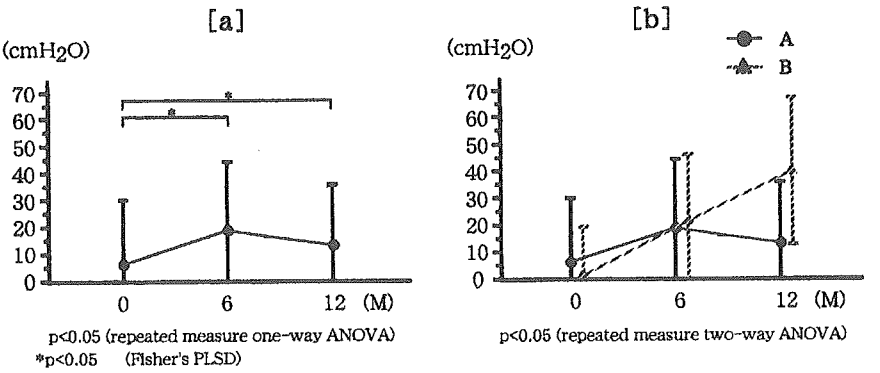

Figure 3. Pabd Qmax. a) Preoperative and postoperative measurement in group A patients. b) Comparison of changes in measurement between groups A and B.

that about 80% of patients with uterine cervical cancer already have some degree of bladder dysfunction before undergoing surgery[9]. It would not be possible to determine whether poor Cves is due to the operation or due to a dysfunction that already existed before the operation, if preoperative tests were not carried out. There has been criticism of studies in which urodynamic analysis was only carried out after the operation[10]. Other important points for urodynamic studies of urinary function after radical hysterectomy are to conduct tests on the subjects at the same time for comparison of results among the subjects and to carry out not only short-term but also long-term assessments. Voiding dysfunction symptoms after radical hysterectomy have been thought to disappear within 6–12 months after the operation[11,12]. However, urodynamic studies have revealed that there does exist underlying bladder dysfunction after 12 months of operation[13].

In this study, the results showed that our technique does not cause a deterioration in Cves and MFR and an increase in residual urine volume at 12 months after the operation, although it does cause some amount of increase in Pabd Qmax and reduction in Pdet Qmax. Actually, there was no patient who complained of urinary difficulty at 12 months after the operation, which was confirmed by a urodynamic study and a questionnaire. That is to say, radical hysterectomy combined with autonomic nerve preservation did not cause significant deterioration in QoL of our patients. There have been very few reports in which the surgical technique used and the timing of tests are clearly described, preoperative and postoperative values for all the patients are compared, and results of both short-term and long-term tests are presented. Such description and presentation of results appear in a report by Scotti *et al.*, but, unfortunately, type II operations were performed on half of their patients[14]. In our experience, bladder dysfunction is rarely a problem in a type II operation.

We employ our technique for autonomic nerve preservation to the uninvaded side for patients with stage IIB uterine cervical cancer. It is reported that the normal urinary function could be maintained when at least one side of the sympathetic nerve was preserved in experimental animals[15]. This data suggest that the normal urinary function can be maintained by applying the operation with autonomic nerve preservation to the noninvaded side in patients with stage IIB cervical cancer who had parametrial invasion only at one side.

Piver's type III (Wertheim–Meigs) operation is the treatment of choice for FIGO stages IB–IIA cervical cancer in Western countries. The standard treatment

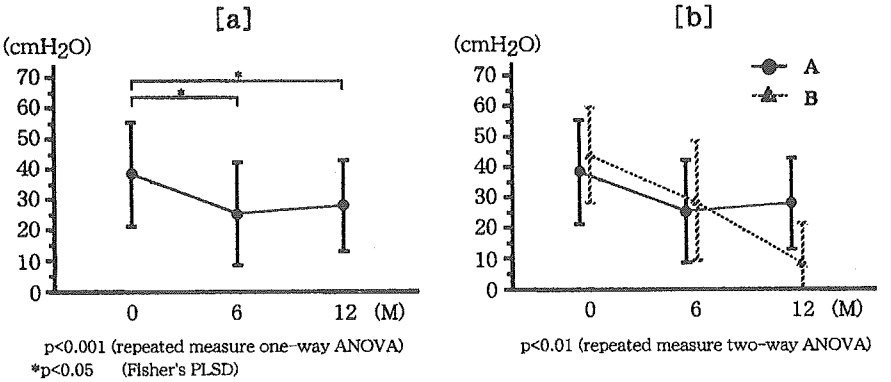

Figure 4. Pde Qmax. a) Preoperative and postoperative measurement in group A patients. b) Comparison of changes in measurement between groups A and B.

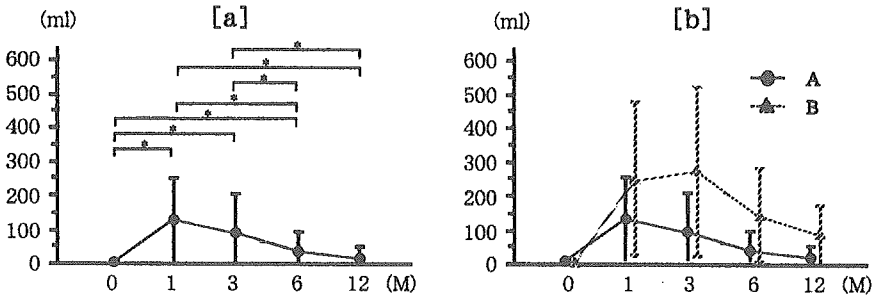

Figure 5. Residual urine volume. a) Preoperative and postoperative measurement in group A patients. b) Comparison of changes in measurement between groups A and B.

for patients with stages IB–IIB cervical cancer in Japan is radical hysterectomy originally described by Okabayashi[16]. This surgery is more radical than Wertheim hysterectomy. According to the recent statistics regarding treatment of cervical cancer announced from the Japan Society of Obstetrics and Gynecology[17], only 4% of patients with stage IIB cervical cancer received primary radiotherapy. Concurrent chemoradiation is not a standard treatment in Japan at this moment. The radiation therapy will cause permanent loss of ovarian function and sexual disturbance due to fibrous stenosis of vaginal canal, and these are quite detrimental for sexually active women. We have performed procedures for preserving ovarian function and preventing shortening of the vagina[8–21] in an attempt to maintain QoL of patients with cervical cancer treated with radical hysterectomy. Regarding chemoradiation, however, a large-scale clinical trial needs to be conducted in Japan too.

In conclusion, our aim seems to have been achieved because the urinary function, both subjectively and objectively, at 12 months after operation is almost normal.

Acknowledgment

The authors wish to thank Dr Kenichi Oguchi for his contribution to introducing urodynamic analysis in the care of patients undergoing radical hysterectomy, without whom our attempts would not have been started and completed.

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Functional analysis of *p53* gene and the prognostic impact of dominant-negative *p53* mutation in endometrial cancer

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In addition to the loss of function, mutant p53 can possess a dominant-negative effect on wild-type p53 and may also exert gain-of-function activity. It is not clear whether the functional status of *p53* mutation contributes to differences in outcome in endometrial cancer. We collected a total of 92 RNA samples of high quality from endometrial cancer tissues, and the samples were subjected to yeast functional assay and sequencing for *p53* mutations. The detected mutant *p53* genes were further investigated for their dominant-negative activity using a yeast-based transdominance assay. *p53* mutation was found in 24 out of 92 (26.1%) tumors, of which 10 exhibited no dominant-negative activity (recessive mutation) and 14 showed dominant-negative activity. Dominant-negative *p53* mutation was related to advanced stages ($p = 0.01$), non-endometrioid type tumors ($p = 0.01$) and grade 3 tumors ($p = 0.04$). The patients with dominant-negative mutation had significantly shorter survival than patients with no mutation ($p < 0.0001$) and those with a recessive mutation ($p = 0.01$) in the *p53* gene. No difference in survival was found between the patients with tumors harboring a recessive *p53* mutation and those with tumors harboring a wild-type *p53*. Multivariate analysis revealed that dominant-negative *p53* mutation ($p = 0.019$), FIGO stage ($p = 0.0037$) and histologic subtype ($p = 0.014$) were independently related to patient survival. Dominant-negative *p53* mutation was the most important prognostic factor for stage III/IV endometrial cancer ($p = 0.0023$). In conclusion, dominant-negative *p53* mutation is often found in advanced stages and aggressive histologic subtypes of endometrial cancer and it is a strong predictor of survival of patients with advanced endometrial cancer. To elucidate further the role of *p53* mutation in endometrial cancer, it is necessary to investigate gain-of-function activity involving dominant-negative p53 mutant proteins.

Key words: endometrial cancer; *p53*; mutation; dominant negative; survival; serous adenocarcinoma

The *p53* tumor suppressor gene is mutated in about 50% of all tumors,[1–3] and more than 19,000 different somatic mutations have been identified.[4] Mutation of the *p53* gene plays a key role in the carcinogenesis and progression of many different malignancies, including endometrial cancer. p53 overexpression has been shown to predict patient survival in endometrial cancer.[5–7] p53 overexpression, as determined by immunohistochemistry (IHC), is a surrogate marker of missense mutation of p53 protein. Because MDM2 is a transcriptional target of p53, loss of p53 function reduces the production of MDM2. MDM2 degrades p53 protein through ubiquitination of the protein, and reduced MDM2 production will lead to an accumulation of p53 protein in the nucleus, which is detected as overexpression by IHC.[8] IHC is a convenient method for the investigation of p53 status. However, it can be affected by many factors, such as antibody used, antigen retrieval technique and subjectivity of criteria for p53 overexpression. p53 overexpression does not necessarily correspond to *p53 gene* mutation. One report showed that only 32% of tumors with exclusively nuclear staining were found to contain a *p53* gene mutation.[6] Dominant-negative *p53* mutation will lead to decreased MDM2 production, irrespective of the status of the second allele of *p53*. *p53* mutations abolishing the production of p53 protein will lead to loss of p53 protein when it is associated with loss of heterozygosity (LOH) of the second allele. Therefore, p53 overexpression can be related to both dominant-negative mutation and recessive missense mutation with LOH. It is therefore reasonable to expect that p53 overexpression does not necessarily correspond to a dominant-negative mutation of *p53*.

At least 2 distinct pathways have been proposed that contribute to cisplatin-induced apoptosis *in vitro*. One involves p53 tumor suppressor protein, and the other is mediated by the p53-related protein p73. Inhibition of p73 function by dominant-negative p73 proteins or by mutant p53 abrogates apoptosis and cytotoxicity induced by these agents.[9] Therefore, investigation into the status of p73 in endometrial cancer is expected to provide further information regarding the response to adjuvant chemotherapy in cases involving this type of cancer.

An understanding of the role played by *p53* mutation in endometrial cancer may lead to tailored treatment planning and more rational targeted approaches for treating this disease. *p53* gene mutation and LOH result in the loss of p53 function. In addition to the loss of function, mutant p53 can possess a dominant-negative effect that suppresses wild-type p53, and which may also exert gain-of-function activity.[10] There have been many studies of the prognostic significance of and/or therapeutic outcome related to the type of *p53* mutations. However, the results of such studies have been inconsistent,[11] and the significance of the dominant-negative function in terms of both prognosis and therapeutic success remains unclear at present.[12]

In order to elucidate the prognostic importance of dominant-negative activity in endometrial cancer, we surveyed the functional status of p53 protein in endometrial carcinoma using a yeast p53 functional assay and a transdominance assay. The yeast p53 functional assay tests the ability of p53 to activate transcription *in vivo* in yeast.[13,14] A modification of this method (transdominance assay) can identify the dominant-negative/recessive properties of the mutant p53.[15] This is the first report to compare directly the functional status of p53 (*i.e.*, the presence or absence of dominant-negative activity) to the survival of patients with endometrial cancer.

Material and methods

Tissue specimens

A total of 92 endometrial carcinoma tissue samples, which were obtained from the resected uterus of patients with endometrial carcinoma treated surgically at the Department of Obstetrics and Gynecology, Mainz University in Mainz, Germany, and at the

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TABLE I – CLINICOPATHOLOGIC CHARACTERISTICS OF PATIENTS WITH ENDOMETRIAL CARCINOMA TREATED IN MAINZ UNIVERSITY AND HOKKAIDO UNIVERSITY

	Mainz, Germany (n = 49)	Sapporo, Japan (n = 43)	p
Age			
< 60	13	26	
≥ 60	36	17	0.001
FIGO stage			
I/II	37	28	
III/IV	12	15	0.27
Histologic subtype			
Endometrioid	44	35	
Nonendometrioid	5	8	0.25
Grade			
1	17	14	
2	19	17	
3	13	12	0.98
Follow-up period (months)	1–130 (median, 58.5)	5–125 (median, 41.0)	0.11

Department of Gynecology, Hokkaido University Graduate School of Medicine and School of Medicine in Sapporo, Japan, were used for this study. Informed consent was obtained from all study participants. From among the 92 samples, 49 were obtained from German patients and 43 from Japanese patients. Surgical treatment was initiated during the period between April 1990 and January 2000 for the German cohort and between February 1990 and December 2002 for the Japanese cohort. The data from 23 patients of the 43 Japanese cohort participants were reported in a previous study.[16] The clinicopathologic variables, namely, FIGO stage, histologic subtype, grade of tumor and follow-up period, did not differ between the 2 cohorts, with the exception of patient age (Table I). The tissue samples included 79 cases of endometrioid-type adenocarcinoma and 13 cases of nonendometrioid adenocarcinoma. The group of nonendometrioid tumors included 11 serous adenocarcinomas, 1 clear cell adenocarcinoma and 1 squamous cell carcinoma. The treatment strategies employed in the 2 institutes were different. At Mainz University, the surgical procedure involved selective lymph node dissection rather than routine systematic lymphadenectomy. At Hokkaido University, routine systematic pelvic and paraaortic lymphadenectomy was carried out. The modality of adjuvant therapy employed at Mainz University was radiotherapy, whereas that used at Hokkaido University was chemotherapy.

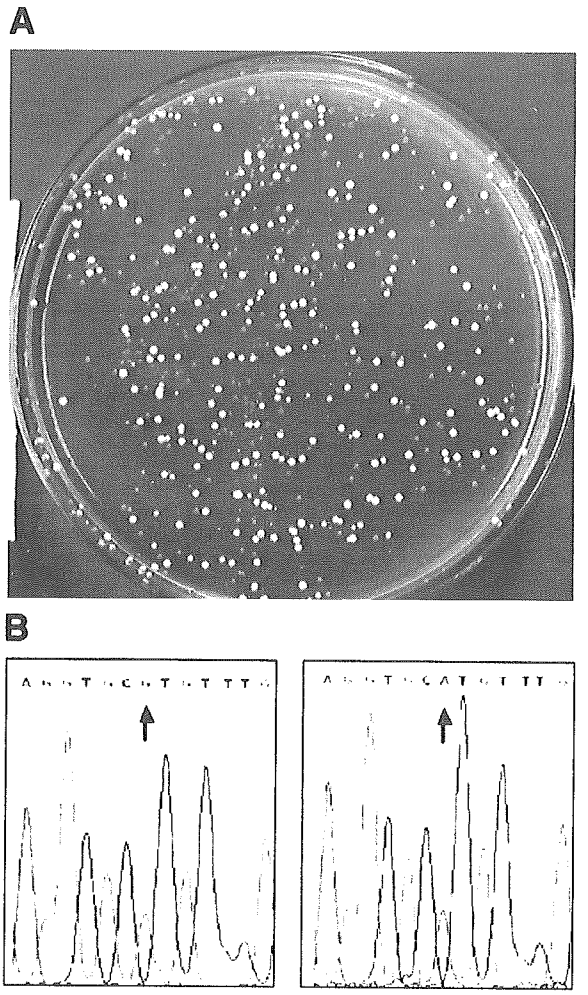

FIGURE 1 – An example of positive yeast p53 functional assay (*a*) and confirmation of p53 mutation by DNA sequencing of the reverse strand: (*b*) wild-type sequence; (*C*) missense mutation of CGT to CAT at codon 273.

RNA extraction and reverse transcription (RT)-PCR

Total RNA was extracted from 100–200 mg of each frozen tissue sample by the guanidinium/phenol/chloroform method (TRIzol reagent; Gibco-BRL, Gaithersburg, MD). RNA integrity was verified by electrophoresis on 1% agarose gel. *p53* cDNA was synthesized at 37°C for 1 hr with 200 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco-BRL) from 1–3 μg of total RNA in 20 μl of RT buffer containing 25 pmol *p53*-specific primer RT-1 (5′-CGGGAGGTAGAC-3′), 7.5 mM dithiothreitol (DTT), 0.5 mM $MgCl_2$ and 0.5 mM of each dNTP. The *p53* cDNA was PCR-amplified in 20 μl of reaction mixture containing 2 μl of RT reaction product, 1.25 units of *Pfu* DNA polymerase (Stratagene, La Jolla, CA), 10% DMSO, 50 μM of each dNTP and 10 pmol of primers P3 [5′-ATTTGATGC-TGTCCCCGGACGATATTGAA(s)C-3′, where (s) represents a phosphorothioate linkage] and P4 [5′-ACCCTTTTTGGACTT-CAGGTGGCTGGAGT(s)G-3′]. PCR was run on a Thermal Cycler Model 2400 (Perkin-Elmer, Chiba, Japan) at 96°C for 1 min, then for 35 cycles of 95°C for 40 sec, 65°C for 70 sec and 78°C for 90 sec, followed by 78°C for 2 min. Satisfactory amplification was confirmed by examining the PCR product in a 1% agarose gel. Each crude PCR product was used for the transformation of yeast.

Plasmids

The yeast expression vector pSS16[17] was digested with excess amounts of *Hind*III and *Stu*I, dephosphorylated with calf intestinal alkaline phosphatase (Takara, Otsu, Japan) and electrophoresed on 1% low-melting-temperature agarose (Sea Plaque agarose; FMC, Rockland, ME). The linearized plasmids were recovered from the gel and purified with Wizard PCR prep kit (Promega, Madison, WI). A gap was created between codons 67 and 347.

Yeast p53 functional assay

The yeast functional assay was performed according to a method described previously.[14,18] The yeast reporter strain yIG397[15] was used throughout the study. The strain yIG397 contains an integrated plasmid with the ADE2 (phosphoribosylaminoimidazole carboxylase, EC 4.1.1.21) open reading frame under the control of a p53-responsive promoter. The genotype is *MAT*a *ade2-1 leu2-3 112trp1-1 his3-11 15can1-100 ura3-1::*[*URA3 3*xRGC-p*CYC1-ADE2*]. When a yeast cell is transformed with a plasmid encoding mutant p53, the cell fails to express ADE2 and forms a red colony due to the accumulation of an oxidized polymerized derivative of phosphoribosyl-aminoimidazole.[19] The yeast was cultured in 100 ml of YPD medium supplemented with 200 μg/ml of adenine until the OD600 value reached 0.8. The cells

TABLE II – DISCOVERED *p53* MUTATIONS AND ITS TRANSDOMINANCE IN 92 ENDOMETRIAL CARCINOMAS

Institute and case number	FIGO stage	Histologic subtype	Grade	Mutation in *p53* gene	Base change	Type of mutation	Transdominance
Hokkaido University							
1	I	Endometrioid adenocarcinoma	2	213 Arg→Stop	CGA→TGA	Nonsense	
2	I	Endometrioid adenocarcinoma	2	321bp deletion (nt 673–993)		Deletion	
3	I	Endometrioid adenocarcinoma	1	133 Met→Arg	ATG→AGG	Missense	
4	I	Endometrioid adenocarcinoma	1	244 Gly→Asp	GGC→GAC	Missense	DN
5	I	Endometrioid adenocarcinoma	2	363bp deletion (nt 385–747)		Deletion	
6	I	Endometrioid adenocarcinoma	1	108 Gly→Ser	GGT→AGT	Missense	
7	I	Endometrioid adenocarcinoma	1	241 Ser→Ala	TCC→GCC	Missense	DN
8	I	Serous adenocarcinoma	3	273 Arg→His	CGT→CAT	Missense	DN
9	II	Serous adenocarcinoma	1	240–243 in frame deletion	AGTTCCTGC ATG→GTG	Deletion	DN
10	III	Endometrioid adenocarcinoma	3	264 Leu→Arg	CTA→CGA	Missense	
11	III	Endometrioid adenocarcinoma	2	280 Arg→Ile	AGA→ATA	Missense	DN
12	III	Endometrioid adenocarcinoma	2	173 Val→Leu	GTG→TTG	Missense	
13	III	Serous adenocarcinoma	2	273 Arg→His	CGT→CAT	Missense	DN
14	IV	Endometrioid adenocarcinoma	3	280 Arg→Gly	AGA→GGA	Missense	DN
15	IV	Serous adenocarcinoma	3	248 Arg→Trp	CGG→TGG	Missense	DN
Mainz University							
1	I	Endometrioid adenocarcinoma	3	306 Arg→Stop	CGA→TGA	Nonsense	
2	I	Endometrioid adenocarcinoma	2	280 Arg→Ser	AGA→AGT	Missense	DN
3	I	Endometrioid adenocarcinoma	3	273 Arg→Cys	CGT→TGT	Missense	DN
4	I	Clear cell adenocarcinoma	3	245 Gly→Val	GGC→GTC	Missense	
5	II	Endometrioid adenocarcinoma	2	257 Leu→Pro	CTG→CCG	Missense	
6	III	Endometrioid adenocarcinoma	3	248 Arg→Gln	CGG→CAG	Missense	DN
7	III	Endometrioid adenocarcinoma	1	273 Arg→His	CGT→CAT	Missense	DN
8	IV	Endometrioid adenocarcinoma	3	175 Arg→His	CGC→CAC	Missense	DN
9	IV	Serous adenocarcinoma	3	273 Arg→His	CGT→CAT	Missense	DN

DN, dominant negative.

were pelleted, washed with LiOAc solution containing 0.1 M lithium acetate, 10 mM Tris-HCl, pH 8.0, and 1 mM $EDTANa_2$; the cells were then pelleted again and resuspended in 500 µl of LiOAc solution. For each transformation, 50 µl of yeast suspension were mixed with 1–5 µl of unpurified *p53* cDNA PCR product, 50–100 ng of linearized plasmid, 5 µl of sonicated single-stranded salmon sperm DNA (10 mg/ml) and 300 µl of LiOAc containing 40% polyethylene glycol 4000 (Kanto, Tokyo, Japan). The mixture was incubated at 30°C for 30 min and heat-shocked at 42°C for 15 min. The yeast was then plated on a synthetic dropout (SD) medium minus leucine plus adenine (5 µg/ml) and was incubated for 48 hr in a 30°C humidified chamber. More than 200 colonies were examined on each culture plate. In this assay system, 16% was the cutoff value for *p53* mutation.[20]

Recovery of p53 plasmids from yeast and DNA sequencing

The yeast was digested with Zymolase-100T (Seikagaku-Kogyo, Tokyo, Japan), and p53 expression plasmids were extracted by the alkaline lysis method (QIAprep plasmid kit; Qiagen, Hilden, Germany) and transfected into XL-1 blue *E. coli* by electroporation. The plasmids were recovered, purified and sequenced with a Dye-Deoxy Terminator Kit (Perkin-Elmer, Urayasu, Japan) on an ABI 377 automated sequencer (Applied Biosystems, Urayasu, Japan) as specified by the manufacturer's protocol and using the following primers: P3seq, 5′-ATTTGATGCTGTCCCCGGACGATATTGAAC-3′; P11seq, 5′-TACTCCCCTGCCCTCAACAAGATG-3′; P12seq, 5′-TTGCGTGTGGAGTATTTGGATGAC-3′; and P13seq, 5′-GCC-CATCCTCACCATCATCACACT-3′.

Transdominance assay

The dominant-negative potential of *p53* mutation was tested using a yeast-based transdominance assay as described previously.[15] Briefly, the yeast functional assay was performed using both a plasmid with wild-type *p53* and a plasmid with mutant *p53* that had been sequence-verified. For each transformation, 50 µl of yeast suspension were mixed with 100 ng of pTSHP53, 100 ng of mutant *p53*-containing pSS16, 50 µg of sonicated single-stranded salmon sperm DNA and 300 µl of LiOAc containing 40% polyethylene glycol 4000. The mixture was incubated at 30°C for 30 min and heat-shocked at 42°C for 15 min. Yeast were then plated on SD medium minus leucine and tryptophan, but which contained a limited amount of adenine (5 µg/ml). The samples were then incubated for 48 hr in a 30°C humidified atmosphere. Double-transformant clones (Leu^+, Trp^+) giving rise to white (Ade^+) or pink/red (Ade^-) colonies were interpreted as expressing recessive and dominant-negative mutations, respectively.

Statistical analysis

The statistical significance of differences between the categorical variables was examined by the chi-square test. Disease-specific survival curves were obtained by the Kaplan-Meier method and the differences between curves were examined by the log-rank test. Independence of prognostic significance was examined using a Cox regression analysis with forward stepwise selection of the variables. $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using the Statview 5.0 software package (SAS Institute, Cary, NC).

Results

p53 status

The wild-type *p53* gene was observed in 68 tumors. A *p53* mutation was found in 24 (26.1%) tumors. An example of a tumor in which the yeast functional assay gave a positive result (*i.e.*, the number of red colonies *vs.* white colonies = 46:54), and in which DNA sequencing analysis revealed a mutation in codon 273, is shown in Figure 1. The *p53* mutations and their respective transdominance property observed here are summarized in Table II. The mutations included 19 missense mutations, 2 nonsense mutations and 3 deletion mutations. Missense mutation accounted for 79% of all the mutations observed in this study. Codon 273 was most frequently mutated (4 273Arg→His and 1 273Arg→Cys),

TABLE III – RELATIONSHIP BETWEEN *p53* MUTATION AND CLINICAL FEATURES OF ENDOMETRIAL CANCER PATIENTS

	All mutations		Dominant-negative mutation	
	Number/total (%)	*p*	Number/total (%)	*p*
Age				
< 60	9/39 (23.1)		5/39 (12.8)	
≥ 60	15/53 (28.3)	0.64	9/53 (17.0)	0.58
FIGO Stage				
I/II	14/65 (21.5)		6/65 (9.2)	
III/IV	10/27 (37.0)	0.19	8/27 (29.6)	0.01
Histologic subtype				
Endometrioid	18/79 (22.8)		9/79 (11.4)	
Nonendometrioid	6/13 (46.2)	0.09	5/13 (38.5)	0.01
Grade				
1, 2	14/67 (20.9)		7/67 (10.4)	
3	10/25 (40.0)	0.06	7/25 (28.0)	0.04
Institute				
Mainz	9/49 (18.4)		6/49 (12.2)	
Sapporo	15/43 (34.9)	0.07	8/43 (18.6)	0.39

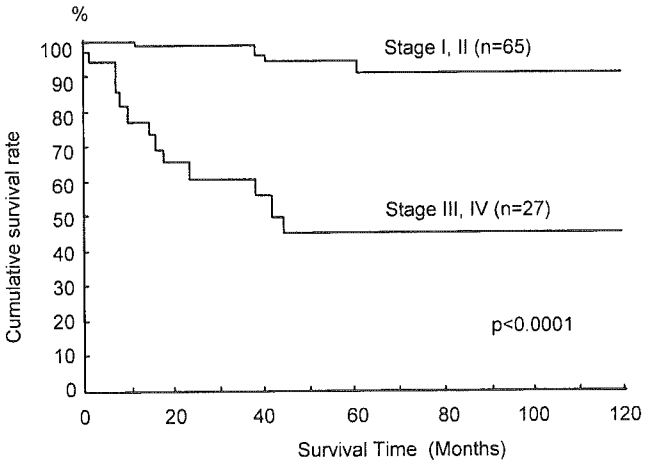

FIGURE 2 – Kaplan-Meier analysis and log-rank test for survival of patients according to FIGO stage.

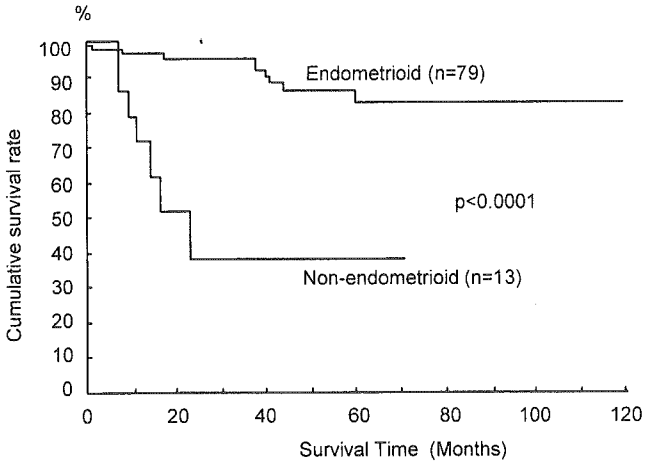

FIGURE 3 – Kaplan-Meier analysis and log-rank test for survival of patients according to histologic subtype of tumors.

followed by codon 280 (280Arg→Ile, 280Arg→Gly, 280Arg→Serand then codon 248 (248Arg→Trp and 248Arg→Gln). Regarding the transdominance of p53 mutation, 10 mutant p53 proteins (10.9%) exhibited recessive activity and 14 mutants

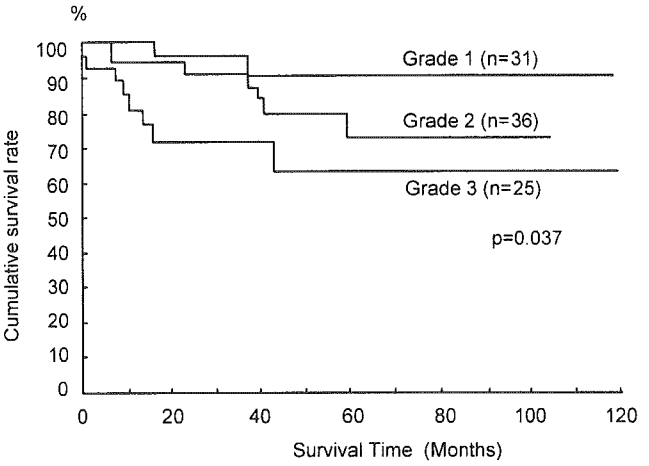

FIGURE 4 – Kaplan-Meier analysis and log-rank test for survival of patients according to grade of tumors.

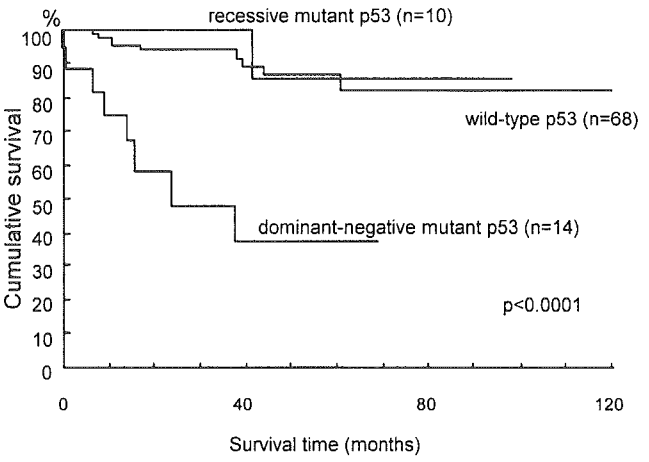

FIGURE 5 – Kaplan-Meier analysis and log-rank test for survival of patients with wild-type, recessive mutant and dominant-negative mutant p53.

(15.2%) showed dominant-negative activity. Not all of the missense mutations had a dominant-negative effect. Only 13 of 19 (68%) missense mutations of *p53* exhibited dominant-negative activity.

p53 mutation was compared to the clinical features (Table III). Total *p53* mutation tended to be related to the following features: nonendometrioid subtype ($p = 0.09$), grade 3 tumors ($p = 0.06$) and Japanese cohort ($p = 0.07$). Dominant-negative mutation was significantly related to advanced FIGO stage ($p = 0.01$), nonendometrioid subtype ($p = 0.01$) and grade 3 tumors ($p = 0.04$).

Survival analysis

The Kaplan-Meier analysis and log-rank test revealed that the survival of patients in this study was significantly related to conventional prognostic factors: FIGO stage ($p < 0.0001$; Fig. 2), histologic subtype ($p < 0.0001$; Fig. 3) and grade of tumor ($p = 0.037$; Fig. 4). As regards *p53* mutation, dominant-negative *p53* mutation was found to be related to poor patient survival ($p < 0.0001$; Fig. 5). Missense *p53* mutation was also significantly related to patient survival ($p = 0.0001$). The age and institute were not related to survival ($p = 0.17$ and 0.52, respectively). When we further examined the functional status of *p53* mutation in relation to patients' survival, the estimated 5-year

TABLE IV – COX REGRESSION ANALYSES FOR PATIENTS WITH ENDOMETRIAL CARCINOMA

Variable	Univariate Cox analysis	Multivariate Cox analysis			
	p	coefficient	Standard error	Hazard ratio	*p*
Dominant negative *p53* mutation	< 0.0001	1.37	0.58	3.9	0.019
FIGO stage	0.0002	1.81	0.62	6.1	0.0037
Histologic subtype	0.0004	1.69	0.58	5.4	0.014
Grade	0.048				NS
Age	0.19				NS
Institute	0.48				NS

survival rate for patients with wild-type p53 (n = 68), recessive *p53* mutation (n = 10) and dominant-negative *p53* mutation (n = 14) was 84.9%, 85.7% and 35.1%, respectively. There was a statistically significant difference in survival between the patients with recessive *p53* mutation and those with dominant-negative *p53* mutation (p = 0.01), as well as between the patients with wild-type *p53* and those with dominant-negative *p53* mutation ($p < 0.0001$). No difference in survival was found between the patients with recessive *p53* mutation and those with wild-type *p53* (Fig. 5). Furthermore, the survival of patients with missense *p53* mutation tended to be related to the transdominance property of mutant *p53*. The 5-year survival rate was 75.0% for patients with recessive missense mutations (n = 10) and 34.6% for patients with dominant-negative missense mutations (n = 14; p = 0.09).

Using multivariate Cox regression analysis, we found that dominant-negative *p53* mutation (p = 0.019), FIGO stage (p = 0.0037) and histologic subtype (p = 0.014) were independent prognostic factor for the patients in this study (Table IV). When only advanced-stage tumors were taken into consideration, dominant-negative *p53* mutation (p = 0.0023) was the most significant predictor of patient survival.

Discussion

The functional activities of mutant p53 proteins have been grouped into 5 categories: retained wild-type activity, loss of function, gain of function, dominant-negative effect and temperature sensitivity.[4] The dominant-negative activity of *p53* mutation corresponds to the capacity of the mutant protein to complex with the product of the remaining wild-type allele to inactivate its function. Thus, dominant-negative *p53* mutation results in the total abrogation of p53 protein function, even if there is still wild-type protein expressed in the cell. Although the importance of analysis of the functional types of *p53* mutation, *i.e.*, the dominant-negative effect and gain of function, in terms of researching carcinogenesis and searching for novel human cancer therapies has been repeatedly emphasized,[10,12,21] it remains unclear whether or not the dominant-negative activity of mutant p53 proteins has a detrimental effect on the survival of cancer patients. Recessive *p53* mutation accompanied by loss of the second allele may be equal to dominant-negative *p53* mutation in terms of loss of function of the gene. Although we did not investigate LOH in this study, the present results did suggest that dominant-negative *p53* mutation is closely related to poor survival of patients with endometrial cancer, even after adjusting for established prognostic factors, that is, tumor stage, grade and histologic type. Future studies including the LOH status of the second allele will be of interest in this context. It is important to investigate whether or not the dominant-negative *p53* mutation exerts an influence on patient survival, not only through a loss of function, but also by other mechanisms attributable to mutant p53 protein such as certain gain-of-function activities.

Also of interest in this context is that dominant-negative *p53* mutation was found to be closely related to the survival of patients with advanced-stage endometrial cancer. Because the prognosis of patients with early-stage endometrial cancer is generally excellent, gynecologic oncologists need to focus increasingly on the survival of patients with advanced endometrial cancer. The histopathologic prognostic factors for endometrial cancer include depth of myometrial invasion, cervical involvement, serosal invasion, adnexal metastasis, positive peritoneal cytology, vaginal metastasis, lymph node metastasis, peritoneal metastasis and bladder/rectal involvement, which are incorporated in the FIGO surgical staging system.[22] The grade of tumor, histologic subtype and lymph-vascular space invasion, which represent the aggressiveness of a tumor, are also important histopathologic prognostic factors that should be taken into consideration in planning treatment for patients with endometrial cancer.[23] The histologic subtypes of serous adenocarcinoma and clear cell adenocarcinoma exhibit more aggressive biologic behavior than common endometrioid adenocarcinoma and has been shown to lead to disproportionate mortality.[24–26] Serous adenocarcinoma is frequently associated with p53 overexpression or *p53* mutation. Our current study has shown that dominant-negative *p53* mutation is an important prognostic factor, in addition to the established predictors of survival, namely, FIGO stage and histologic subtype, in cases of endometrial cancer. This suggests that dominant-negative *p53* mutation may be a reasonable target for a novel therapy for cases of endometrial cancer with a poor prognosis.

In the present study, only 68% of the missense mutations in the cases of endometrial cancer studied here exhibited dominant-negative activity. This finding suggests that determining the dominant-negative activity of a *p53* mutation is more important than merely determining the presence or absence of a *p53* mutation as part of a tailored treatment or rational targeted treatment for endometrial cancer. Because of the high frequency of *p53* mutations in human cancers, and due to the pivotal role of p53 in regulating growth, apoptosis and DNA repair, the introduction of the wild-type p53 gene has been regarded as a reasonable strategy for a gene therapy designed to restore the lost activity of p53.[27] However, this approach has achieved substantial effectiveness to date.[28] A possible reason for the unsatisfactory results may be the accumulation of dominant-negative mutant p53, which results in a high amount of mutant protein, which would override the effects of the wild-type protein introduced by gene therapy. This explanation may in part account for the recently reported failure of gene therapy in the study of ovarian cancer.[29] In addition to overriding wild-type p53, some dominant-negative mutants are known to cause a gain of function related to tumor progression.[10,12,30] Such mutants include 175 Arg→His, 273 Arg→His and 248 Arg→Trp, which accounted for 6 of the 14 dominant-negative mutants identified in the present study. The gain-of-function property of the p53 mutants is considered to lend further malignant phenotypes to the tumor cells, such as enhancement of tumorigenicity, metastatic potential and therapy resistance. These properties may account for the extremely poor survival of endometrial cancer patients with dominant-negative p53 mutations. The 6 cases with gain-of-function mutations in this study included 1 case of stage I serous adenocarcinoma (case 8 in the Japanese cohort), 1 case of stage III endometrioid adenocarcinoma (cases 7 in the German cohort), 1 case of stage III serous adenocarcinoma (case 13 in the Japanese cohort), 1 case of stage IV endometrioid adenocarcinoma (case 9 in the German cohort) and 2 cases of stage IV serous adenocarcinoma (case 15 in the Japanese cohort and case 9 in the German cohort). The gain-of-function mutation appears to be related to

advanced-stage tumors, although the number of cases studied was not large enough to draw any conclusions.

In summary, this study indicates that dominant-negative mutation of *p53* gene is often found in the advanced stages and aggressive histologic subtypes of endometrial cancer. Moreover, such mutation is a strong predictor of the survival of patients with advanced endometrial cancer. Further investigation will be needed in order to clarify whether or not identification of the dominant-negative property of *p53* mutation may be useful for tailoring the treatment of endometrial cancer.

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SURGEON'S CORNER

A systematic nerve-sparing radical hysterectomy technique in invasive cervical cancer for preserving postsurgical bladder function

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Abstract. Sakuragi N, Todo Y, Kudo M, Yamamoto R, Sato T. A systematic nerve-sparing radical hysterectomy technique in invasive cervical cancer for preserving postsurgical bladder function. *Int J Gynecol Cancer* 2005;**15**:389–397.

The objective of this study is to describe a technique for preserving the autonomic nerve systematically, including the hypogastric nerves, pelvic splanchnic nerves, and pelvic plexus and its vesical branches, based on anatomic considerations for the autonomic nerves innervating the urinary bladder, in radical hysterectomies and to assess postsurgical bladder function. A nerve-sparing radical hysterectomy was carried out on 27 consecutive patients with uterine cervical cancer treated between 2000 and 2002. The FIGO stages of the disease consisted of 10 stage Ib1, 6 stage Ib2, 3 stage IIa, and 8 stage IIb. The nerve-sparing procedure was successfully completed in 22 of the 27 patients (81.5%) in the study. At 1 year after the operation, bladder symptoms were significantly improved in the nerve-sparing group compared to the non–nerve-sparing group. Urinary incontinence and abnormal (diminished) bladder sensation were observed in three of the five patients (two patients had both symptoms), for whom the nerve-sparing procedure could not be performed, but none of the 22 patients for whom the nerve-sparing procedure was performed had incontinence, and only two patients had abnormal (increased) bladder sensation ($P = 0.0034$ for incontinence and $P = 0.030$ for abnormal bladder sensation). The patients' survival was not adversely affected by the nerve-sparing procedure. Although it is still preliminary, the surgical technique described in this report is thought to be effective for preserving bladder function, and thus, the quality of life could be improved for patients with cervical cancer who are treated with a radical hysterectomy. For further evaluation of the efficacy of nerve-sparing radical hysterectomy, a prospective randomized trial needs to be performed.

KEYWORDS: autonomic nerve, bladder function, cervical cancer, nerve sparing, quality of life (QOL), radical hysterectomy.

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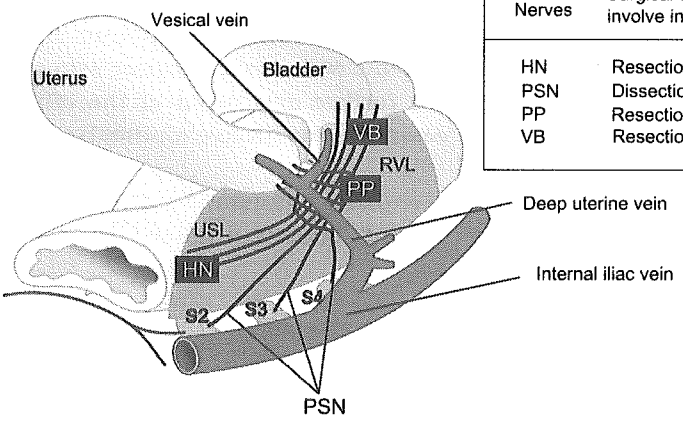
When treating cancer patients, it is important to consider how to minimize deterioration of their quality of life (QOL). Radical hysterectomies are widely performed to treat invasive cervical cancer. Intraoperative and postoperative morbidity includes urinary tract fistula, ileus, thromboembolism, lymphocyst, lymphedema, and bladder dysfunction (neurogenic bladder). Difficulty in urination after the operation impairs the QOL of patients by causing both physical and mental stress. Piver *et al.* described five classes of extended radical hysterectomy[1]. Type III (Wertheim–Meigs) operation is the treatment of choice for FIGO stages Ib–IIa cervical cancer in Western countries and for stages Ib–IIb in Japan. The steps of classical radical hysterectomy at which autonomic nerves may be injured are as follows: (1) hypogastric (sympathetic) nerves at resection of the uterosacral ligament at the posterior pelvic wall, (2) pelvic splanchnic (parasympathetic) nerves in dissection of lymph nodes medial to the internal iliac vein and around the deep uterine vein, (3) vesical branches of the pelvic plexus at resection of the vesico-uterine ligament, and (4) pelvic plexus at resection of the uterosacral and rectovaginal ligaments and resection of the vagina (Fig. 1). In order to maintain bladder function, those nerve networks should be preserved intact as much as possible unless these attempts sacrifice the therapeutic role of surgery. Various attempts have been made to preserve urinary function, including recently proposed autonomic nerve–preserving radical hysterectomy techniques[2–6]. A detailed anatomic study of the pelvic autonomic nerves was conducted by one of the authors (T.S.) and his colleagues[7], providing us with clues on how to develop a theoretical approach for preserving the autonomic nerves when performing a radical hysterectomy. In this preliminary report, we describe a technique for systematic autonomic nerve preservation, which was developed by our institute based on anatomic considerations.

Materials and methods

A total of 27 patients who underwent radical hysterectomies during the period from January 2000 to December 2002 were included in the study. The patients were at the following FIGO stages: 10 at stage Ib1, 6 at stage Ib2, 3 at stage IIa, and 8 at stage IIb. Nineteen of the 27 patients had squamous cell carcinoma, 2 had adenocarcinoma, 5 had adenosquamous carcinoma, and 1 had small-cell carcinoma. A pelvic and para-aortic lymphadenectomy, at least to the level of the inferior mesenteric artery, was carried out in all patients as previously reported[8]. The diameter of the tumor and the length of the resected vagina were measured on the extirpated uterus specimen. Postoperative whole-pelvic external radiation therapy (50 Gy) was employed when there was lymph node metastasis or histologically confirmed parametrial invasion. When the tumor had invaded the lymphatic or vascular channels, we treated the patient with cisplatin-based chemotherapy for three to six cycles, unless the patient refused this treatment. The follow-up period ranged from 12 to 48 months (median 29 months).

Statistical analysis

Categorical variables were analyzed using the Chi-square test or Fisher's exact test. The median values of the continuous variables were compared by the Mann–Whitney *U* test. Disease-free survival was calculated



Nerves	Surgical steps that may involve injuries to the nerves
HN	Resection of USL
PSN	Dissection of LN
PP	Resection of RVL and Vagina
VB	Resection of VUL

Figure 1. Autonomic nerves that may be injured in radical hysterectomy. HN, hypogastric nerves; PSN, pelvic splanchnic nerves; PP, pelvic plexus; VB, vesical branches of PP; USL, uterosacral ligament; LN, lymph nodes; RVL, rectovaginal ligament; VUL, vesicouterine ligament.

according to the Kaplan–Meier method. The statistical significance level was set at $P < 0.05$.

The systematic autonomic nerve preservation technique

Bladder function is controlled by the sympathetic nerves (mainly the hypogastric nerves) and the parasympathetic nerves (pelvic splanchnic nerves). These two nerve fibers intermingle to form the pelvic plexus (Fig. 1). The bladder is innervated by nerve fibers branching from the pelvic plexus (Fig. 2A,B). The following surgical procedure, which was based on anatomic considerations for the autonomic nerves innervating the urinary bladder[7], was used. The pelvic plexus and branches innervating the bladder are the most important nerves to preserve in a nerve-sparing radical hysterectomy, as shown in the autopsy of cadavers (Fig. 2B). Before the hysterectomy, the pelvic lymph nodes were removed. The uterosacral ligaments and rectal pillars (rectovaginal ligament) were then dissected. The first step for preserving the autonomic nerves was to identify and lateralize the hypogastric nerves and the proximal part of the pelvic plexus during the dissection of the uterosacral ligament and rectovaginal ligament. The peritoneum of the cul-de-sac was incised, and the prerectal space was developed, exposing the rectovaginal ligament between the prerectal space and the pararectal space. The hypogastric nerves and pelvic plexus are located laterally, attached to the rectovaginal ligament (Fig. 2A). After lateralizing the hypogastric nerves and the proximal part of the pelvic plexus, the nerve tissue can be preserved by selective resection of the exposed uterosacral and rectovaginal ligaments.

The next step for preserving the autonomic nerves is to identify the pelvic splanchnic nerves fusing to the pelvic plexus. The cardinal ligament lymph nodes were dissected to clearly skeletonize the deep uterine vein, using a suction apparatus. We carefully preserved the pelvic splanchnic nerves arising from the sacral surface. Then, the anterior part of the vesico-uterine ligament was dissected, and the ureteral tunnel was developed. Since the vesical vein drains from the bladder to the deep uterine vein coursing through the posterior part of the vesico-uterine ligament, separation and cutting of the vesical vein is required in order to resect the uterus (Fig. 3). Then, the fatty connective tissue of the posterior part of the vesico-uterine ligament was dissected, without disturbing the main part of the vesical nerve branches of the pelvic plexus, using Kelly forceps introduced from the ventral to dorsal direction (Fig. 4). A small portion of the vesical branches around the ureter may be sacrificed at this step. This enabled identification of the plane between the pelvic plexus and the paracolpium (Fig. 5A,B). Next, the blood vessels of the cardinal ligament were resected at their origin from the internal iliac vein. Careful rubbing of the deep uterine vein in an upward (ventral) direction to its point of attachment to the paracolpium enabled the lower (dorsal) nerve tissue to be spared (Fig. 6). The preserved pelvic splanchnic nerves arise from the sacral surface and fuse to the pelvic plexus parallel to the rectovaginal ligament that composes the medial side of the pararectal space. The space between the pelvic plexus and the paracolpium was developed anteroposteriorly by using Kelly forceps or Metzenbaum scissors (Fig. 7). Using this approach, the pelvic plexus was put to the

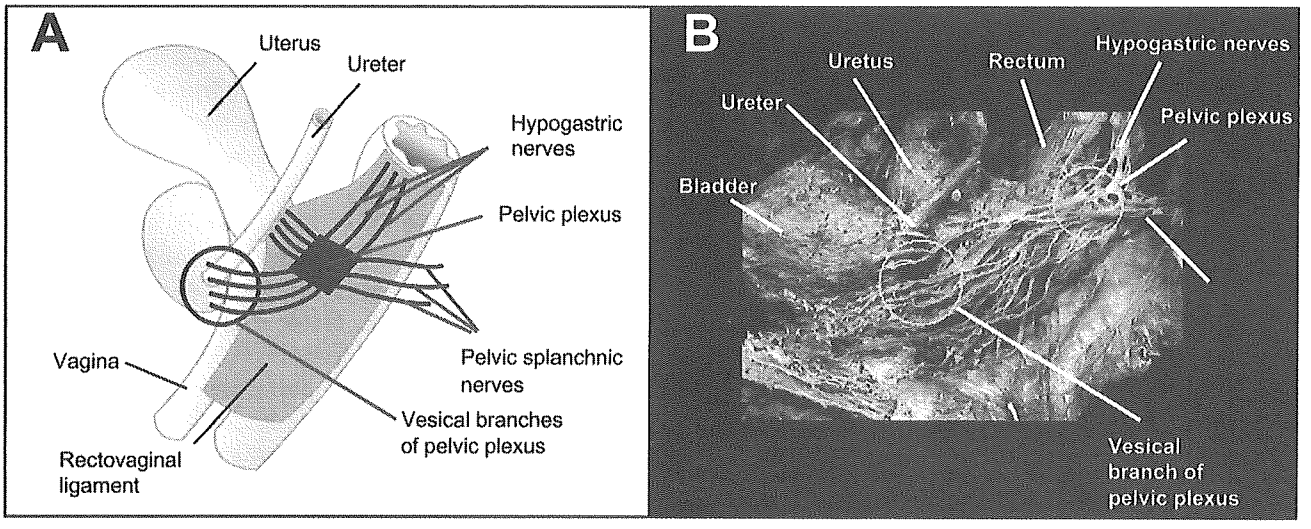


Figure 2. A) Illustration of autonomic nerves that control bladder function and B) anatomic distribution of autonomic nerves in cadavers.

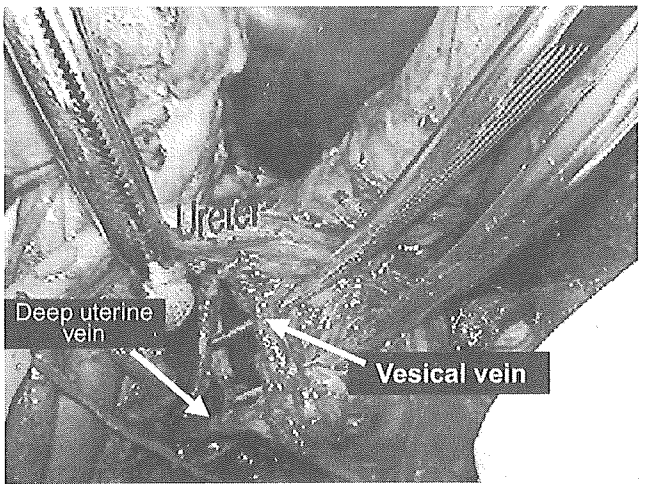


Figure 3. Separation and cutting of the vesical vein in the posterior part of the vesico-uterine ligament. It should be noted that the deep uterine vein had not been cut yet at this step.

side. Then, the uterine branch of the pelvic plexus was cut, which enabled dissection of the paracolpium without involving the pelvic plexus (Fig. 8). Finally, a sufficient length of the vagina was cut by pulling the uterus upward and lateralizing the pelvic plexus. These procedures enabled systematic preservation of the autonomic nerves (Fig. 9).

Results of nerve-sparing radical hysterectomy

The autonomic nerves were completely preserved, at least on one side, in 22 of the 27 patients (group A). For 5 of the 27 patients, the space between the pelvic plexus and the paracolpium could not be developed and therefore the pelvic plexus was not put laterally. The vaginal canal was cut without selective dissection of the uterine branches of the pelvic plexus for these patients (group B). The nerve-sparing procedure in these patients was unsuccessful due to bleeding from the paracolpium and the surgeon's inexperience in performing the procedure. The completion rate of the procedure was 81.5%. The surgical and postsurgical clinicopathologic details on the 27 patients are shown in Table 1. The 22 patients in group A ranged in age from 35 to 60 years (median age 43 years), and the 5 patients in group B ranged in age from 31 to 64 years (median age 46 years). In the group A patients, there were six with stage Ib1, six with stage Ib2, three with stage IIa, and seven with stage IIb cancer. For the stage IIb patients, the nerve-sparing procedure was employed on the uninvaded side only. In the group B patients, there were four with stage Ib1 and one patient with stage IIb. The stage distribution in each group was not significantly different ($P = 0.15$). The tumor diameter in the group A and B patients ranged from 11 to 70 mm (median 39 mm) and from 12 to 50 mm (median 34 mm), respectively. There was no statistically significant difference between the two groups ($P = 0.57$). The length of the resected vagina in the patients in each group ranged from 20 to 45 mm (median 30 mm) and from 25 to 45 mm (median 35 mm), respectively, with no significant difference between the two groups ($P = 0.30$). Therefore, the inability to complete the nerve-sparing procedure does not seem to be related to the patient's age, tumor stage, tumor diameter, or length of the resected vagina.

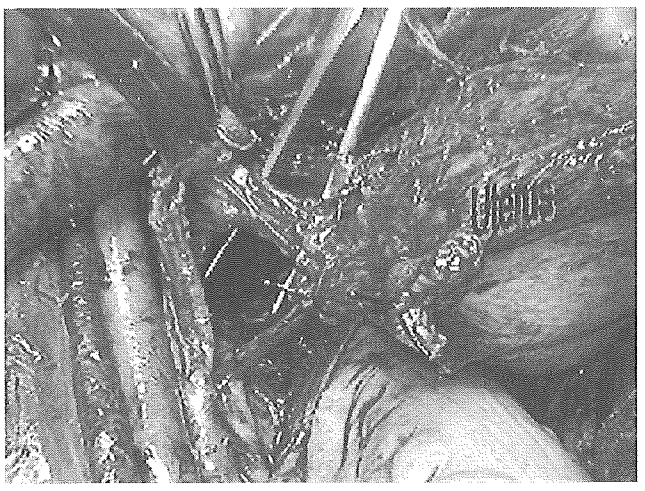


Figure 4. Dissection of fatty connective tissue (arrowhead) of the posterior part of the vesico-uterine ligament. A portion of the vesical branches of the pelvic plexus, which pass around the ureter, may be sacrificed at this step.

The length of operation time for nerve-sparing and non–nerve-sparing radical hysterectomy ranged from 387 to 791 min (median 515 min) and from 345 to 648 min (median 370 min), respectively. The difference was not statistically significant ($P = 0.13$) although the length of operation time for the nerve-sparing group was about 2 h longer than that for the non–nerve-sparing group. Our university hospital is a central teaching hospital, and this may partly explain the wide distribution of operation time. Blood loss in nerve-sparing and non–nerve-sparing radical hysterectomy ranged from 640 to 4185 mL (median 1400 mL) and from 450 to 2400 mL (1160 mL), respectively. The difference was not significant ($P = 0.62$).

Radiation therapy was performed postoperatively in one patient from group A. Radiation therapy combined with chemotherapy was carried out for two patients from group A and none from group B. Chemotherapy was used postoperatively in 15 patients from group A and 3 from group B. None of the

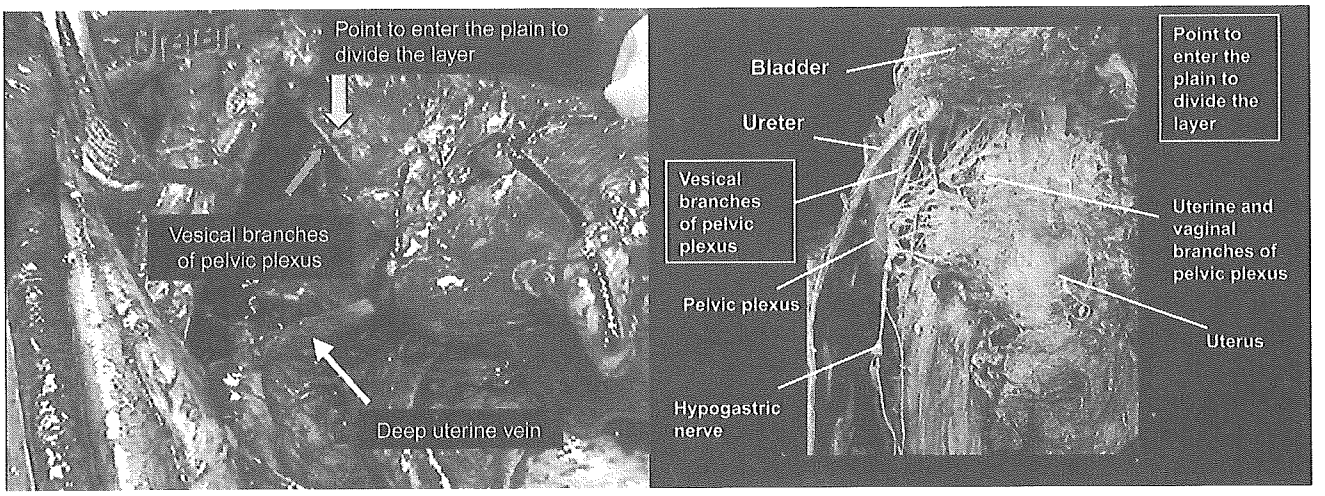


Figure 5. A) Vesical branches of the pelvic plexus after dissection of the vesico-uterine ligament and the point at which the plane must be entered to divide the vesical branches/pelvic plexus and the paracolpium and B) the same point at which the plane must be entered to divide the layers in the cadaver.

patients took cholinergic agents or α1 blocker during the observation period. The duration of disease-free survival in the group A patients ranged from 13 to 48 months and in the group B patients, from 12 to 36 months. One stage IIb patient in group A suffered a recurrence in the pelvis 13 months after the operation but was successfully treated with radiation therapy. The cumulative disease-free survival rate for the group A and group B patients at 24 months was 95.5% and 100%, respectively (Fig. 10).

At 1 year postsurgery, three patients from group B had stress urinary incontinence, but none from group A had urinary incontinence ($P = 0.0034$). In group A, there were 20 patients with normal bladder sensation and two with increased bladder sensation, but none of the patients had a reduced desire to void. In group B, there were three patients (60%) with a reduced desire to void. Abnormal bladder sensation was more frequently observed in the group B patients ($P = 0.030$).

Discussion

Bladder dysfunction, typical of vesicovaginal fistula, ureterovaginal fistula, or urination difficulty in patients who have undergone radical hysterectomy, causes deterioration in the patients' QOL due to physical and mental stress. Many gynecologic oncologists have become knowledgeable about the anatomic

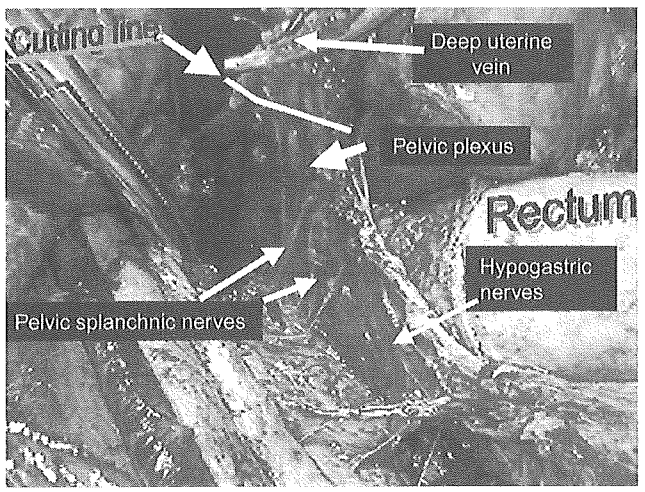


Figure 6. The cutting line between the pelvic plexus and its uterine branch, which is shown after resecting and pulling upward of deep uterine vein.

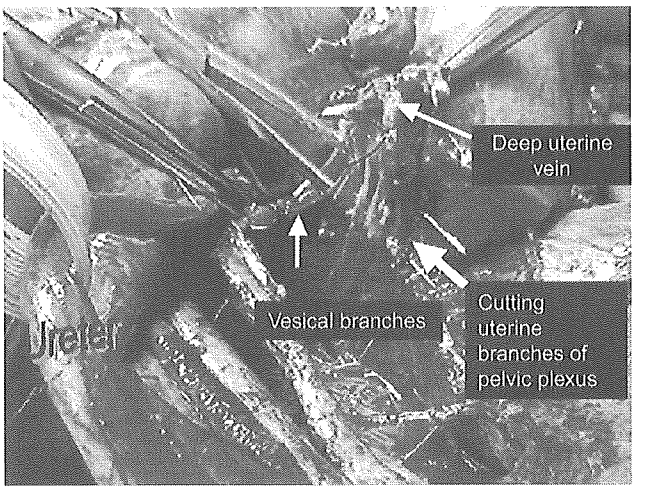


Figure 7. Separation of the pelvic plexus from the rectovaginal ligament.

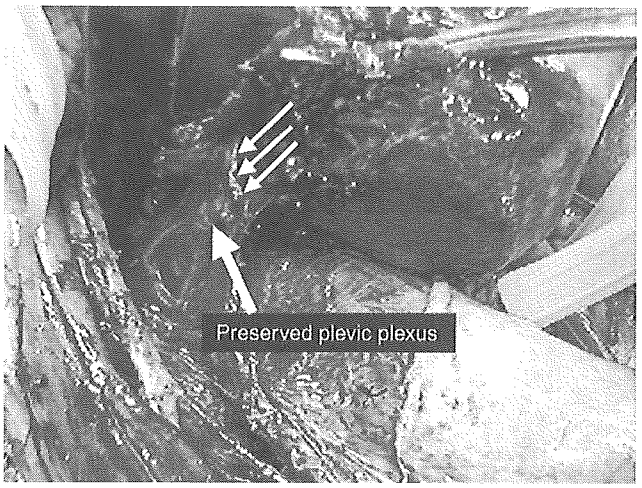


Figure 8. Separated and retracted pelvic plexus after the autonomic nerve preservation.

distribution of the nerves controlling bladder function, and recently, a great deal of interest has been shown in autonomic nerve–sparing surgical techniques[2–6]. It has been known for a long time that the hypogastric nerves, pelvic splanchnic nerves, pelvic plexus, and the distal part of the pelvic plexus (the vesical nerve branch) are important in urination physiology[9,10], and many studies have shown that bladder dysfunction can be reduced by minimizing the extent of the radical hysterectomy[11–13]. However, gynecologic oncologists should balance the cure of disease and QOL, namely, oncologic priorities of removal of disease and all its potential routes of local spread, and bladder function. We have attempted to establish a surgical technique that will preserve the autonomic nerves without sacrificing the radicality of the radical hysterectomy procedure.

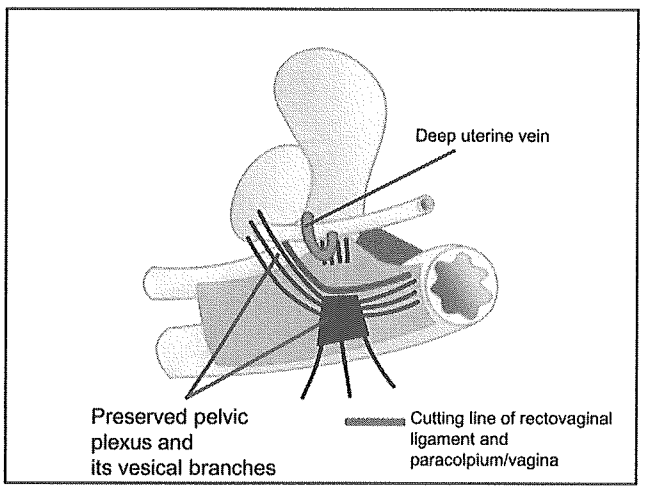


Figure 9. Illustration of systematic autonomic nerve preservation and the cutting line of the rectovaginal ligament, paracolpium, and vagina.

Piver *et al.* proposed five classes of extended radical hysterectomy[1], and these classes are often used now to discriminate between the types of surgery needed. The surgical technique of Wertheim–Meigs is considered to correspond to type III[14]. In a radical hysterectomy, the uterosacral ligament is resected at the posterior pelvic wall. Hypogastric nerves should be separated from the uterosacral ligaments before cutting the ligament deeply at the posterior pelvic wall in radical hysterectomy to preserve the hypogastric nerves[15]. This technique was also previously described by Sakamoto and Takizawa[16] and is the popular technique employed by many gynecologic oncologists in Japan. The uterosacral ligament may contain lymphatic channels draining caudally to sacral nodes and common iliac nodes. We routinely dissect sacral nodes and common iliac nodes including those of the medial area of common iliac arteries. We previously reported the results of systematic lymphadenectomy for cervical cancer[8]. The incidence of sacral node metastasis was only 1.9%. For the sake of oncologic pertinence in nerve-sparing radical hysterectomy, we have employed systematic lymphadenectomy including sacral nodes, medial part of the common iliac nodes, and para-aortic nodes, and applied a nerve-sparing technique to stage Ib disease and to the uninvaded side of stage II disease.

In a radical hysterectomy, the pelvic plexus is in close proximity to the paracolpium at the depth at which the vagina should be dissected. If separation of the pelvic plexus from the paracolpium is insufficient, the pelvic plexus will be injured when the vagina is amputated. Possover *et al.* recently reported that preservation of the pelvic splanchnic nerves and pelvic plexus, with the middle rectal artery serving as a landmark for identification, is important for preserving bladder function[6]. If the cardinal ligament below the middle rectal artery is dissected, the pelvic splanchnic nerves will be injured. Moreover, if the uterosacral ligaments and rectovaginal ligaments are excised deeply at the posterior wall of the pelvis, the hypogastric nerves and pelvic plexus may also be excised. Therefore, it is not clear, just from hearing that a Piver's type III operation has been performed, whether or not the hypogastric nerves, pelvic splanchnic nerves, and pelvic plexus have been preserved. Thus, accurate evaluation of bladder dysfunction after radical hysterectomy is not possible without detailed information on the surgical procedure used.

The most important step that must be performed in our technique is to separate the pelvic plexus from the

Table 1. Surgical and postsurgical information on the 27 patients with cervical cancer who underwent a radical hysterectomy intended to preserve the autonomic nerves

Case	Age	Stage (histotype)	Diameter of tumor (mm)	Length of RV (mm)	Nerve preservation	LNM	LV	RT	Symptom (1 year after operation)		DFS (months)
									Incontinence	Bladder sensation	
Group A											
1	39	Ib1 (S)	20	40	+	−	+	−	−	Normal	36
2	42	Ib1 (S)	11	30	+	−	−	−	−	Normal	33
3	36	Ib1 (S)	23	20	+	−	−	−	−	Normal	19
4	38	Ib1 (S)	39	40	+	−	+	−	−	Increased	48
5	39	Ib1 (AS)	39	42	+	−	+	−	−	Normal	36
6	57	Ib1 (SM)	18	20	+	−	+	−	−	Normal	15
7	39	Ib2 (S)	45	26	+	−	+	−	−	Normal	39
8	36	Ib2 (S)	45	35	+	+	+	+	−	Increased	37
9	60	Ib2 (S)	70	20	+	−	+	−	−	Normal	20
10	44	Ib2 (S)	50	25	+	−	+	−	−	Normal	19
11	35	Ib2 (AS)	40	25	+	−	−	−	−	Normal	18
12	49	Ib2 (A)	60	35	+	−	−	−	−	Normal	17
13	44	IIa (S)	30	45	+	−	+	−	−	Normal	29
14	35	IIa (S)	25	32	+	+	+	−	−	Normal	18
15	54	IIa (AS)	35	30	+	−	+	−	−	Normal	17
16	44	IIb (S)	55	40	+	−	−	+	−	Normal	46
17	52	IIb (AS)	17	32	+	−	+	−	−	Normal	44
18	38	IIb (S)	25	30	+	+	+	+	−	Normal	41
19	45	IIb (A)	50	30	+	+	+	−	−	Normal	14
20	54	IIb (S)	37	30	+	−	+	−	−	Normal	13[a]
21	42	IIb (S)	20	30	+	−	+	−	−	Normal	31
22	49	IIb (S)	50	25	+	−	+	−	−	Normal	24
Group B											
23	40	Ib1 (S)	35	30	−	−	−	−	+	Reduced	32
24	61	Ib1 (S)	25	40	−	−	+	−	+	Reduced	35
25	64	Ib1 (S)	34	25	−	−	+	−	−	Reduced	36
26	46	Ib1 (S)	12	30	−	−	−	−	−	Normal	12
27	31	IIb (AS)	50	40	−	−	+	−	+	Normal	28

RV, resected vagina; LNM, lymph node metastasis; LV, lymph-vascular space invasion; RT, radiation therapy; DFS, disease-free survival; S, squamous cell carcinoma; AS, adenosquamous carcinoma; SM, small-cell carcinoma; A, adenocarcinoma.

[a]Patient had a recurrence in the pelvis, which was successfully controlled by radiotherapy.

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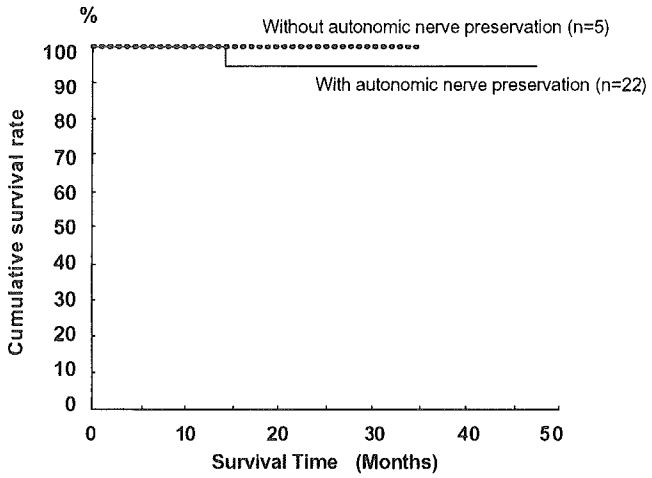

Figure 10. Disease-free survival of patients treated with radical hysterectomy with or without systematic preservation of the autonomic nerves.

paracolpium and to selectively dissect the uterine branch of the pelvic plexus. The sympathetic nerves (hypogastric nerves) and parasympathetic nerves (pelvic splanchnic nerves) fuse to form the pelvic plexus. This important anatomic structure spreads its branches to the bladder. After separating the pelvic plexus from the paracolpium and the rectovaginal ligament (paracervical tissues), we can remove a sufficient length of the vagina, without involving the pelvic plexus. Therefore, we evaluated bladder function by comparing the group in which only the paracolpium was selectively dissected after the uterine branch of the pelvic plexus was cut with the group in which the paracolpium was dissected without dissection of the uterine branches. This study has shown that the bladder function of the group in which only the paracolpium was dissected after dissection of the uterine branches of the pelvic plexus was better preserved than that of the group in which the paracolpium was dissected without dissection of the uterine branches.

We employed our technique for autonomic nerve preservation to the uninvaded side in patients with stage IIb uterine cervical cancer. It has been reported, in experimental animals, that normal urinary function could be maintained when at least one side of the sympathetic nerve was preserved[17]. These data suggest that normal urinary function can be maintained by applying the operation with autonomic nerve preservation to the uninvaded side in patients with stage IIb cervical cancer, who have parametrial invasion only on one side.

In conclusion, our technique to preserve the pelvic autonomic nerves, which is based on a detailed anatomic study, is relatively easy to perform and is a feasible technique to employ in the treatment of invasive cervical carcinoma. Our aim of improving the long-term prognosis of bladder function seems to have been achieved because 1 year after the operations, the patients' urinary function is almost normal. A detailed urodynamic study on patients treated with our nerve-sparing radical hysterectomy will be reported in a further paper. The nerve-sparing procedure was thought to give patients better QOL with regard to bladder function, with no additional adverse effects on radical hysterectomy. For further evaluation of the efficacy of nerve-sparing radical hysterectomy, a prospective randomized trial needs to be performed.

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Combined use of magnetic resonance imaging, CA 125 assay, histologic type, and histologic grade in the prediction of lymph node metastasis in endometrial carcinoma

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OBJECTIVE: The aim of this study was to predict retroperitoneal lymph node metastasis during the preoperative examination of patients with endometrial carcinoma and to determine whether lymphadenectomy must be performed.

STUDY DESIGN: This study was carried out on 214 patients with endometrial carcinoma. Preoperative evaluators were volume index, depth of myometrial invasion (as assessed by magnetic resonance imaging), serum CA 125 level, histologic type, and histologic grade. With the use of receiver operating characteristic curves, cutoff values of volume index and serum CA 125 levels were determined. The relationships of these evaluators with pelvic lymph node metastasis were investigated by multivariate analysis with a logistic regression model. The relationships of these evaluators with para-aortic lymph node metastasis were investigated in the same way.

RESULTS: Histologic type, volume index, histologic grade, and serum CA 125 level were found to be independent risk factors for pelvic lymph node metastasis; serum CA 125 level and volume index were found to be independent risk factors for para-aortic lymph node metastasis. Among 110 cases with no risk factors for pelvic lymph node metastasis, pelvic lymph node metastasis was observed in 4 cases (3.6%). On the other hand, only 1 case of 128 cases (0.7%) with no risk factors for para-aortic lymph node metastasis actually had metastasis.

CONCLUSION: Careful consideration of the possibility of the elimination of the requirement of retroperitoneal lymphadenectomy is needed in cases with no risk factors for lymph node metastasis. However, our results suggest that para-aortic lymphadenectomy may not be necessary in cases with no risk factors for para-aortic lymph node metastasis. (Am J Obstet Gynecol 2003;188:1265-72.)

Key words: Endometrial carcinoma, lymph node metastasis, magnetic resonance imaging, CA 125

Retroperitoneal lymph node metastasis is an important prognostic factor in endometrial carcinoma.[1] Known risk factors for retroperitoneal lymph node metastasis include histologic grade and myometrial invasion.[2,3] However, there is no method for determining directly the presence and depth of myometrial invasion in a preoperative setting. Therefore, myometrial invasion is evaluated indirectly by magnetic resonance imaging (MRI) in many institutions. Although the accuracy of the evaluation of myometrial invasion by MRI has been improved recently by the combined use of gadolinium-enhanced contrast imaging, the level of accuracy still has limitations. There have been many studies on MRI-based evaluation of myometrial invasion, but few studies on the usefulness of MRI for the prediction of lymph node metastasis. On the other hand, it has been reported, however, that the serum CA 125 level is related to retroperitoneal lymph node metastasis and prognosis.[4,5]

Serum CA 125 levels in addition to MRI findings and pathological factors that can be evaluated before an operation were taken into account in multivariate analysis for our study to determine the risk factors for lymph node metastasis.

Material and methods

Among patients with endometrial carcinoma who were treated in the Department of Obstetrics and Gynecology, Hokkaido University Hospital, and two affiliated hospitals during the period of 1993 to 2000, 214 patients underwent pelvic MRI, endometrial biopsy, and serum CA 125 determination as preoperative examinations and hys-

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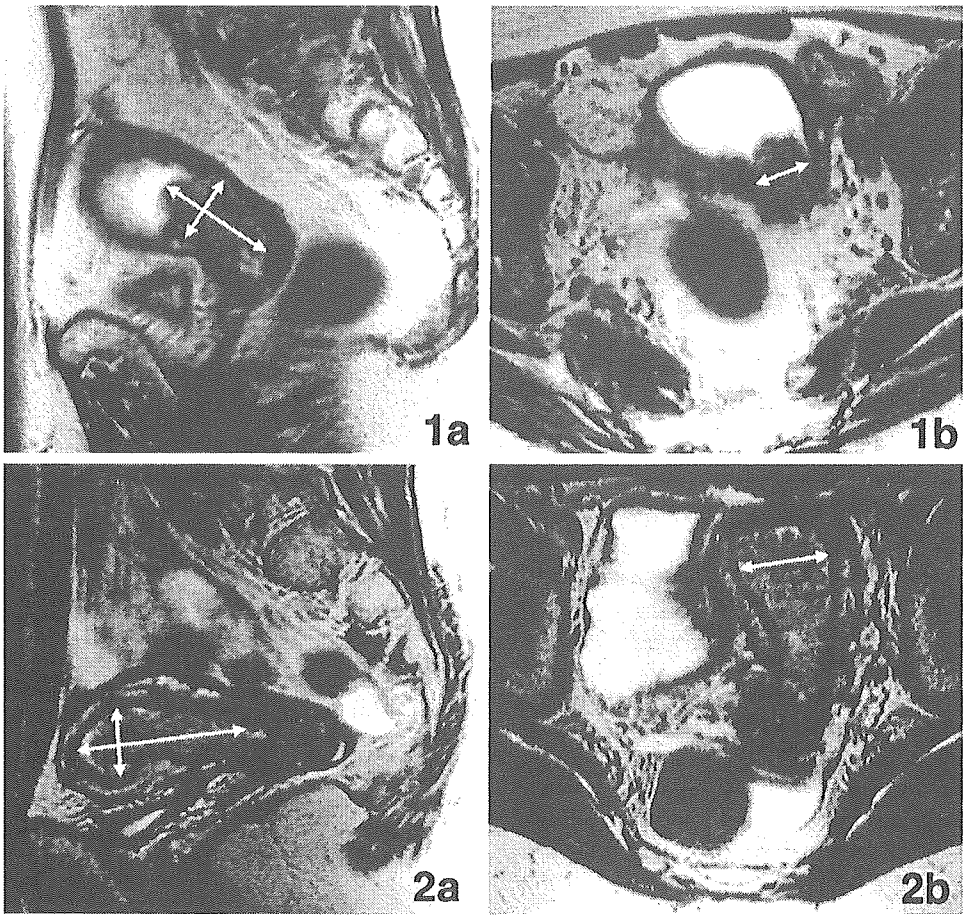

Fig 1. *Volume index,* defined as product of maximum longitudinal diameter along uterine axis, maximum anteroposterior diameter (thickness) in sagittal section image **(1a, 2a),** and maximum horizontal diameter in horizontal section image **(1b, 2b).** Volume index of upper image **(1a, 1b)** was 25 and of the lower image **(2a, 2b)** 40.

terectomy, bilateral salpingo-oophorectomy, and retroperitoneal lymphadenectomy as initial treatment. 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. For medical reasons, such as advanced age and complications, para-aortic lymphadenectomy was not performed in 2 patients. The ages of the patients ranged from 23 to 80 years (mean age, 55.9 years). The clinicopathologic characteristics of the patients are shown in Table I.

Volume index was evaluated in either of T_2-weighted MRIs or gadolinium-enhanced T_1-weighted images, which show tumor lesions more clearly. *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 (Fig 1). We considered this to be a reliable substitute for tumor volume, and we obtained receiver operating characteristic curves with the use of measured values (Fig 2). When determined on the curves, cutoff values for pelvic lymph node metastasis and para-aortic lymph node metastasis were 25 and 40, respectively.

Myometrial invasion was categorized into three levels: (1) no invasion when a clear junctional zone could be identified in a T_2-weighted image and when the border between the endometrium and the myometrium was smooth and clear, (2) invasion of less than one half the myometrium when a partially ruptured junctional zone was identified or when the border between the endometrium and the myometorium was irregular, with tumor signals remaining in one half of the myometirum, and (3) invasion of more than one half the myometrium when a partially interrupted junctional zone was identified or when the border between the endometrium and

Table I. Clinicopathologic characteristics of 214 patients with endometrial adenocarcinoma

Clinicopathologic characteristic	*No.*	*Pelvic lymph node metastasis*	*Para-aortic node metastasis*
International Federation of Gynecologists and Obstetricians stage (1988)			
I	147	0	0
II	10	0	0
III	52	24	16
IV	5	5	3
Histologic type			
Endometrioid	205	26	17
Mucinous	1	0	0
Serous	6	3	2
Clear	2	0	0
Histologic grade (postoperative)			
G1	130	9	4
G2	53	9	5
G3	31	11	10
Total	214	29	19

myometrium was irregular, with tumor signals in more than one half the myometrium. Cases in which the degree of invasion could not be determined precisely were classified into a deeper invasion category.

The serum CA 125 level was determined with a radioimmunoassay (RIA) kit (Fujirebio Diagnostics, Malvern, Pa). A population should be divided into the premenopausal and postmenopausal groups to determine the relationships between measured serum CA 125 levels and pathologic factors because the serum CA 125 level is affected by ovarian hormones and aging.[5-8] In the current study, the patient population was divided into two groups by age. The results of the measurement were used to obtain receiver operating characteristic curves for retroperitoneal lymph node metastasis (Fig 3). With use of these curves, the cutoff points in each groups were set. Two cutoff values (28 U/mL for patients aged <50 years and 70 U/mL for patients aged ≥50 years) divided patients into low and high CA 125 groups for pelvic lymph node metastasis. Another two cutoff values (30 U/mL for patients aged <50 years and 90 U/ml for patients aged ≥50 years) also divided patients into low and high CA 125 groups for para-aortic lymph node metastasis.

Preoperative endometrial biopsy specimens were evaluated for histologic type and histologic grade. Postoperative pathologic specimens were evaluated for histologic type, histologic grade (three grades according to the 1988 International Federation of Gynecologists and Obstetricians criteria), myometrial invasion (no invasion, invasion of less than one half of the myometrium, or invasion of one half or more of the myometrium), and retroperitoneal lymph node metastasis (present or absent).

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

Table II. Evaluation of myometrial invasion by MRI

	MRI-based myometrial invasion	
	<½	*≥½*
Myometrial invasion: <½	102	35
After operation: ≥½	16	61

Table III. Preoperative histologic grade evaluation

	Preoperative histologic grade		
Postoperative histologic grade	*HG1*	*HG2*	*HG3*
HG1	117	12	1
HG2	15	37	1
HG3	4	9	18
Totals	136	58	20

HG, Histologic grade (International Federation of Gynecologists and Obstetricians, 1988).

Results

Table II shows the results of myometrial invasion evaluation with the use of MRI. Cases with invasion of less than one half the myometrium had a sensitivity of 79.2%, a specificity of 74.4%, a positive predictive value of 63.5%, a negative predicative value of 86.4%, and an accuracy of 76.1%. Table III shows the results of histologic grade evaluation with preoperative endometrial biopsy specimens and specimens obtained from hysterectomy. Fourteen percent of G1 cases that were diagnosed on the basis of results of an examination of preoperative biopsy specimens were upgraded to G2 or G3 adenocarcinoma after