analysis in which LNM including both pelvic LNM and para-aortic LNM was used as a dependent variable.

Table 1
Characteristics of a cohort of 214 patients with endometrial carcinoma to produce LNM score in the pilot study

Study design	Retrospective cohort study
Number of institution	3
Number of patients	214
Age, median (range)	56 (23–80)
Number of resected lymph nodes, mean±SD	71.2±34.0

	No.	LNM	PLNM	PANM
pT (TNM classification)				
1a	54	0	0	0
1b	62	5	5	1
1c	44	8	8	6
2a	10	3	2	1
2b	8	5	5	4
3	31	5	4	4
4	5	5	5	3
Histologic type (preoperative diagnosis)				
Endometrioid	207	27	25	17
(G1)	(134)	(10)	(9)	(7)
(G2)	(55)	(10)	(10)	(4)
(G3)	(18)	(7)	(6)	(6)
Serous	7	4	4	2

These data were not published in a previous paper [4].
LNM: lymph node metastasis, PLNM: pelvic lymph node metastasis, PANM: para-aortic lymph node metastasis.

The impact of the LNM score was determined according to the number of independent risk factor which was confirmed in Table 2. All patients were classified into low risk group (with no risk factor), intermediate risk group (with one risk factor), high risk group (with two risk factors) and extremely high risk group (with all risk factors). Of the 214 patients, 116 (54%) were included in the low risk group, 52 (24%) were included in the intermediate risk group, 36 (17%) were included in the high risk group and 10 (5%) were included in the extremely high risk group. Based on the LNM score, the estimated rates of LNM (para-aortic LNM) were 3.4% (0.0%) in the low risk group, 7.7% (5.8%) in the intermediate group, 44.4% (30.6%) in the high risk group and 70.0% (50.0%) in the extremely high risk group.

Validation study of LNM score

Among patients with endometrial carcinoma treated in the Department of Obstetrics and Gynecology, Hokkaido University Hospital and twelve affiliated hospitals during the period from July 2000 to April 2005, 216 patients who underwent extensive surgical staging including systematic lymphadenectomy were entered in this study. The patients underwent pelvic MRI, endometrial biopsy, and serum CA125 level determination as preoperative examinations. Although lymphadenectomy was not carried out in some affiliated hospitals for some patients who were preoperatively diagnosed as having grade 1 tumor without myometrial invasion, all patients entered in the validation study underwent hysterectomy, bilateral salpingo-oophorectomy, and systematic lymphadenectomy as initial treatment. For a medical complication, para-aortic lymphadenectomy was not performed in one patient. Five cases with an admixture of sarcomatous component and carcinomatous component in a preoperative endometrial biopsy were excluded from this study. A total of 211 patients were included in the study. The clinicopathologic characteristics of those patients are shown in Table 3. The ages of the patients ranged from 24 to 77 years (median age, 57 years). 21 patients were in pT1a (TNM classification), 91 were in pT1b, 50 were in pT1c, 4 were in pT2a, 17 were in pT2b, 26 were in pT3, and 2 were in pT4. The lymphadenectomy specimens included a median of 77 lymph nodes (mean±SD, 79.4±29.6) evaluated for each patient. 36 (17.1%)

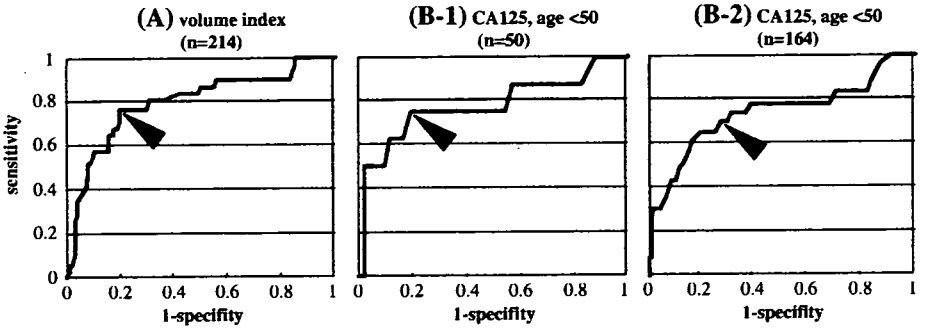

Fig. 1. (A) Receiver operating characteristic curve obtained from the relationships between volume index and LNM in the pilot study. Cut-off value (arrow) for LNM was 36. (B) Receiver operating characteristic curves obtained from the relationships between serum CA125 level and LNM. Cut-off value (arrow) for LNM was 70 U/ml for patients aged less than 50 years. Cut-off value (arrow) for LNM was 28 U/ml for patients aged 50 years or over. (These data were not published in a previous paper [4].)

patients had LNM and 26 (12.3%) had para-aortic LNM. Logistic regression analysis was used to determine whether the factors incorporated into the model of LNM score are still valid in a validation study. Volume index, MRI-based myometrial invasion, serum CA125 level, and tumor grade/histology were used as independent variables, and LNM was used as a dependent variable. The 211 patients were classified into a low risk group, an intermediate risk group, a high risk group and an extremely high risk group. The actual rates of LNM were compared to the estimated rates of LNM according to the model of LNM score.

Statistical analysis

Logistic regression analysis was used to select the risk factors for LNM. Variables that achieved statistical significance in univariate analysis were subsequently included in a multivariate analysis. The statistical significance level was set at .05. Statistical analyses were performed with StatView J-5.0 PPC (SAS Institute, Cary, NC).

Results

Cases with invasion of less than one half the myometrium had a sensitivity of 83%, a specificity of 83%, and an accuracy of 83%. Cases with the diagnosis of G1/G2 histopathology had a sensitivity of 97%, specificity of 74% and accuracy of 92%. Only 5.7% of cases with G1/G2 histopathology diagnosed by endometrial biopsy were upgraded to G3/serous adenocarcinoma after hysterectomy.

Table 4 shows the results of logistic regression analysis. Univariate analysis revealed that all factors were significantly related to LNM. Multivariate analysis confirmed that only high volume index, high serum CA125, and G3/serous adenocarcinoma were independent risk factors for LNM for this cohort of patients. MRI-based myometrial invasion had an odds ratio of 5.4 (95% confidence interval [CI]=2.4–11.9) before adjusting other factors but an odds ratio of 2.7 (95% CI=1.1–6.5) after adjusting volume index. Eventually it had an odds ratio of 2.0 (95% CI=0.8–5.3) after adjusting volume index, serum CA125, and tumor grade/histology.

LNM frequencies according to the LNM score are shown in Table 5. The rates of LNM were 3.2% (3/95) in the low risk group, 15.3% (9/59) in the intermediate group, 30.2% (13/43) in the high risk group and 78.6% (11/14) in the extremely high risk group. The rates of para-aortic LNM were 1.0% (1/95) in the low risk group, 11.9% (7/59) in the intermediate group, 23.8% (10/42) in the high risk group and 57.1% (8/14) in the extremely high risk group.

7 (6.2%) of the 113 patients who had G1/G2 tumor as assessed by endometrial biopsy and invasion of less than half of the myometrium as assessed by MRI had LNM. On the other hand, 3 (3.2%) of the 95 patients with low risk according to the LNM score had LNM. 5 (4.4%) of the 113 patients who had G1/

Table 2
Correlation between the factors incorporated into the LNM score in the pilot study

Factor	LNM		Univariate analysis	Multivariate analysis			
	n/N	%	*p*-value	*β*	SE	OR (95% CI)	*p*-value
Preoperative tumor grade/histology							
G1/G2	20/189	10.6					
G3/serous	11/25	44.0	<0.0001	1.2	0.55	3.5 (1.2–10.2)	<0.05
Volume index							
<36	7/153	4.6					
≥36	24/61	39.3	<0.0001	1.8	0.53	5.7 (2.0–16.1)	<0.001
MRI myo-invasion							
<1/2	7/118	5.9					
≥1/2	24/96	25.0	<0.0005	0.7	0.53	2.1 (0.7–5.8)	NS
Serum CA125 level							
Low	9/146	6.2					
High	22/68	32.4	<0.0001	1.2	0.49	3.5 (1.3–9.1)	<0.05

These data were not published in a previous paper [4].
LNM: lymph node metastasis, PLNM: pelvic lymph node metastasis, PANM: para-aortic lymph node metastasis.

Table 3
Characteristics of a cohort of 211 patients with endometrial carcinoma included in the validation study

Study design	Retrospective cohort study			
Number of institution	13			
Number of patients	211			
Age, median (range)	57 (24–77)			
Number of resected lymph nodes, mean±SD	79.4±29.6			
	No.	LNM	PLNM	PANM
pT (TNM classification)				
1a	21	0	0	0
1b	91	11	10	10
1c	50	15	12	7
2a	4	1	1	1
2b	17	4	4	3
3	26	3	3	3
4	2	2	2	2
Histologic type (preoperative diagnosis)				
Endometrioid	205	33	30	24
(G1)	(124)	(13)	(12)	(8)
(G2)	(51)	(7)	(6)	(5)
(G3)	(30)	(13)	(12)	(11)
Serous	6	3	2	2

LNM: lymph node metastasis, PLNM: pelvic lymph node metastasis, PANM: para-aortic lymph node metastasis.

G2 tumor as assessed by endometrial biopsy and invasion of less than half of the myometrium as assessed by MRI had para-aortic LNM. On the other hand, only 1 (1.0%) of the 95 patients with low risk according to LNM score had para-aortic LNM.

Fig. 2 shows LNM frequencies for the combined cohorts of 425 patients with endometrial carcinoma according to LNM score. The rates of LNM were 3.3% (95% CI=0.9–5.7) in the low risk group, 11.7% (95% CI=5.7–17.7) in the intermediate group, 36.7% (95% CI=26.1–47.3) in the high risk group and 75.0% (95% CI=57.7–92.3) in the extremely high risk group. The rates of para-aortic LNM were 0.5% (95% CI=0.0–1.4) in the low risk group, 9.0% (95% CI=3.7–14.3) in the intermediate group, 27.6% (95% CI=17.6–37.7) in the high risk group and 54.2% (95% CI=34.2–74.1) in the extremely high risk group. Of the 211 patients in low risk group, 1.7% (95% CI=0.0–4.1) with grade 1 carcinoma and invasion of less than half of the myometrium as assessed by MRI had LNM. On the other hand, 5.2% (95% CI=0.8–9.7) with grade 2 carcinoma or invasion of more than half of the myometrium as assessed by MRI had LNM.

Table 5
LNM frequencies according to LNM score

LNM score		LNM (%)		PLNM (%)		PANM (%)	
		Pilot study	Validation study	Pilot study	Validation study	Pilot study	Validation study
Low risk	RF=0	3.4	3.2	3.4	3.2	0.0	1.0
Intermediate risk	RF=1	7.7	15.3	7.7	15.3	5.8	11.9
High risk	RF=2	44.4	30.2	38.9	23.3	30.6	23.8
Extremely high risk	RF=3	70.0	78.6	70.0	71.4	50.0	71.4

LNM: lymph node metastasis, PLNM: pelvic lymph node metastasis, PANM: para-aortic lymph node metastasis, RE: risk factor (MM tumor volume, serum CA125 level, tumor grade/histology).

Discussion

(G1) The FIGO 2001 annual report showed that the 5-year overall survival rate of patients with carcinoma of the endometrium has increased by 13.5% in the past 30 years [5]. The main change in the therapeutic paradigm for endometrial cancer in the past 30 years is the introduction of surgical staging. About 91% of patients have been surgically staged [5]. The treatment strategy including surgical staging may have resulted in improvement of the 5-year survival rate. Recent scientific publications have confirmed the relative safety of surgical staging including lymphadenectomy when performed by subspecialty trained surgeons, and this procedure has become accepted as the standard treatment for women with endometrial cancer [6]. However, there are still some issues of debate about lymphadenectomy in endometrial cancer. There has not been a gold-standard method for selecting patients who do not need lymphadenectomy in a preoperative setting, although many gynecological oncologists would agree that patients with endometrial carcinoma who have grade 1 tumor with no myometrial invasion do not need lymphadenectomy. A consensus

Table 4
Correlation between the factors incorporated into the LNM score and LNM in the validation study

Factor	LNM		Univariate analysis	Multivariate analysis			
	n/N	%	*p*-value	*β*	SE	OR (95% CI)	*p*-value
Preoperative tumor grade/histology							
G1/G2	20/175	11.4					
G3/serous	16/36	44.4	<0.0001	1.6	0.47	4.8 (1.9–12.0)	<0.001
Volume index							
<36	9/137	6.6					
≥36	27/74	36.5	<0.0001	1.2	0.51	3.4 (1.3–9.4)	<0.05
MRI myo-invasion							
<1/2	10/128	7.8					
≥1/2	26/83	31.3	<0.0001	0.7	0.49	2.0 (0.8–5.3)	NS
Serum CA125 level							
Low	11/134	8.2					
High	25/77	32.5	<0.0001	1.0	0.47	2.7 (1.1–6.9)	<0.05

LNM: lymph node metastasis, PLNM: pelvic lymph node metastasis, PANM: para-aortic lymph node metastasis.

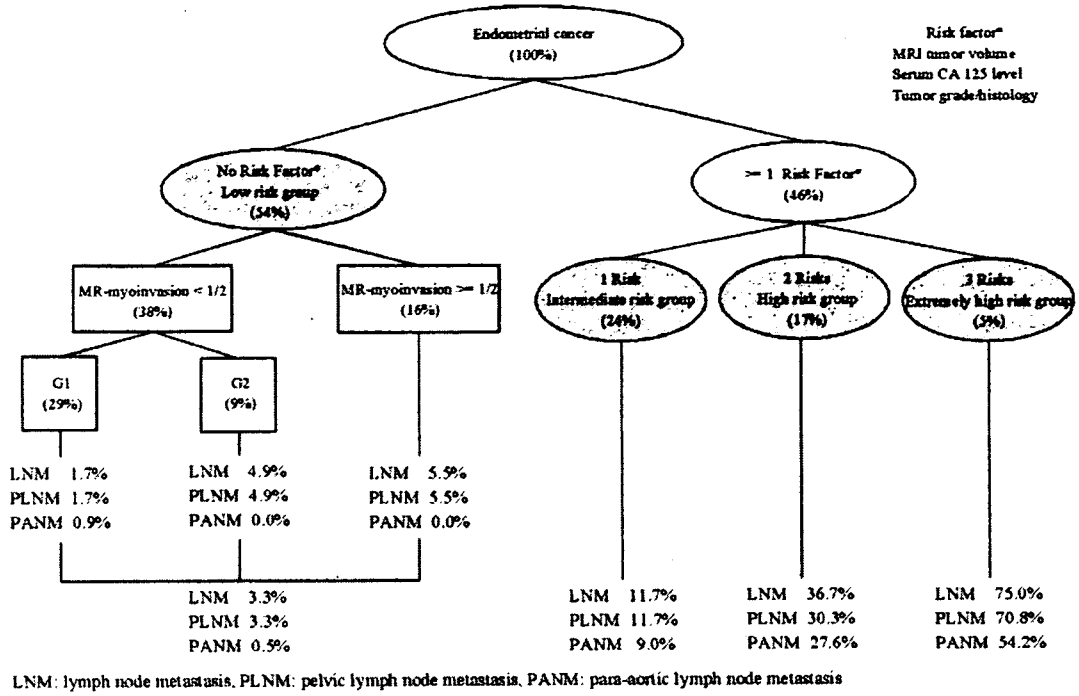

Fig. 2. Rate of LNM for the combined cohorts of 425 patients with endometrial carcinoma according to LNM score and distribution map of patients estimated from a previous paper [4].

has not been reached regarding the issue of whether to extend the application field of lymphadenectomy to the para-aortic area, although it has been suggested para-aortic lymphadenectomy has a potential therapeutic role in node-positive endometrial cancer. The establishment of a gold-standard method for selecting patients with endometrial carcinoma who have little risk for para-aortic LNM would be clinically very useful.

Classically, the risk of LNM has been classified according to the depth of myometrial invasion and histological grade. The methods used to evaluate those parameters in a preoperative setting are MRI and endometrial biopsy. Patients without myometrial invasion who have histologic grade 1 would be candidates for elimination of lymphadenectomy. Although myometrial invasion is indirectly evaluated by MRI in many institutions in Japan, the level of accuracy still has limitations. MRI-based evaluation used for diagnosis of deep invasion in a multi-institutional cooperative study had a sensitivity of 54% and specificity of 89%, indicating that results of previous single institutional studies might have been biased [7]. The level of accuracy for diagnosis of the presence or absence of myometrial invasion using MRI is poorer than the level of accuracy for diagnosis of deep (>1/2) invasion. Pathological examination using frozen sections obtained during the operation improves the level of accuracy [8,9], but even its level of accuracy is not sufficient. It has been reported that only 60%–72% of patients who were diagnosed as having no myometrial invasion by pathological examination using frozen sections obtained during the operation were diagnosed as having no myometrial invasion by examination of resected specimens [8,9]. There is also the question of whether myometrial invasion is an independent risk factor of LNM. Since it is difficult to evaluate tumor volume in the resected uterus, tumor volume has not been included in previous histopathologic analyses to determine independent risk factors of LNM. However, tumor volume can be estimated by using MRI. There has not been sufficient study to determine which is a more significant risk factor of LNM, myo-invasion or tumor volume. We showed in this validation study that myometrial invasion assessed by MRI might be a confounding factor of the volume index but not an independent risk factor for LNM, as we found in our previous study [4]. Although histological grade is evaluated by endometrial curettage in many institutions, the level of accuracy also has limitations. Larson et al. reported that the use of office endometrial biopsy had a sensitivity of 67%, specificity of 73% and accuracy of 70% for the diagnosis of G1 histopathology and that 37% of cases with G1 histopathology diagnosed by office endometrial biopsy were upgraded to G2/G3 adenocarcinoma after examination of specimens obtained from hysterectomy [10]. However, the level of accuracy for diagnosis of histologic grade would be higher and the number of upgraded cases would decrease if the category of diagnosis including both G1 and G2 is investigated. Larson et al. reported that the use of endometrial biopsy had an accuracy of 86% for the diagnosis of G1/G2 histopathology and that only 1.7% of cases with G1/G2 histopathology diagnosed by office endometrial biopsy were upgraded to G3 adenocarcinoma after examination of specimens obtained from hysterectomy [10].

According to our LNM score, the rate of para-aortic LNM in the low risk group was 0.5%. If treatment strategy including para-aortic lymphadenectomy rescued all patients with para-aortic LNM, the results shown in Fig. 2 suggest that performing para-aortic lymphadenectomy in all cases of endometrial cancer would result in improvement of the 5-year survival rate by about 10%. On the other hand, only 0.5% of patients with no risk factor would benefit from para-aortic lymphadenectomy. According to our

LNM score, the rate of LNM in the low risk group with histologic grade 1 and with invasion of less than half of the myometrium as assessed by MRI was only 1.7%. The rate of LNM increases in proportion to the number of resected lymph nodes [11]. Conversely, cases false-negative for LNM will increase if there are only a few resected lymph nodes. Since the number of resected lymph nodes in this study was much more than that in previous studies, the reported rates of LNM may be reliable.

To conclude, our LNM score may offer useful information for stratification of risk of LNM, and our results suggest that para-aortic lymphadenectomy can be eliminated in cases with no risk factors for LNM in the LNM score and that lymphadenectomy itself may be eliminated in some cases with no risk factors for LNM in the LNM score.

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ORIGINAL ARTICLE

Masashi Takano · Toru Sugiyama · Nobuo Yaegashi · Mitsuaki Suzuki · Hiroshi Tsuda · Satoru Sagae · Yasuhiro Udagawa · Kazuo Kuzuya · Junzo Kigawa · Satoshi Takeuchi · Hitoshi Tsuda · Takuya Moriya · Yoshihiro Kikuchi

Progression-free survival and overall survival of patients with clear cell carcinoma of the ovary treated with paclitaxel-carboplatin or irinotecan-cisplatin: retrospective analysis

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Abstract

Background. Irinotecan hydrochloride, a topoisomerase I inhibitor, has been preliminarily recognized as an effective agent against clear cell carcinoma of the ovary (CCC), but there are few clinical data. Our aim was to compare progression-free survival (PFS) between patients treated with irinotecan hydrochloride and cisplatin (CPT-P) and those with treated with paclitaxel and carboplatin (TC).

Methods. One hundred and seventeen patients at International Federation of Gynecology and Obstetrics (FIGO) stages Ic (ascites/malignant washing) – IV were identified by scanning the medical records of ten Japanese hospitals. After complete surgical staging procedures including lymphadenectomy, 35 patients received CPT-P and 82 patients received TC. The PFS and overall survival of the two groups were compared using the Kaplan-Meier method.

Results. There was no significant difference in median age, performance status, FIGO stage, rate of optimal cytoreduction, or follow-up period between the CPT-P and TC groups. Two-year and 5-year PFS was 48% and 40%, respectively, in the TC group and 55% and 55%, respectively, in the CPT-P group ($P = 0.31$). Multiple regression analysis revealed that only residual tumor was an independent prognostic factor for PFS ($P < 0.01$).

Conclusion. CPT-P showed a potential therapeutic effect, at least no less than that of TC therapy. Although there was no significant survival benefit in the present retrospective analysis, we recommend that the CPT-P regimen be evaluated in a larger, prospective, clinical trial.

Key words Ovarian cancer · Clear cell carcinoma · Irinotecan · Adjuvant chemotherapy · Paclitaxel · Progression-free survival

M. Takano (✉) · Y. Kikuchi
Department of Obstetrics and Gynecology, National Defense Medical College, Namiki 3-2, Tokorozawa, Saitama 359-8513, Japan
Tel. +81-4-2995-1687; Fax +81-4-2996-5213
e-mail: mastkn@ndmc.ac.jp

T. Sugiyama
Department of Obstetrics and Gynecology, Iwate Medical University, Morioka, Japan

N. Yaegashi
Department of Obstetrics and Gynecology, Tohoku University, Sendai, Japan

M. Suzuki
Department of Obstetrics and Gynecology, Jichi Medical College, Tochigi, Japan

H. Tsuda
Department of Obstetrics and Gynecology, Osaka City General Hospital, Osaka, Japan

S. Sagae
Department of Obstetrics and Gynecology, Sapporo Medical University, Sapporo, Japan

Y. Udagawa
Department of Obstetrics and Gynecology, Fujita Health University, Toyoake, Japan

K. Kuzuya
Department of Gynecology, Aichi Cancer Center Hospital, Nagoya, Japan

J. Kigawa
Department of Obstetrics and Gynecology, Tottori University, Yonago, Japan

S. Takeuchi
Department of Obstetrics and Gynecology, Kobe National Hospital, Kobe, Japan

H. Tsuda
Departmnet of Basic Pathology, National Defense Medical College, Tokorozawa, Japan

T. Moriya
Pathology Laboratory of Central Clinical Facilities, Tohoku University, Sendai, Japan

Introduction

Clear cell carcinoma of the ovary (CCC) was initially termed "mesonephroma ovarii" by Schiller in 1939,[1] and in 1973 it was strictly defined by the World Health Organization as lesions characterized by clear cells growing in solid/tubular or glandular patterns, as well as hobnail cells.[2] Many publications have identified the distinctive behavior of CCC. The

most distinctive characteristics recognized are that patients with CCC had worse prognoses compared with those with other pathological types of epithelial ovarian carcinomas[3,4] and that CCC showed resistance to conventional platinum-based chemotherapy.[5–8]

Since the establishment of paclitaxel and carboplatin (TC) as the "gold standard" regimen for epithelial ovarian cancer,[9,10] the regimen has been widely used for all histological subtypes of ovarian tumors. But response in measurable CCC cases treated with TC was relatively low, ranging from 22% to 56%.[11–13] The survival benefit of the regimen is also controversial; one study showed superior survival benefit,[14] and another implied no survival benefit in either early or advanced cases.[15]

As irinotecan hydrochloride, a semisynthetic derivative of camptothecin, has been reported to have additive and synergistic effects in combination with cisplatin in vitro,[16–18] combination therapy with irinotecan and cisplatin (CPT-P) has been used clinically for patients with various solid tumors. Especially, a large clinical trial revealed that CPT-P had significant activity for extensive small-cell lung cancer.[19] Moreover, CPT-P has been reported to be effective in first-line and second-line chemotherapy for the treatment of CCC.[20–22] The aim of the present retrospective study was to compare the survival benefit of combination therapy with CPT-P with that of TC.

Patients and methods

A retrospective review of patients with CCC seen at ten Japanese hospitals from January 1, 1992 to December 31, 2003 was done. Of all the patients treated at these hospitals, the following patients were selected: (a) patients who underwent complete surgical staging procedures, including hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing, omentectomy, pelvic lymphadenectomy, and para-aortic lymphadenectomy; (b) patients whose tumor specimens were confirmed as CCC by two pathologists in a central pathological review; (c) patients who were at International Federation of Gynecology and Obstetrics (FIGO) stages Ic (ascites/malignant washing), II, III, and IV; (d) patients treated with six courses of combination chemotherapy using CPT-P, or six courses of TC; (e) age 75 years or less; (f) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 2 or less; (g) pretreatment leukocyte count of 4000/mm^3 or more, platelet count of 100 000/mm^3 or more, hemoglobin, 9.0 g/dl or more, serum creatinine, less than 1.5 mg/dl, creatinine clearance, 60 ml/min or more, and GOT and GPT less than twice the upper limit of normal at the hospitals. The study was approved by the Ethics Committee at each hospital.

One cycle of the CPT-P regimen consisted of a drip infusion of 50–60 mg/m^2 of cisplatin on day 1 and 50–60 mg/m^2 of irinotecan on days 1, 8, and 15, and 1 week off and it was repeated every 4 weeks. The TC regimen consisted of a drip infusion of 175–180 mg/m^2 of paclitaxel and carboplatin (AUC, 5–6).

The time to progression was defined as the interval from the date of primary surgery until the date of recurrence or tumor progression. Survival duration was determined as the time from the date of primary surgery until death or the date of last follow-up contact. The Kaplan-Meier method was used for the 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 less than 0.05 was considered statistically significant. Stat View software version 5.0 (SAS, Cary, NC, USA) was used to analyze the data.

Results

In all, 35 patients with the CPT-P regimen and 82 with the TC regimen were enrolled in the present retrospective study. The characteristics of the patients are outlined in Table 1. There was no significant difference in median age, performance status, FIGO stage, residual tumor diameter,

Table 1. Characteristics of the patients

	Irinotecan plus cisplatin	Paclitaxel plus carboplatin	P value
Patients (n)	35	82	
Median age, years (range)	52 (34–69)	54 (38–74)	0.79
Performance status			0.12
0	18 (51%)	45 (55%)	
1, 2	17 (49%)	37 (45%)	
FIGO stage			0.94
Ic (Ascites/malignant washing)	13 (37%)	28 (34%)	
II	6 (17%)	15 (18%)	
III	13 (37%)	34 (41%)	
IV	3 (9%)	5 (6%)	
Residual tumor diameter			0.62
0 cm	23 (66%)	52 (63%)	
<1 cm	5 (14%)	8 (10%)	
>1 cm	7 (20%)	22 (27%)	
Follow-up period (months)			0.28
Median	17	21	
Range	5–83	3–73	

Table 2. Multiple regression survival analysis for stage Ic (ascites/malignant washing)-IV patients with clear cell carcinoma of the ovary

Variables (number of patients)	Hazard ratio	95% Confidence interval	*P* value
Age (years)			0.35
<50 (n = 38)	1		
>51 (n = 79)	1.33	0.73; 2.42	
Performance status			0.61
0 (n = 63)	1		
1, 2 (n = 54)	1.17	0.64; 2.14	
FIGO stage			0.16
Ic (Ascites/malignant washing), II (n = 62)	1		
III, IV (n = 55)	1.70	0.81; 3.56	
Residual tumor			<0.01
None (n = 75)	1		
<1 cm (n = 13)	2.54	1.16; 5.57	
>1 cm (n = 29)	3.17	1.35; 7.40	
Chemotherapy			0.21
Irinotecan and cisplatin (n = 35)	1		
Paclitaxel and carboplatin (n = 82)	1.55	0.79; 3.03	

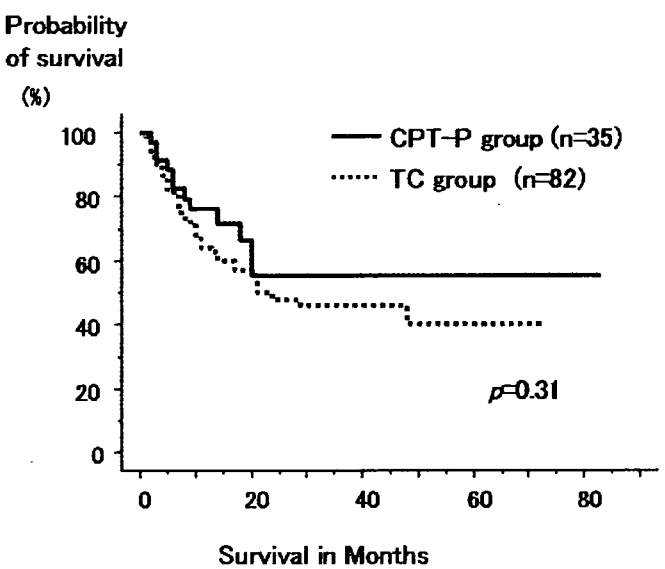

Fig. 1. Kaplan-Meier curves comparing the progression-free survival (PFS) of stage Ic (ascites/malignant washing) – IV patients according to adjuvant chemotherapy. The 2-year and 5-year PFS was 55% and 55%, respectively, in the irinotecan and cisplatin (*CPT-P*) group, and 48% and 40% in the paclitaxel and carboplatin (*TC*) group (P = 0.31)

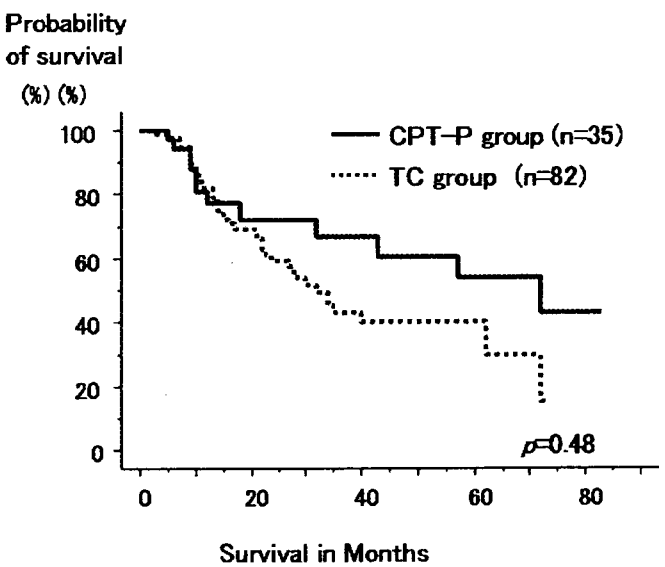

Fig. 2. Kaplan-Meier curves comparing the overall survival of all the patients treated with the combination of irinotecan and cisplatin (*CPT-P*) and those treated with paclitaxel and carboplatin (*TC*; P = 0.48). The 2-year and 5-year overall survival was 72% and 54%, respectively, in the CPT-P group and 60% and 43% in the TC group

or follow-up period between the CPT-P group and the TC group. The median age was 52 years in the CPT-P group and 54 years in the TC group. The CPT-P group included 13 patients (37%) at stage Ic (ascites/malignant washing), 6 (17%) at stage II, 13 (37%) at stage III, and 3 (9%) at stage IV. In the TC group, 28 patients (34%) were at stage Ic, 15 (18%) at stage II, 34 (41%) at stage III, and 5 (6%) at stage IV. Optimal cytoreduction (residual tumor diameter <1 cm) with the initial surgery was achieved in 80% (28/35 patients) in the CPT-P group and 73% (60/82 patients) in the TC group. In patients with tumors at FIGO stages III and IV, the rate of optimal surgery was 56% (9/16 patients) in the CPT-P group and 46% (18/39 patients) in the TC group. The median follow-up period was 17 months in the CPT-P group and 21 months in the TC group.

The 2-year and 5-year progression-free survival (PFS) rates were 55% and 55%, respectively, in the CPT-P group and 48% and 40% in the TC group (Fig. 1; P = 0.31). The 2-year and 5-year overall survival rates were 72% and 54%, respectively, in the CPT-P group and 60% and 43% in the TC group (Fig. 2; P = 0.48). Multiple regression analysis revealed that only residual tumor was an independent prognostic factor for PFS (P < 0.01; Table 2). Age, performance status, and FIGO stage were not significant prognostic factors. Additionally, chemotherapy was also not an independent factor for PFS in the CCC patients in the present

study (TC compared with CPT-P: hazard ratio, 1.55; 95% confidence interval, 0.79 to 3.03, $P = 0.21$).

Discussion

It has been well recognized that CCC has low sensitivity to conventional platinum-based chemotherapy.[3,4,7] But it is still uncertain which regimen would be the best candidate for CCC. Some reports have indicated a survival benefit of paclitaxel and platinum therapy in comparison with platinum-based chemotherapy.[10,12] A larger study implied that a combination with paclitaxel and platinum had almost the same impact on survival as conventional platinum-based chemotherapy in both early- and advanced-stage patients.[15]

The CPT-P regimen was initially introduced as a treatment for platinum-refractory ovarian cancer.[22] Since then, the regimen has been used for the treatment of CCC as first-line chemotherapy and has shown moderate activity against CCC.[20,21] The present study implies that the survival of patients treated with CPT-P might be improved compared with the survival of those treated with TC. However, our study was a limited retrospective study and failed to prove the superiority of the CPT-P regimen. The effectiveness of irinotecan as well as paclitaxel against CCC was also confirmed in vitro.[23] Combined with mitomycin C, irinotecan also showed higher activity than conventional platinum-based chemotherapy.[24] Chemotherapeutic regimens including irinotecan have been suggested to have a potential antitumor effect against CCC as first-line chemotherapy.

CCC has been reported to have distinct molecular characteristics compared with other histological subtypes. The overexpression of hepatocyte nuclear factor-1 beta[25] and that of ABCF2, a member of the ATP-binding cassette gene superfamily[26] were observed in CCC. These molecules might be another or additive target in the treatment of CCC.

Although there was no statistically significant difference in survivals between the CPT-P and TC regimens in our study, CPT-P was shown to have the same chemotherapeutic benefit in the survival of CCC patients as TC. Therefore, we recommend that the CPT-P regimen be tested in a large-scale prospective study.

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Treatment of squamous cell carcinoma of the uterine cervix with radiation therapy alone: long-term survival, late complications, and incidence of second cancers

T Ota*,[1], N Takeshima[1], T Tabata[1], K Hasumi[1] and K Takizawa[1]

[1]*Department of Gynecology, Cancer Institute Hospital, Ariake 3-10-6, Koutou-ku, Tokyo 135-8550, Japan*

The objective of this retrospective study was to determine the survival rate, incidence of late complications, and incidence of second cancers when radiation therapy alone is used for carcinoma of the uterine cervix. Between 1971 and 1995, 1495 patients with squamous cell carcinoma of the uterine cervix (stages I–IV) were treated with radiation therapy alone in our hospital. Radiation therapy consisted of a combination of high-dose-rate intracavitary brachytherapy and external beam radiotherapy. The cumulative 5-year survival rates for stages Ib, II, and III/IVa carcinoma were 93.5, 77.0, and 60.3%, respectively, and the 10-year survival rates were 90.9, 74.5, and 56.1%, respectively. Local control rates for stages Ib, II, and III/IVa carcinoma were 92.0, 79.4 and 64.2%, respectively. Eighty-two (5.5%) patients suffered grade III/IV or V (fatal) complications. A second cancer developed in 13 (0.87%) patients. Second cancers were observed most frequently in the rectum (five cases), colon (three cases), and uterine body (two cases). Long-term follow-up data revealed that our method of radiation therapy alone for locally advanced carcinoma of the uterine cervix is effective, with low incidences of late complications and second cancers.

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Cervical cancer is one of the most common cancers in women worldwide. Despite worldwide efforts at screening with the aim of detecting cervical cancer in the early stage, many cancers are not discovered until already advanced. Traditionally, radical hysterectomy or radiation therapy alone has been accepted as standard treatment for early-stage invasive cervical cancer, and locally advanced cancer has been treated by radiotherapy alone consisting of a combination of high-dose-rate intracavitary brachytherapy (ICBT) and external beam radiotherapy (EBRT) (Coia *et al*, 1990; Komaki *et al*, 1995; Barillot *et al*, 1997). In the past few years, substantial advances in the management of locally advanced cervical cancer have been reported. Five randomised trials showed improved survival and local control when cisplatin-based chemotherapy was added concurrently to radiation treatment in patients with locally advanced cervical cancer (Keys *et al*, 1999; Morris *et al*, 1999; Rose *et al*, 1999; Whitney *et al*, 1999; Peters *et al*, 2000). This combined modality approach produced an absolute increase in 5-year survival of 12% as compared with radiation therapy alone and resulted in a sudden change in the standard of care for this disease. Now that concurrent treatment with cisplatin-based chemotherapy and radiotherapy is the standard treatment for locally advanced cervical cancer, the main issue in treatment is how chemotherapy is used, not much attention is given to the radiotherapy method.

For more than 30 years, we have treated locally advanced cervical cancer by radiation therapy alone. In this study, we retrospectively analysed 1495 cases of cervical carcinoma treated at our institution from 1971 to 1995 by a unique combination EBRT/ICBT regimen. We were interested in determining the long-term survival rate as well as the incidences of late complications and second cancers.

*Correspondence: Dr T Ota; E-mail: tsuyoshi.ota@jfcr.or.jp
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METHODS

Study population

From 1971 through 1995, a total of 1600 new patients with primary invasive cervical carcinoma were treated by radiotherapy alone at the Cancer Institute Hospital, Tokyo, Japan. Of these patients, 1495 (93.4%) had pure squamous cell carcinoma (SCC). The remaining 105 patients with non-SCC were excluded from this study. After an initial clinical examination, all patients underwent a complete staging workup, including a complete blood count, blood chemistry tests, chest radiography, and biopsy of the cervical tumour. Cystoscopy and drip infusion pyelography were performed in all patients; only in suspicious case was sigmoidoscopy performed.

The numbers of patients are shown by cancer stages in Table 1. Stages were determined according to the International Federation of Gynecology and Obstetrics criteria. Patients with distant metastasis (ie stage IVb cervical carcinoma) before treatment and patients treated by chemotherapy before radiation were excluded.

Treatment schedule

The radiation treatment schedule is shown in Figure 1. A combination of EBRT and ICBT was used in all patients according

Table 1 Cervical carcinomas treated with radiotherapy alone

Clinical stage	No. patients	%	
Stage Ib	174	11.6	
Stage IIa	25	1.7	40.7
Stage IIb	583	39.0	
Stage IIIa	16	1.1	45.1
Stage IIIb	658	44.0	
Stage IVa	39	2.6	
Total	1495	100	

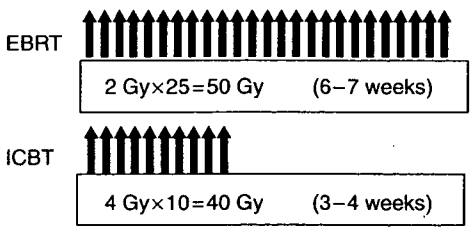


Figure 1 Treatment schedule for radiation therapy.

to a regimen that is unique to our institution. For all patients, including those with stage III or IV disease, it was planned that EBRT and ICBT would begin simultaneously. However, in 431 patients (28.9%) we were unable to insert the uterine applicator at the time of radiotherapy was initiated because of the tumour bulk, thus, EBRT was started alone without the use of a midline shield until 20 Gy was delivered; ICBT was performed later. In patients who initially received 20 Gy with unshielded EBRT, reduction of one to three fractions of ICBT was considered, depending on the radiation effect. Occasionally, ICBT could not be applied even after EBRT. Indeed, in 13 patients, radiotherapy was achieved with EBRT alone. Overall treatment time ranged from 6 to 7 weeks.

External beam radiotherapy to a total dose of 50 Gy was delivered in 2 Gy fractions per day, 5 days a week, by using an isocentric technique via a pair of parallel opposed anterior and posterior ports and 18-MV X-ray beam. Both pelvic and para-aortic fields were irradiated except in patients with non-bulky stage I disease; only pelvic radiation was performed in these patients. The superior margin of the para-aortic radiation field was the L2–3 intervertebral space, the lateral limit of each pelvic field was 2 cm lateral to the most lateral point of the pelvic wall, and the lower limit was the caudal pole of the obturator foramen. A midline shield was inserted throughout the external radiation period (except in patients in whom EBRT was started before ICBT) regardless of the stage of cervical carcinoma. The midline shield was 4 cm wide at the central axis.

High-dose-rate remote afterloading ICBT was performed with a uterine applicator and two vaginal applicators used with ^{60}Co sources (2–4 Ci). Low-dose consecutive brachytherapy was prescribed to all patients. A dose of 4 Gy in one fraction was routinely prescribed to point A, which was located 2 cm superior to the cervical ostium and 2 cm lateral to the central axis of the uterus. Intracavitary brachytherapy was performed with two or three insertions per week, for a total of 10 fractions. When ICBT was applied to a bulky tumour at the start of treatment, the dose of radiation delivered to point A was gradually adjusted in parallel with the reduction in tumour size. Because ICBT was divided into 10 fractions, this process was accomplished without severe morbidity. Orthogonal radiographs were obtained for each insertion, and each treatment session was planned. In the majority of cases, neither anaesthesia nor sedation was necessary during ICBT. EBRT and ICBT were not performed on the same day. When ICBT was performed, EBRT was performed the next day.

Survival

Survival of each patient was calculated from the date therapy was started to the date of the last follow-up examination. Survival curves were drawn according to the Kaplan–Meier method. The log-rank test was used for univariate analysis. *P*-values of less than 0.05 were considered statistically significant.

Complete response was defined as absence of cancer cells as determined by smear cytology and biopsy of the uterine cervix. Recurrence was defined as reappearance of cancer cells.

Local control rate

Complete response was defined as no remaining cancer cells according to cytologic and histologic assessment for over 3 months after radiotherapy. Local control was defined as absence of recurrence in the pelvic cavity.

Late complications

Late rectal and bladder complications and non-rectal gastrointestinal sequelae (small bowel complications) were graded according to the Radiation Therapy Oncology Group (*RTOG*)/the European Organization for Research and Treatment of Cancer (*EORTC*) scoring system (Cox *et al*, 1995).

Second cancers

Cancer in the radiation field that differed histologically from the primary cancer, as distinguished from recurrence or metastasis from the primary tumour, was considered a second cancer. The person-years proposed by Schoenberg and Myer (1972) was used for statistical analysis. The disease expectancy table used for the present study was the one provided by the Center for Cancer Control and Information Services, National Cancer Center, Japan, which calculated expected ratios from 1995.

Follow-up

Follow-up examinations were done every 3 months during the first 5 years after treatment and then at 6-month intervals for the next 5 years. All follow-up examinations included pelvic examination with cytologic assessment of the uterine cervix and tumour marker SCC antigen, and identification of late complications. Every 6 months, we obtained a computed tomography scan of the abdomen and a chest X-ray firm. All patients were followed up for more than 10 years after radiation therapy. Our hospital is one of a few institutions that are permitted to access the family registry database. We consulted the district legal affairs bureau for survival information or the cause of the death pertaining to each patient, so our records were complete; no patient was lost to follow-up. Of the total 1495 patients, 1224 (81.9%) were followed up directly at the hospital and 271 (18.1%) were followed up indirectly through the district legal affairs bureau.

RESULTS

Study population

Patient numbers by cancer stage of the study are shown in Table 1. Mean age of the patients was 60.6 years (range, 29–93 years). Of the total 1495 patients, the numbers of patients with stage Ib, II, and III/IVa disease were 174 (11.6), 608 (40.7), and 713 (47.7%), respectively.

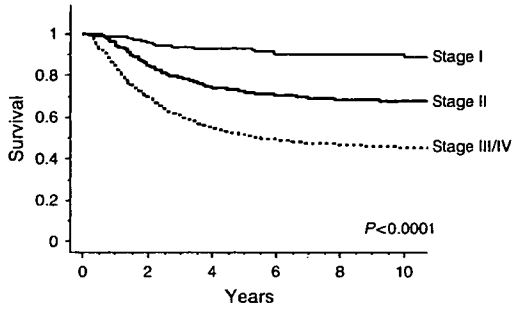
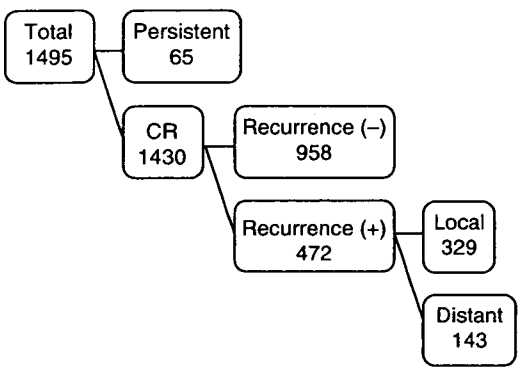


Figure 2 Disease-specific survival per clinical stage for patients with cervical carcinoma treated with radiotherapy alone.



Control rate: (958+143)/1495=73.6%

Figure 3 Local control of cervical carcinoma treated by radiotherapy alone.

Survival

Disease-specific survival curves are shown according to cancer stages in Figure 2. The cumulative 5-year survival rates for stages Ib, II, and III/IVa disease were 93.5, 77.0, and 60.3%, respectively. Ten-year survival rates for stages Ib, II, and III/IVa disease were 90.9, 74.5, and 56.1%, respectively.

Local control

Local control and recurrence are shown in Figure 3. Of the total 1495 patients, 1430 patients had a complete response, as shown by cytologic and histologic assessment. Of these 1430 patients, 472 patients suffered recurrence (local recurrence, 329 patients; distant metastasis, 143 patients). Thus, the local control rate was 73.6%. Local control rates for stages Ib, II, and III/IVa disease were 92.0, 79.4, and 64.2%, respectively. The most common sites of recurrence were the lung (55 patients) and supraclavicular lymph nodes (35 patients).

Late complications

Late complications are presented in Table 2. According to the *RTOG/EORTC* scoring system, grade III, IV, or V (fatal) late complications involving the rectum, small bowel, or urinary tract were observed in 97 (6.5%) cases. Sixty-one cases (4.1%) involved stage III disease. The crude incidences of grade III, IV, and V (fatal) rectal complications were 1.1% (17 patients), 1.5% (22 patients), and 0.07% (1 patient), respectively. One patient with a rectal complication died of uncontrolled rectal bleeding 8 years after radiation therapy. The crude incidences of grade III and IV small bowel complications were 0% (0 patients) and 0.7% (10 patients), respectively. The crude incidences of grade III and IV urinary tract complications were 1.5% (23 patients) and 0.8% (12 patients), respectively. The most common grade III complications were haematuria (1.5%) and proctitis (1.1%). The most common grade IV complications were rectovaginal fistula (1.1%) and vesicovaginal fistula (0.8%). Nine patients (0.6%; one with stage I disease, two with stage II disease, five with stage III disease, and one with stage IV disease) required reconstruction of both the urinary tract and lower gastrointestinal tract.

Table 2 Grades of late complications according to site

	Grade III	Grade IV	Grade V (fatal)
Rectum	13 (0.9%)	21 (1.4%)	1 (0.07%)
Small bowel	11 (0.7%)	—	—
Bladder	17 (1.1%)	9 (0.6%)	—
Combined	—	10 (0.7%)	—
Total	41 (2.7%)	40 (2.7%)	1 (0.07%)

Total 82 cases (5.5%).

Table 3 Second cancers after treatment with radiotherapy alone

Site	Observed	Expected	O/E
Rectum	5	1.9	2.6
Colon	3	3.3	0.9
Uterine body	2	0.7	2.9
Ovary	1	1.0	1.0
Acute leukaemia	1	0.5	1.9
Pelvic MFH	1	—	—

Abbreviation: MFH = malignant fibrous histiocytoma.

Second cancers

The incidence of second cancers and observed/expected ratio of incident cancers are shown in Table 3. Second cancers were observed in 13 cases (0.87%). The most frequent sites were the rectum (five cases), colon (three cases), and uterine body (two cases). Analysis on a site-by-site basis revealed an excess of second cancers in the rectum and uterine body and of occurrences of acute leukaemia.

DISCUSSION

Our radiation therapy regimen differs from the international standard. The concept is unique in that the primary tumour is treated by intracavitary irradiation, and the pelvic and para-aortic lymph nodes are treated by external beam irradiation to a total 50 Gy in 25 fractions. Intracavitary brachytherapy, at a dose of 4 Gy in one fraction routinely prescribed to point A, was performed with two or three insertions per week, for a total of 10 fractions in total. It is essential that both radiation therapies are started simultaneously and that EBRT is performed with a midline shield, no matter what the disease stage. It is difficult to insert the uterine applicator into a bulky tumour at the beginning of treatment. Skill is needed. A midline shield was used throughout the external radiation period, so it is possible that dose of radiation delivered to point A was higher. The other important factor is that the overall treatment time is 7 weeks or less.

Our regimen yielded good results and decreased the incidence of complications for a number of reasons. First, the overall treatment period is short. Several studies have shown that overall treatment time is a significant prognostic factor for patients with cervical cancer treated with radiation therapy alone (Fyles *et al*, 1992; Girinsky *et al*, 1993; Lanciano *et al*, 1993; Perez *et al*, 1995;