Table 5 Treatment for seminoma and NSGCT

	Stage I seminoma	Stage I NSGCT	Metastatic seminoma	Metastatic NSGCT
No. patients	502	165	143	237
Chemotherapy	13 (2.6%)	15 (9.1%)	87 (60.8%)	220 (92.8%)
CDBCA monotherapy	9	1	1	
BEP/EP/Alterations	4	14	84	204
VIP			1	14
Unknown/others			1	2
Radiation therapy	79 (5.7%)	5 (3.0%)	43 (30.0%)	5 (2.1%)
Extirpation surgery	2 (0.4%)	3 (1.8%)	21 (14.7%)	23 (9.7%)
Surveillance alone	408 (81.3%)	142 (86.1%)		

Multimodal treatment for metastatic seminoma in eight patients. Multimodal treatment for metastatic NSGCT in 11 patients.

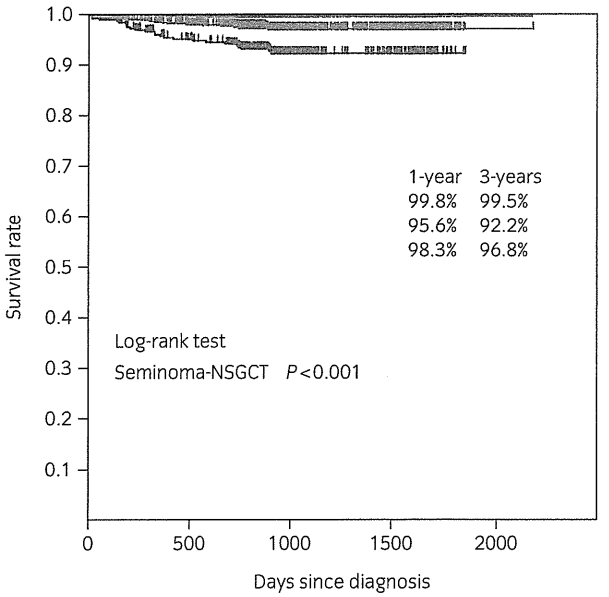

Fig. 1 Overall survival in seminoma and NSGCT. ——, Seminoma (n = 657); ——, NSGCT (n = 377); ——, overall (n = 1034).

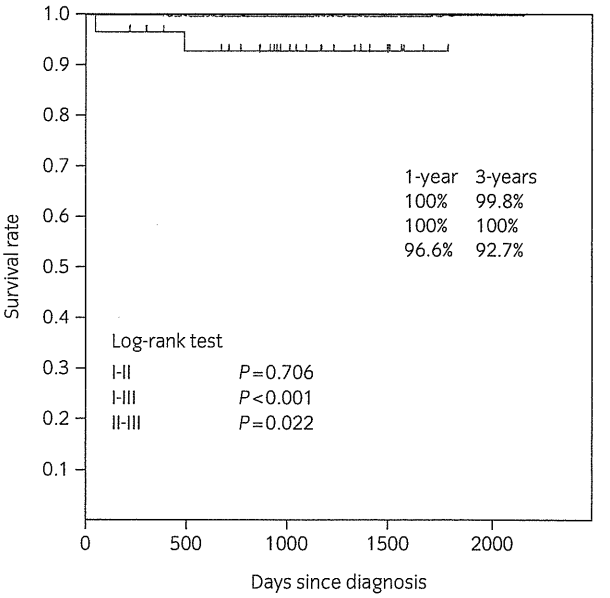

Fig. 2 Overall survival in seminoma stratified by JUA classification. ——, Stage I (n = 552); ——, stage II (n = 76); ——, stage III (n = 31).

and 99.8% in stage I seminoma, 100% and 100% in stage II seminoma, 96.6% and 92.7% in stage III seminoma, 99.3% and 98.5% in stage I NSGCT, 96.9% and 95.1% in stage II NSGCT, and 91.0% and 83.7% in stage III NSGCT, respectively (Figs 3,4). According to the IGCCC, 1- and 3-year overall survival rates were 99.4% and 99.1% in the good prognosis group, 100% and 100% in the intermediate prognosis group, and 83.7% and 79.9% in the poor prognosis group (Fig. 4).

Discussion

The current study was carried out by the JUA to comprehensively aggregate the national registration for testicular cancer, and was the first attempt to evaluate the distribution of histology and clinical stages as well as treatment variation for testicular cancer using a large cohort of patients. For the treatment of patients with testicular cancers in clinical practice, guidelines from the JUA, EAU or National Comprehensive Cancer Network are currently updated,[4,6,7] but the diagnosis and treatment policy for testicular cancer might be varied between facilities. In fact, various kinds of test kits are available to measure HCG levels. The measurement of HCG activity shown by mIU/mL is possible using two types of commercially available measurement kits, which include intact HCG (measuring the bound units of α and β chains) and total HCG (measuring free β-HCG, HCG cutting type, cutting-free β-HCG in addition to intact HCG). These measurements were recommended as the general rule for clinical and pathological studies on testicular tumor published in 2005. In the present study, these measurements were reported in approximately 40% of patients. In contrast, the use of free β-HCG assay kits (units: ng/mL) was more prevalent in even 60% of patients. In fact, the present study fails to evaluate the IGCCC in all patients because of the difficulty in adjustments of these assays.

High orchidectomy was carried out in 98.2% of patients as the treatment for primary lesion. This could be because the importance of this procedure not only for surgical resection, but also for correct histological diagnosis is understood. Histopathological findings showed a high prevalence of pure seminoma, accounting for approximately 60%, which was

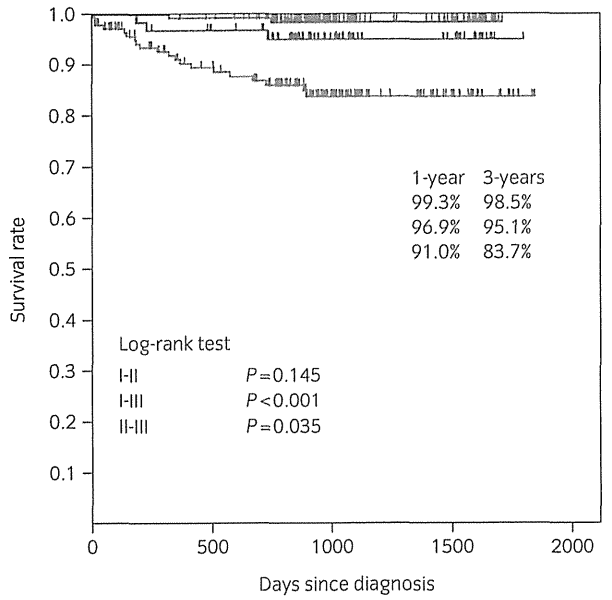

Fig. 3 Overall survival in NSGCT stratified by JUA classification. ——, Stage I (n = 162); ——, stage II (n = 68); ——, stage III (n = 145).

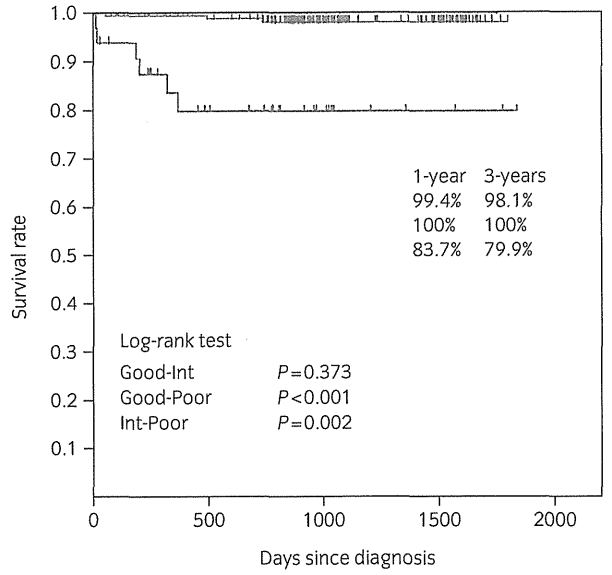

Fig. 4 Overall survival stratified by IGCCC. ——, Good (n = 119); ——, intermediate (n = 82); ——, poor (n = 57).

consistent with a historical report.[8] The most frequent metastatic sites were the retroperitoneal lymph node and lung in the present study.

The 25-Gy irradiation to the pelvis and para-aortic lymph node regions reportedly lowers the relapse rate for stage I seminoma by approximately 5%.[9] In the present study registered in 2005 and 2008, radiation therapy for stage I seminoma was still a common procedure reported in 15.7% of patients, which might have since been reduced because of the recent guideline clearly highlighting the risk of infertility and increased incidence of secondary malignancies.[6,7]

The introduction of chemotherapy has made testicular cancer a treatable disease. Even in patients with metastases, modern chemotherapy offers a cure rate of at least 80%.[10] The combination of BEP chemotherapy was reported in 1987, and became a standard treatment for metastatic testicular cancers.[11] Three or four courses of BEP are recommended depending on the risk groups.[12] Four courses of EP and three courses of BEP chemotherapy are equally recommended for patients with good prognosis.[13] BEP and EP therapy were widely used for both seminoma and NSGCT in more than 90% of patients in this survey. Other combinations, such as VIP, were used as initial chemotherapy for testicular cancer, which remained at approximately 6% of cases of NSGCT.

As for the survival rate, the Japan National Cancer Center reported a 5-year overall survival rate of 92% based on 369 patients with testicular cancer registered in six centers from 1993 to 1999. The 5-year survival rates according to progression of disease was shown to be 97.8% in patients without metastasis, 100% in patients with regional lymph node metastasis and 70.7% in patients with distant metastasis.[8] The current survey showed 3-year overall survival of more than 95% in all patients and 92.7% 3-year survival rate even in stage III patients. Although it is difficult to compare two cohorts with different observation periods, the survival rates in the present survey might be consistent with or better than the data from the previous report. The original paper from the IGCCC reported 5-year survival rates in the good, intermediate and poor prognosis groups of 91%, 79%, and 48%, respectively.[5] Even in the selected patients who had the proper HCG measurement, the IGCCC in the present study documented 3-year overall survival rates in the good, intermediate and poor prognosis groups of 99.1%, 100%, and 79.9%. This dramatic improvement in the treatment of testicular cancer can be attributed to accurate diagnosis by using available tumor markers and sophisticated imaging modalities, as well as improved surgical techniques and effective chemotherapy.

The present report is the first large-scale study of the characteristics and survival of testicular cancer patients in Japan based on multi-institutional registry data, and showed a good prognosis even at an advanced stage. The improved survival was attributed to substantially by accurate diagnosis and effective multimodal treatment.

Acknowledgments

These clinicopathological statistics are based on the results from a number of institutions in Japan (Table S1). We are grateful for the cooperation of many Japanese urologists.

Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Institutions that were registered.

Appendix I

JUA staging system for testicular cancer (2005; established based on Boden–Gibb's staging system)
Stage I: Confined to testis
Stage II: RPLN involved below diaphragm
  IIA: RPLN <5 cm
  IIB: RPLN 5 cm or more
Stage III: Distant metastasis
  III0: No evident radiographic metastasis with elevated tumor marker
  IIIA: LN above diaphragm without visceral organs involved
  IIIB: Lung metastasis
    IIIB1: 4 or less lung metastasis, all <2 cm
    IIIB2: 5 or more lung metastasis or >2 m in size
  IIIC: Visceral metastasis other than lung

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RESEARCH

Immunophenotype and Human Papillomavirus Status of Serous Adenocarcinoma of the Uterine Cervix

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Abstract Serous adenocarcinoma of the cervix (SACC) is a very rare tumor. Our study aimed to characterize the immune profile and human papillomavirus (HPV) status of SACC, in comparison with other serous adenocarcinomas arising in the female genital tract. The pathological specimens obtained from 81 patients with serous carcinoma of the uterine cervix (n=12), 29 endometrium, 20 ovary and 20 patients with mucinous carcinoma of the uterine cervix were reviewed. We assessed the expression of WT-1, p53, p16, HER2, CEA, and CA125 by immunohistochemistry and HPV DNA by PCR in 12 SACC samples. Their immune profile was compared with that of uterine papillary serous carcinoma (UPSC), ovarian serous adenocarcinoma (OSA), and mucinous endocervical adenocarcinoma (MEA). WT-1 and HER2 were expressed in very few SACC samples (0 and 0 %, respectively), but p16, CA125, CEA and p53 were present in 100, 92, 58 and 50 %, respectively. The difference in WT-1 expression between SACC and UPSC, MEA is not significant, but SACC differ significantly from OSA (p<0.01). HPV DNA (type 16 or 18) was detected in 4 of the 12 SACC. The immunophenotype of SACC was similar to UPSC, whereas the frequency of expression of WT-1 was significantly lower in SACC than OSA. It appeared that p53 expression was associated with worse clinical outcome in patients with SACC, and that HPV infection was related to its occurrence.

Keywords Serous adenocarcinoma · Uterine cervix · Immunohistochemical features · Human papillomavirus

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Introduction

Serous adenocarcinomas are one of the most aggressive gynecological cancer types, very rare in the uterine cervix, but common in the ovary, fallopian tube, and peritoneum. It represents <10 % of endometrial carcinomas [1]. Zhou et al. [2] reported the first detailed clinicopathological features of 17 cases of serous adenocarcinoma of the uterine cervix (SACC). Our previous retrospective study assessed the clinicopathological features and prognosis of 12 patients with SACC who underwent hysterectomy [3]. However, little is known about the immunoprofile and human papillomavirus (HPV) involvement in SACC.

In the present study, we characterized the immunohistochemical features of 12 SACCs, using seven antibodies against WT-1, p53, p16, human epidermal growth factor receptor 2 (HER2), carcinoembryonic antigen (CEA), and CA125 and compared the immunoprofile with uterine papillary serous carcinoma (UPSC), ovarian serous adenocarcinoma (OSA) and mucinous endocervical adenocarcinoma (MEA). In this way, we aimed to characterize the immunoprofile and HPV status of SACC, in comparison with other serous adenocarcinomas arising in the female genital tract and MEA.

Published online: 05 November 2014

Materials and Methods

Patients and Tissue Samples

We reviewed the medical records and pathological specimens obtained from 81 patients with serous carcinoma of the uterine cervix (n=12), 29 endometrium, 20 ovary and 20 patients with mucinous carcinoma of the uterine cervix. All 81 patients were treated in the Department of Gynecology and diagnosed in the Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan, between 1985 and 2005 and underwent surgical staging according to the International Federation of Gynecology and Obstetrics (FIGO) system. All patients with serous carcinomas of the cervix had radical hysterectomy and bilateral salpingo-oophorectomy in order to exclude a primary neoplasm in the corpus, ovary or tube. All hematoxylin-eosin-stained slides were reviewed in all cases, and final diagnoses were confirmed by two observers (S.T. and Y.S.).

The diagnosis of SACC was made only when an invasive endocervical adenocarcinoma exhibited a prominent papillary structure and/or slit-like glandular spaces, and usually moderate to marked cytologic atypia (Fig. 1a) without either intra- or extra-cytoplasmic mucin. Absence of concurrent or previous primary endometrial, ovarian, fallopian tubal or peritoneum serous carcinoma was a prerequisite for the diagnosis of SACC. At least 10 % of the tumor area had to be of papillary serous type for inclusion as a SACC in this study. Seven of 12 SACC cases were classified as pure serous adenocarcinomas, and the other 5 were mixed serous adenocarcinoma. Uterine and ovarian serous adenocarcinoma and mucinous endocervical adenocarcinoma were diagnosed according to the World Health Organization International Histological Classification of Tumors [4]. Eleven cases of mixed types were included among the 29 UPSCs, but mixed serous adenocarcinoma and mucinous adenocarcinomas were excluded from this study among the cases of OSA. All cases were included only when destructive or frank stromal invasion was observed.

Immunohistochemistry

All tumor tissue specimens having been fixed in formalin and embedded in paraffin were cut into 4-μm-thick serial sections for immunohistochemical staining, in addition to the usual hematoxylin and eosin staining. This study was performed with the approval of the Internal Review Board on ethical issues. Antibodies used for immunohistochemistry were WT-1 (clone 6 F-H2, 1:50, Dako, Glostrup, Denmark), p53 (clone DO-7, 1:100, Dako), p16 (clone 16P07, 1:100, Neomarkers Inc., Fremont, CA), HercepTest (Dako), CEA (polyclonal, 1:5,000, Dako), and CA125(clone M11, 1:20, Dako). Immunohistochemical staining for WT-1, p53, p16, HER2, CEA, and CA125 products was performed with an autoimmunostainer (Autostainer Link 48, Dako) according to the manufacturers' instructions.

Scoring of the Results

The results of the immunohistochemical staining were evaluated as the percentage of positively stained neoplastic cells. In mixed type tumors, only the serous component was evaluated

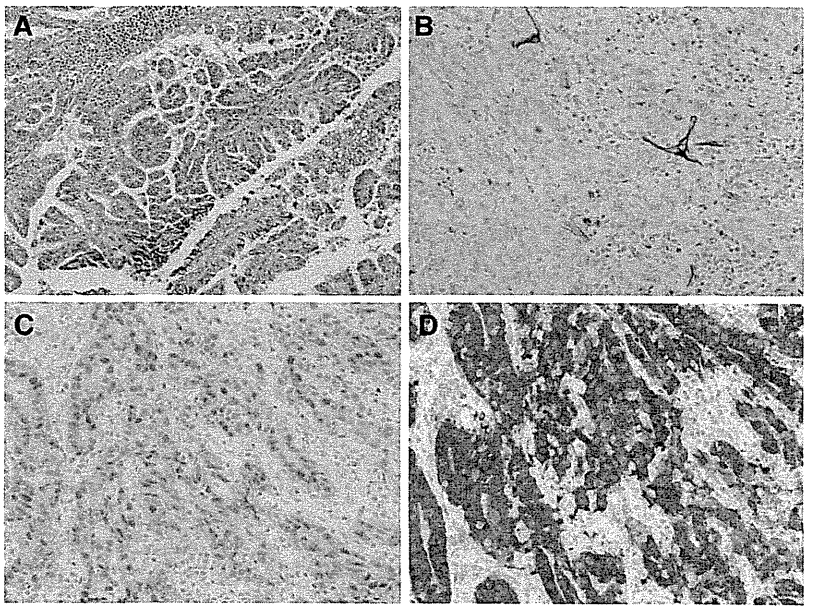

Fig. 1 Histopathological presentation of SACC. **a** H&E staining. (x100). **b** Immunohistochemical staining showing lack of WT-1 expression (x200). **c** Immunohistochemical staining showing expression of p53 (x200). **d** Immunohistochemical staining showing expression of p16 (x200)

in this study. For all antibodies except HER2, the level of expression was graded according to the percentage of immunoreactive neoplastic cells of the serous carcinoma component as follows: 0, <10 %; 1+, 10–25 %; 2+, 26–50 %; 3+, >50 %. Tumors with >10 % stained cells were considered positive for expression of that antigen. Immunoreactivity for HER2 was scored semiquantitatively as follows: 0, no immunostaining, or membrane staining in <10 % of cells; 1+, weak or barely perceptible staining in ≥10 % of cells, the cells stained in only part of the membrane; 2+, weak or moderate staining in the whole membrane in ≥10 % of tumor cells; 3+, strong staining in the whole membrane in ≥10 % of tumor cells [5]. We defined cases scoring 2+ and 3+ as HER2–positive. The immunohistochemical evaluation was performed by two observers (S.T. and Y.S.) separately, and the median value was used.

Polymerase Chain Reaction and Sequencing Analysis

DNA samples were extracted from paraffin embedded sections using the QIAamp DNA FFPE tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCR was performed using HPV consensus primers GP5+/6+ as previously described [6]. DNA samples obtained from cervical squamous cell carcinoma with HPV 16 infection and from the HeLa cell line, which is positive for HPV 18 DNA, were used as positive controls. *GAPDH* was amplified to ensure proper DNA extraction using the primer pair 5′-GCAG TGGGGACACGGAAGGC-3′ and 5′-ACTGTGGATG GCCCCTCGG-3′. The PCR products were electrophoresed in a 2 % (w/v) agarose gel and visualized under ultraviolet light with ethidium bromide staining.

The PCR products were purified using QIAquick Spin (Qiagen) and bidirectionally sequenced with the same primers as used for amplification. Sequence data were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistics

Inter-group comparisons were made by Fisher's exact test. $P<0.05$ was considered statistically significant.

Results

Expression of WT-1, p53, p16, HER2, CEA, and CA125 in Cancer

Table 1 shows a comparison of staining with each antibody in SACC, UPSC, OSA and MEA. WT-1 and p53 staining was nuclear, p16 staining was both cytoplasmic and nuclear, HER2, CEA and CA125 staining was only at the membrane.

Table 1 Immunohistochemical findings

Molecule	Number of cases (%)			
	Immunohistochemistry score			
	0 (0–9 %)	1 (10–25 %)	2 (26–50 %)	3 (50 % <)
SACC (n=12)				
WT-1	12 (100)	0 (0)	0 (0)	0 (0)
p53	6 (50)	2 (17)	0 (0)	4 (33)
p16	0 (0)	0 (0)	2 (17)	10 (83)
HER2	11 (92)	1 (8)	0 (0)	0 (0)
CEA	5 (42)	3 (25)	1 (8)	3 (25)
CA125	0 (0)	1 (8)	0 (0)	11 (92)
UPSC (n=29)				
WT-1	23 (80)	2 (7)	1 (3)	3 (10)
p53	6 (21)	0 (0)	3 (10)	20 (69)
p16	1 (3)	1 (3)	2 (7)	25 (87)
HER2	23 (80)	1 (3)	1 (3)	4 (14)
CEA	20 (69)	4 (14)	2 (7)	3 (10)
CA125	1 (3)	0 (0)	2 (7)	26 (90)
OSA (n=20)				
WT-1	0 (0)	0 (0)	0 (0)	20 (100)
p53	4 (20)	0 (0)	0 (0)	16 (80)
p16	0 (0)	5 (25)	1 (5)	14 (70)
HER2	18 (90)	0 (0)	1 (5)	1 (5)
CEA	18 (90)	2 (10)	0 (0)	0 (0)
CA125	0 (0)	0 (0)	0 (0)	20 (100)
MEA (n=20)				
WT-1	20 (100)	0 (0)	0 (0)	0 (0)
p53	18 (90)	1 (5)	0 (0)	1 (5)
p16	1 (5)	1 (5)	0 (0)	18 (90)
HER2	19 (95)	1 (5)	0 (0)	0 (0)
CEA	1 (5)	4 (20)	1 (5)	14 (70)
CA125	1 (5)	0 (0)	1 (5)	18 (90)

SACC Serous adenocarcinoma of the cervix, *HER2* human epidermal growth factor receptor 2, *CEA* carcinoembryonic antigen, *UPSC* uterine papillary serous carcinoma, *OSA* ovarian serous adenocarcinoma, *MEA* endocervical adenocarcinoma

Serous Adenocarcinoma of the Uterine Cervix (SACC)

Representative immunohistochemical stainings for each antibody are shown in Figs. 1 and 2. WT-1 and HER2 were negative in all SACC (Figs. 1b and 2a). Six SACC cases (50 %) were positive for p53 (Fig. 1c), with 4 of them showing strong (3+) expression. In contrast, p16 had intermediate (2+) or strong expression in all 12 SACC (Fig. 1d), and 11/12 were strongly positive for CA125 (Fig. 2d).

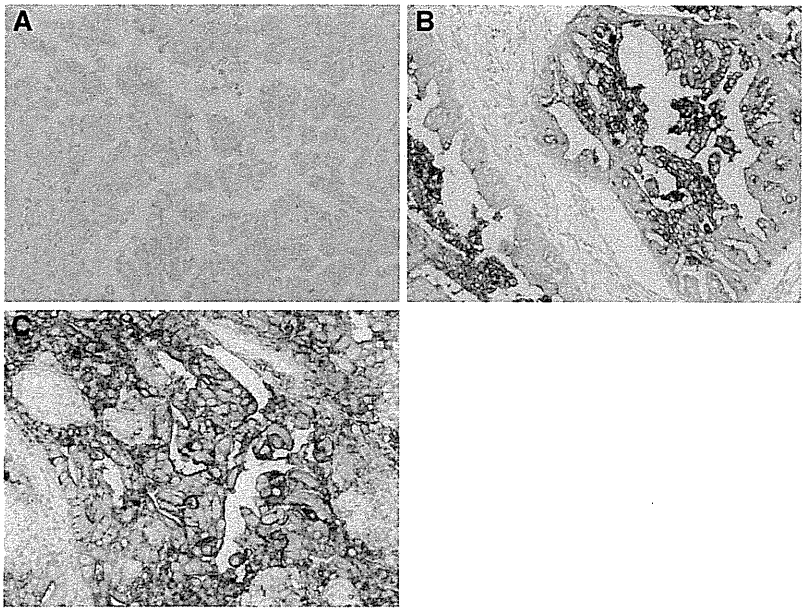

Fig. 2 Histopathological presentation of SACC. (**a**) Immunohistochemical staining showing negative expression of HER2. (x200). Immunohistochemical staining showing expression of (**b**) CEA (x200), (**c**) CA125 (x200)

Uterine Papillary Serous Carcinoma (UPSC)

Representative immunohistochemical stainings for each antibody are shown in Fig. 3. Only 21 % (6/29) of UPSC cases were positive for WT-1 (Fig. 3b) and only one case of UPSC was CK5/6-positive (Fig. 3b). Similarly, 17 % (5/29) of UPSC s were positive for HER2 (Fig. 3d), with 4 cases having strong expression. In contrast, p16 and CA125 showed intermediate or strong positive expression in the majority of cases (93 % and 97 %, respectively). These findings in UPSC are thus similar to SACC. p53 was positive in 23 cases of UPSC (79 %)(Fig. 3c), and strongly positive in most of these (69 %).

Ovarian Serous Adenocarcinoma (OSA)

Representative immunohistochemical stainings for each antibody are shown in Fig. 4. WT-1 (Fig. 4b) and CA125 were positive in all OSA. Thus, the frequency of WT-1 expression was significantly higher in OSA than SACC ($p<0.01$). p53 and p16 were strongly positive in 80 % (16/20) and 70 % (14/20) of OSA cases, respectively (Fig. 4c and d). In contrast,

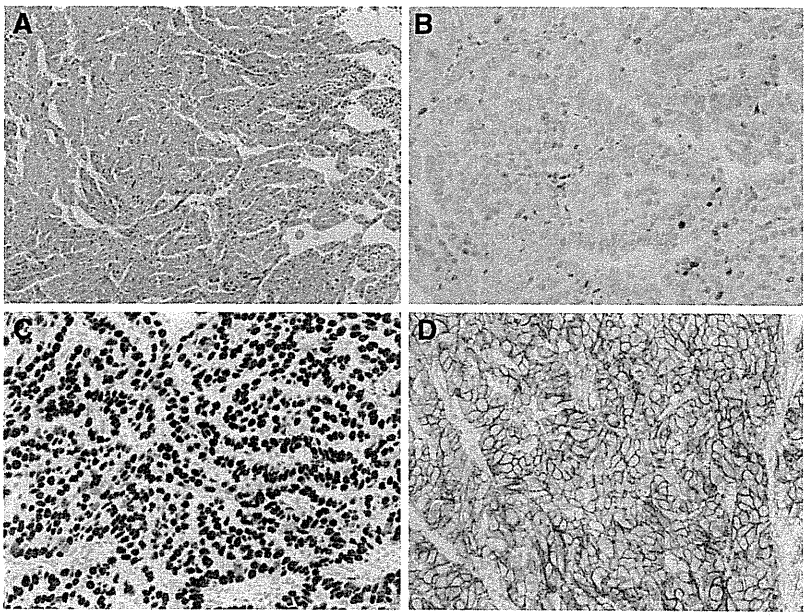

Fig. 3 Histopathological presentation of UPSC. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) WT-1 (x200), (**c**) p53 (x200), (**d**) HER2 (x200)

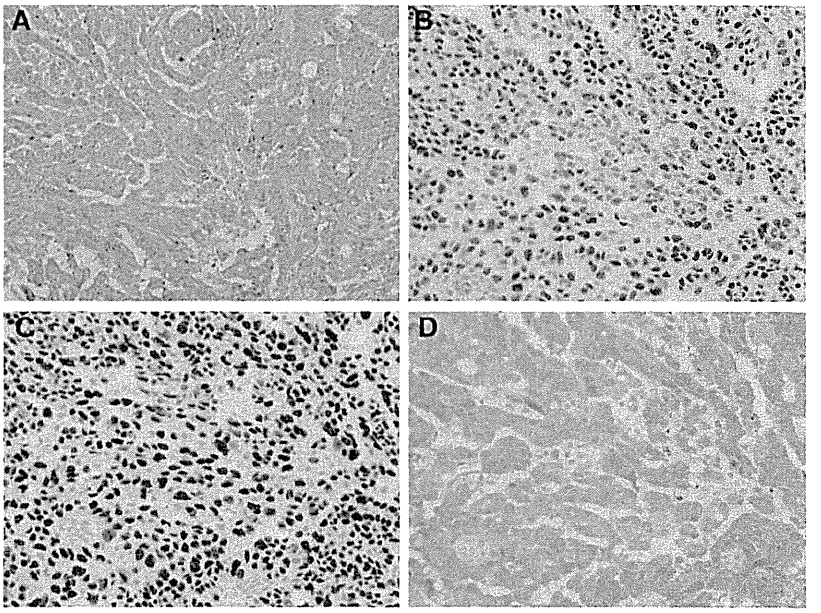

Fig. 4 Histopathological presentation of OSA. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) WT-1 (x200), (**c**) p53 (x200), (**d**) p16 (x200)

HER2 and CEA expression was rare, at only 10 % (2/20) each.

Mucinous Endocervical Adenocarcinoma (MEA)

Representative immunohistochemical stainings for each antibody are shown in Fig. 5. WT-1 and HER2 were negative in all MEA, p53 expression was rare, at 10 % (2/20). In contrast, p16 and CA125 both showed strong positive expression in 90 % (18/20) of cases (Fig. 5b and d).

We suggested binarizing the immunostaining results as positive vs negative, and comparing using the Fisher's exact test. WT-1 and p53 appear to show differences in percent of cases expressing these proteins between SACC, UPSC, OSA and MEA. The difference in WT-1 expression between SACC and UPSC, MEA is not significant, but SACC differ significantly from OSA ($p<0.01$). In the case of p53, overexpression (3+ staining) is seen in 4/12 SACC which differs significantly compared to either endometrial (20/29) or ovarian (16/20) serous carcinomas. There is a tendency between recurrence and p53

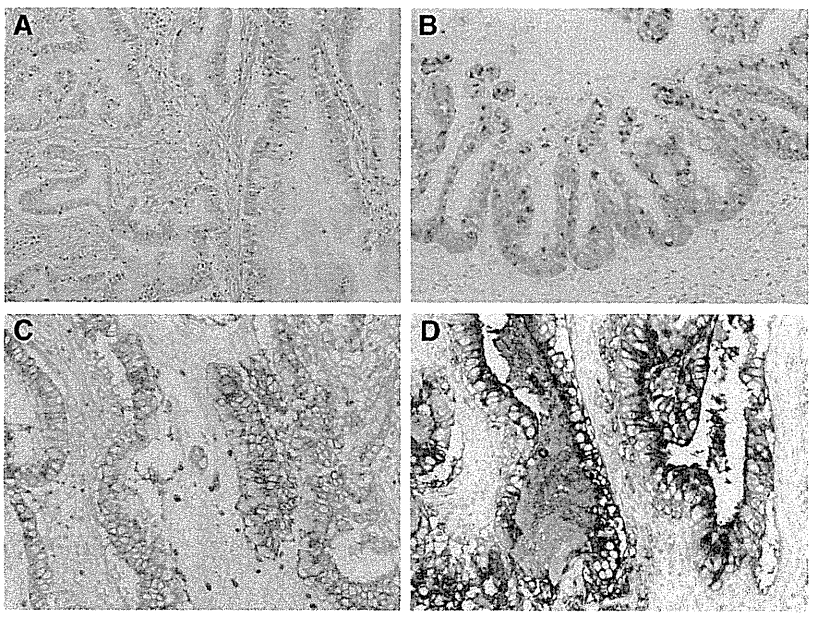

Fig. 5 Histopathological presentation of MEA. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) p16 (x200), (**c**) CEA (x200), (**d**) CA125 (x200)

overexpression (p=0.06). The differences in frequency of HER2 expression are not significant.

HPV Infection

GAPDH was negative in the PCR for 2 of 12 SACC (cases 2 and 6), suggesting poor DNA preservation. These were excluded from further analysis. Among the remaining ten cases, four (cases 1, 3, 7 and 9) were positive when using the HPV consensus primers GP5+/6+ (Fig. 6). Sequencing of the PCR products showed that they had been derived from HPV16 in two samples (cases 1 and 9) and from HPV18 in the other two (cases 3 and 7).

Discussion

The p53 tumor suppressor gene plays a major role in cell cycle control and growth arrest following DNA damage. Mutations of this gene are the most common genetic alterations in human cancers [7]. Overexpression of p53, as detected by immunohistochemistry, has been proposed to indicate a worsened prognosis in some malignancies [8]. In our study, 50 % (6/12) cases of SACC were positive for p53, among them 4 with strong expression. In contrast, 90 % of MEA were negative for p53. Hunt et.al. [8] reported that p53 was not expressed in 86 % (30/35) of their uterine cervical adenocarcinomas, implying a difference in the pathogenetic mechanisms between SACC and MEA in the context of p53 inactivation. However, there is a report that the rates of p53 gene mutation and p53 nuclear immunoreactions in adenocarcinomas of the uterine cervix are relatively high, at 46 % and 32 %, respectively [9]. Therefore, further studies are needed to clarify differences in the pathogenetic mechanisms in SACC and MEA.

Interestingly, 3 of 4 SACC cases which showed strong p53 expression had died, and there is a tendency between recurrence and p53 expression (p=0.06). Zhou et al. [2] reported that nuclear immunoreactivity for p53 was present in 5 of 12 SACC cases, and, of the five patients with p53 positive tumors, 4 developed metastases. Batistatou et al. [10] reported a deceased case of SACC with p53 expression. Recently,

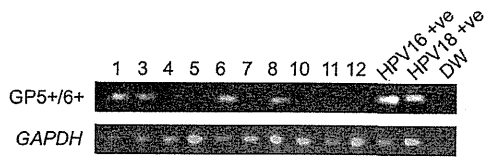

Fig. 6 Detection of HPV DNA by polymerase chain reaction, Four cases (cases 1, 3, 7 and 9) were positive using the HPV consensus primers GP5+/6+. DW, distilled water (no DNA template); +ve, positive control. *GAPDH* served as a positive control

Nofech-Mozes et al. [11] reported p53 immunostaining in 9 of 10 SACC cases, of which 3 had strong expression (>50 % of cells positive). That study [11] included 3 deceased cases of SACC, of whom two had strong expression (>50 % of cells). Thus, the strong p53 expression in SACC seemed to be associated with worse clinical outcome. Establishing p53 status may therefore contribute to prognostic indicators in this disease.

In addition, overexpression of p16 induced by HPV has been found to be associated with cervical squamous carcinoma [12–15]. It was also reported to be expressed in cervical adenocarcinoma [16–18]. In our study, 90 % (18/20) of MEA and 83 % (10/12) of SACC were strongly positive for p16, with no significant difference between SACC and MEA. Chiesa-Vottero et al. [19] reported that p16 overexpression was present in uterine and ovarian high grade serous adenocarcinomas. In our study, both OSA and UPSC showed p16 expression, with no significant differences among serous adenocarcinomas arising from different female genital tract organs and MEA.

Some studies have shown that persistent infection with high risk HPV is an important etiological factor for the occurrence of cervical adenocarcinoma [20, 21]. In the present study, 2 cases had HPV 16 DNA and 2 had HPV 18 DNA (both high risk HPV types). Nofech-Mozes et al. [22] reported that high-risk HPV DNA was found in 3 of 4 SACCs. Another study reported that HPV was detected in 1 of 3 SACCs [23]. We suggest that high risk HPV infection affects the occurrence of SACC.

Despite different origins, serous adenocarcinomas of the female genital tract show similar morphologic features, characterized by the presence of a prominent papillary structure and/or slit-like glandular spaces, and usually moderate to marked cytologic atypia. It has been shown that among the various histological types of ovaian carcinoma, the incidence of WT-1 positive tumors is highest in ovarian serous adenocarcinoma [24–27]. Nofech-Mozes et al. [28] concluded that strong WT-1 expression was associated with OSA rather than UPSC. In our study, WT-1 was positive in all OSA, whereas only 3 of 29 (10 %) UPSC showed strong expression of this antigen. In addition, WT-1 was negative in all SACC in this study. Nofech-Mozes et al. [11] also reported that only two cases of 10 SACC showed immunoreactivity to WT-1 where staining was seen in <50 % of all neoplastic cells. This is similar to the findings in our study. We suggest that SACC has biological features similar to UPSC. These may be associated with its embryologic developmental origin, in that both the uterine cervix and the uterine corpus are derived from the mullerian duct. On the other hand, the ovary is derived from indifferent gonad.

Generally, a majority of endocervical adenocarcinomas is CEA positive [29]. Alkushi et al. [30] reported that all endocervical type cervical adenocarcinomas expressed CEA

as detected by the polyclonal antibody. In our study, 95 % (19/20) of MEA were positive (>10 % of cells) and 58 % (7/12) of SACC were also positive (>10 % of cells). Zhou et al. [2] reported that 50 % (6/12) of SACC were positive for CEA, but Nofech-Mozes et al. [11] reported that only 30 % (3/10) were. Another two SACC cases were reported to be negative for CEA. The frequency of CEA expression in SACC tended to be low compared with MEA. Therefore, it was thought to be useful to distinguish SACC from MEA with respect to CEA immunostaining.

Although CA125 is a valuable serum marker for gynecologic cancer, its utility as an immunohistochemical marker is limited. Zhou et al. [2] reported that 75 % (9/12) of SACC were positive for CA125, and, in our study, 92 % (11/12) of SACC were strongly positive. Similarly, all OSA, 90 % (26/29) of UPSC, and 90 % (18/20) of MEA showed strong expression of CA125. There was no significant difference in CA125 expression among serous adenocarcinomas arising in different female genital tract organs and MEA.

HER2 immunostaining is associated with poor patient outcome in UPSC [31–33]. However, 92 % (11/12) of SACC were negative for HER2, and the differences in frequency of HER2 expression are not significant. HER2 expression appears not to be associated with poor prognosis in SACC, but this may due to the small number of SACC patients studied.

In summary, we have found that p53, p16 and CA125 expression is common in SACC. p53 expression seems to be associated with worse clinical outcome, and HPV infection is related to its pathogenesis. The immunohistochemical expression pattern in SACC samples was similar to UPSC. Although SACC is a very rare tumor, it is hoped that appropriate immunoprofiling will contribute to the introduction of improved management of patients with this tumor.

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Conflict of Interests The authors declare no conflicts of interest.

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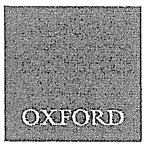
Clinical Trial Note



Clinical Trial Note

Non-randomized confirmatory trial of modified radical hysterectomy for patients with tumor diameter 2 cm or less FIGO Stage IB1 uterine cervical cancer: Japan Clinical Oncology Group Study (JCOG1101)

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Abstract

A non-randomized confirmatory trial was started in Japan to evaluate the efficacy of modified radical hysterectomy in patients with tumor diameter 2 cm or less FIGO Stage IB1 uterine cervical cancer, for which the current standard is radical hysterectomy. This study began in January 2013 and a total of 240 patients will be accrued from 37 institutions within 3 years. The primary endpoint is 5-year survival. The secondary endpoints are overall survival, relapse-free survival, local relapse-free survival, percent completion of modified radical hysterectomy, percent local relapse, percent pathological parametrial involvement, days until self-urination and residual urine disappearance, blood loss, operation time, percent post-operative radiation therapy, adverse events and severe adverse events. This trial was registered at the UMIN Clinical Trials Registry as UMIN 000009726 (http://www.umin.ac.jp/ctr/).

Key words: FIGO stage IB1 (≤2 cm) uterine cervical cancer, modified radical hysterectomy, clinical trials, Phase III

Introduction

Uterine cervical cancer (UCC) is the fifth most common disease for women in Japan after breast cancer, colon cancer, gastric cancer and lung cancer. Both the prevalence and the mortality of UCC are currently increasing in women in their 20 and 30 s. Stage IB1 comprises 36% of cases of invasive cancer (Stages IB–IV) in Japan (1).

The standard surgery for Stage IB1 UCC is radical hysterectomy (RHx). The frequency of pathological parametrial invasion and

lymph node (LN) metastasis is reportedly very low and relapse-free survival is good enough, especially when the tumor diameter is 2 cm or less (TD ≤ 2 cm) (2–4). However, RHx causes loss of desire to void because of damage to the pelvic splanchnic nerve due to surgery (5). Self-catheterization is required in some patients for the rest of their life, which often impairs quality of life, especially for young women. Persistent urination disorder can secondarily cause chronic urinary tract infection and renal failure. Because of such disadvantages for patients, a less invasive surgery that does not impair the prognosis should be pursued.

Modified radical hysterectomy (MRHx) has been a standard treatment for minimally invasive cancer of the uterine cervix. Compared with RHx, pelvic splanchnic nerves can be preserved in MRHx and it can prevent urination disorder. It has been reported that MRHx could reduce the number of days until self-urination from catheter removal and the number of days until residual urine disappearance compared with RHx (6,7). Yang et al. reported that MRHx shortened mean time to voiding and reduced post-operative voiding difficulty (8). However, the prognosis of Stage IB1 UCC treated with MRHx has yet to be well examined.

From 2008 to 2009, the Gynecologic Cancer Study Group of the Japan Clinical Oncology Group (JCOG) performed an observational study (JCOG0806-A) of surgical procedures and pathological findings for Stage IB1 UCC (Tomoyasu Kato, unpublished data). Five-year overall survival (OS) was 95.8% and the proportion of pathological parametrial involvement was 1.9% in patients with TD ≤ 2 cm Stage IB1 UCC treated by RHx. Five-year OS was 97.0% in patients with TD ≤ 2 cm Stage IB1 UCC treated by MRHx. From the results of this study, MRHx is considered to be a promising treatment, even in terms of the efficacy, but this should be confirmed in a prospective study.

A randomized controlled trial (RCT) is the gold standard to establish a new standard treatment. However, the five-year OS of RHx is known to be as high as 95% and this high proportion would not differ even if we conduct a RCT. On the other hand, if the 5-year OS of MRHx is proven to be similarly high, it can be accepted as the new standard because the complications of MRHx are definitely less than those of RHx. Therefore, we set this trial as a non-randomized confirmatory trial to evaluate the efficacy of MRHx in patients with TD ≤ 2 cm Stage IB1 UCC (Fig. 1).

The Protocol Review Committee of JCOG approved the protocol in November 2012 and the study was activated in January 2013. This trial was registered at the UMIN Clinical Trials Registry as UMIN 000009726 (http://www.umin.ac.jp/ctr/index.htm].

Protocol digest of JCOG 1101

Purpose

The aim of this study is to evaluate the efficacy of MRHx compared with RHx in patients with TD ≤ 2 cm FIGO Stage IB1 UCC.

Study setting

A multi-institutional non-randomized Phase III study.

Endpoints

The primary endpoint is 5-year OS, which is defined as days from enrollment to death from any cause and censored at the latest day without events. The secondary endpoints are OS, relapse-free survival, local relapse-free survival, percent completion of modified radical hysterectomy, percent local relapse, percent pathological parametrial involvement, days until self-urination and residual urine disappearance, blood loss, operation time, percent post-operative radiation therapy, adverse events and severe adverse events. Relapse-free survival is defined as days from enrollment to any disease relapse or death from any cause and censored at the latest date when the patient is alive. Local relapse-free survival is defined as days from enrollment to local disease relapse or death from any cause and censored at the latest date when the patient is alive. Days until self-urination and residual urine disappearance are defined as days from post-operative urethral catheter removal to self-urination and residual urine disappearance (residual urine 50 cc or less and self-urination volume more than residual urine).

Eligibility criteria

Inclusion criteria

For inclusion in the study, the patient must fulfill all of the following criteria:

(i) Any one of the following histologies in the primary lesion located at the uterine cervix.
   1. Squamous cell carcinoma (keratinizing or non-keratinizing type)
   2. Adenosquamous carcinoma (except for glassy cell carcinoma)
   3. Adenocarcinoma (endocervical-type mucinous adenocarcinoma, intestinal-type mucinous adenocarcinoma or endometrioid adenocarcinoma)

(ii) Clinical Stage IB1 fulfilling (1 or 2) (The General Rules for Clinical and Pathological Management of Uterine Cervical Cancer in Japan, 3rd edition, 2012)
   1. To fulfill (a or b), in the case that a tumor with maximum diameter (MD) of 2 cm or less is confirmed in a pelvic magnetic resonance image (MRI) within 56 days before registration
      (a) When diagnostic conization of the cervix is performed within 28 days before registration after MRI, MD is confirmed as 2 cm or less (a colposcopy is not essential)
      (b) When diagnostic conization of the cervix is not performed, an invasive cancer is not detected or the length

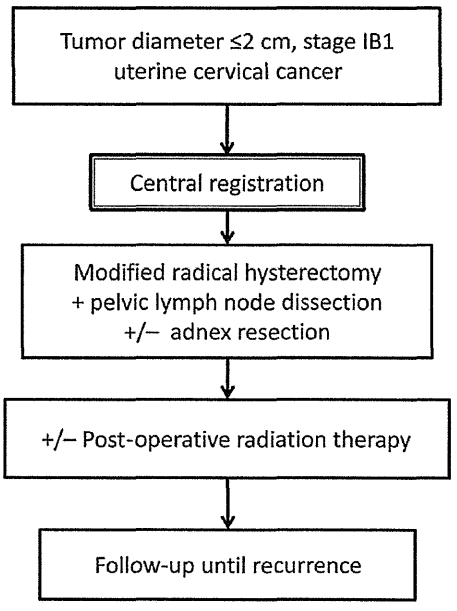

Figure 1. Schema of the study.

of invasion is confirmed as 2 cm or less in a colposcopy within 28 days before registration
2. To fulfill (a or b), in the case that a tumor is not detected in a pelvic MRI within 56 days before registration
   (a) When an invasive tumor is not confirmed in a colposcopy within 28 days before registration, the length of invasion is confirmed as 2 cm or less by diagnostic conization of the cervix within 28 days before registration
   (b) When an invasive tumor is confirmed in a colposcopy within 28 days before registration, the length of invasion is confirmed as 2 cm or less in the colposcopy

(iii) Neither distant LN metastasis nor distant metastasis in abdominal and pelvic computed tomography (CT) within 28 days before registration
(iv) Aged 20–70 years old
(v) ECOG performance status (PS) of 0 or 1
(vi) No following prior treatment
   1. Surgery except for cervical conization for UCC
   2. Surgery for lower abdominal or pelvic malignancy
   3. Radiation therapy or chemotherapy for other malignancies
(vii) Adequate organ functions
(viii) Written informed consent

Exclusion criteria

Patients are excluded if they meet any of the following criteria:

(i) Simultaneous or metachronous (within 5 years) double cancers except carcinoma *in situ* or intramucosal tumor
(ii) Active infectious disease to be treated
(iii) Body temperature of 38°C or more
(iv) Women during pregnancy or breastfeeding
(v) Psychiatric disease
(vi) Systemic and continuous steroid medication
(vii) Uncontrolled diabetes mellitus
(viii) Uncontrolled hypertension

Treatment methods

The protocol treatment consists of MRHx and post-operative radiation therapy.

Modified radical hysterectomy

RHx is a method of hysterectomy defined as being intermediate between simple hysterectomy and radical hysterectomy. The surgical procedure must be compliant with the following (The General Rules for Clinical and Pathological Management of Uterine Cervical Cancer, 3rd edition): the protocol surgery is to cut the anterior layer of the vesicouterine ligament of the uterus, mobilize the ureter laterally, and remove the part of the parametrial tissue and vaginal wall away from the uterine cervix. The posterior layer of the vesicouterine ligament must not be resected. It is recommended that bilateral adnexa be resected, but they can be preserved if the following criteria are met: (i) premenopausal patients who wish for their ovaries to be preserved, (ii) pre-operative histological diagnosis is squamous cell carcinoma and (iii) no macroscopic metastasis.

Dissection of regional LNs (common iliac LNs, external iliac LNs, internal iliac LNs, obturator LNs, sacral LNs and cardinal ligament LNs) must be carried out. Para-aortic LN biopsy must be performed when macroscopic metastasis is suspected.

Laparoscopic and robotic surgeries are not allowed.

Radiation therapy

Whole-pelvic irradiation is administered when pelvic LNs are positive, parametric invasion is positive, or depth in the cervical wall is 2/3 or more in the post-operative pathological diagnosis. Irradiation of the para-aortic LN region is added when the para-aortic LN is positive in the post-operative pathological diagnosis. Intracavitary brachytherapy for the vaginal cuff is administered when cancer is positive within 1 cm of the vaginal end. Radiation therapy is administered from Day 21–42 after surgery.

Whole-pelvic irradiation comprises a total dose of 50.4 Gy in a fraction of 1.8 Gy five times a week. In case irradiation for para-aortic LNs is added to that for the whole-pelvic region, irradiation comprises a total dose of 45 Gy in a fraction of 1.8 Gy five times a week. Intracavitary brachytherapy for the vaginal cuff comprises a total dose of 30 Gy in a fraction of 6 Gy once a week by itself, a total dose of 8 Gy in a fraction of 4 Gy once or twice a week when combined with whole-pelvic irradiation, and a total dose of 12 Gy in a fraction of 4 Gy once or twice a week when combined with irradiation for the whole-pelvic and para-aortic LN region. The dose of intracavitary brachytherapy is prescribed at a depth of 0.5 mm from the vaginal surface.

For external beam irradiation, CT simulation is mandatory. The gross tumor volume is not defined in this trial because the macroscopic site of disease is resected before irradiation. The clinical target volume (CTV) includes CTV vaginal cuff, CTV paracolpium and CTV LN subclinical. The planning target volume (PTV) includes PTV vaginal cuff, PTV paracolpium and PTV LN subclinical. PTV vaginal cuff and PTV paracolpium are defined as 1–1.5 cm margins for anterior and posterior directions and 0.5–1 cm margins for the lateral direction around the CTV vaginal cuff and paracolpium, respectively, to compensate for setup variations and internal organ motion. PTV LN subclinical is defined as 0.5–1 cm margins for all directions around CTV LN subclinical to compensate for setup variations and internal organ motion.

Follow-up

All enrolled patients are followed up for at least 5 years. Tumor marker, vaginal stump cytology, physical examination and safety are to be evaluated at least every 3 months for the first 3 years and every 6 months in the fourth and fifth years. Chest X-ray and abdominal and pelvic CT are to be evaluated at least every 6 months for the first 3 years and every 12 months in the fourth and fifth years.

Study design and statistical analysis

This trial is a non-randomized confirmatory trial designed to evaluate the efficacy of RHx in patients with tumor diameter 2 cm or less FIGO stage IB1 UCC.

In our previous observational study (JCOG0806-A), the 5-year survival of the current standard procedure, radical hysterectomy, was 95.8%. Thus, we anticipate in this study that the expected 5-year survival of RHx is also 95.8%. RHx is a less toxic procedure in terms of urination disorder than radical hysterectomy, and it would be a non-inferiority trial if we conducted a RCT to compare both procedures. Considering the difference of toxicity, we would set the non-inferiority margin as 5%. Thus, we set the threshold 5-year survival of this study as 90.8%.The planned accrual period is 3 years, and the follow-up period is 5 years after completion of accrual. In this trial, the planned sample size is 240 patients, which was calculated based on an expected 5-year survival of 95.8% and a threshold of 90.8%, with a one-sided alpha error of 0.05 and a beta error of 0.1.

Interim analysis and monitoring

In this trial, an interim analysis is not planned because this is a single-arm trial and it is not appropriate to judge that the primary endpoint is met or not based on preliminary data. However, when the efficacy is unexpectedly low, patient accrual should be stopped and the result should be published early. Therefore, the Data and Safety Monitoring Committee will check monitoring reports issued twice a year and discuss early termination when tumor recurrence is observed in >24 patients, 10% of the total of 240 registered patients.

Central monitoring will be performed every 6 months by the JCOG Data Center to evaluate study progress and improve study quality.

Participating institutions (from North to South)

Hokkaido University Hospital, Sapporo Medical University Hospital, Iwate Medical University Hospital, Tohoku University Hospital, Tsukuba University Hospital, National Defense Medical College, Saitama Cancer Center, Saitama Medical Center, Saitama Medical University, Jikei Kashiwa Hospital, National Cancer Center Hospital, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Keio University Hospital, Jikei University Hospital, Cancer Institute Hospital, The University of Tokyo Hospital, Juntendo University Hospital, NTT Medical Center Tokyo, Kitasato University School of Medicine, Niigata Cancer Center Hospital, Shinshu University, Shizuoka Cancer Center, Aichi Cancer Center Hospital, Nagoya University School of Medicine, Kyoto University Hospital, Osaka City University Hospital, Kinki University School of Medicine, Osaka Prefectural Hospital Organization Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City General Hospital, Hyogo Cancer Center, Tottori University, Shikoku Cancer Center, Kyushu Cancer Center, Kurume University School of Medicine, Kyushu University Hospital, Saga University, Kumamoto University Medical School and Kagoshima City Hospital.

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Conflict of interest statement

None declared.

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Vaginal tolerance of CT based image-guided high-dose rate interstitial brachytherapy for gynecological malignancies

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Abstract

Background: Purpose of this study was to identify predictors of vaginal ulcer after CT based three-dimensional image-guided high-dose-rate interstitial brachytherapy (HDR-ISBT) for gynecologic malignancies.

Methods: Records were reviewed for 44 female (14 with primary disease and 30 with recurrence) with gynecological malignancies treated with HDR-ISBT with or without external beam radiation therapy. The HDR-ISBT applicator insertion was performed with image guidance by trans-rectal ultrasound and CT.

Results: The median clinical target volume was 35.5 ml (2.4-142.1 ml) and the median delivered dose in equivalent dose in 2 Gy fractions (EQD_2) for target volume D_{90} was 67.7 Gy (48.8-94.2 Gy, doses of external-beam radiation therapy and brachytherapy were combined). For re-irradiation patients, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 91.1 Gy, 100.9 Gy, 260.3 Gy, 212.3 Gy, 170.1 Gy, 117.1 Gy, 105.2 Gy, and 94.7 Gy, respectively. For those without prior radiation therapy, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 56.3 Gy, 54.3 Gy, 147.4 Gy, 126.2 Gy, 108.0 Gy, 103.5 Gy, 94.7 Gy, and 80.7 Gy, respectively. Among five patients with vaginal ulcer, three had prior pelvic radiation therapy in their initial treatment and three consequently suffered from fistula formation. On univariate analysis, re-irradiation and vaginal D_{2cc} in EQD_2 was the clinical predictors of vaginal ulcer ($p = 0.035$ and $p = 0.025$, respectively). The ROC analysis revealed that vaginal wall D_{2cc} is the best predictor of vaginal ulcer. The 2-year incidence rates of vaginal ulcer in the patients with vaginal wall D_{2cc} in EQD_2 equal to or less than 145 Gy and over 145 Gy were 3.7% and 23.5%, respectively, with a statistically significant difference ($p = 0.026$).

Conclusions: Re-irradiation and vaginal D_{2cc} is a significant predictor of vaginal ulcer after HDR-ISBT for gynecologic malignancies. Three-dimensional image-guided treatment planning should be performed to ensure adequate target coverage while minimizing vaginal D_{2cc} in order to avoid vagina ulcer.

Keywords: Gynecologic brachytherapy, High-dose-rate brachytherapy, Interstitial brachytherapy, Vaginal ulcer

Introduction

High-dose rate intracavitary brachytherapy (HDR-ICBT) is an established method in the management of gynecological malignancies, especially in cervical cancer. However, in patients with a narrow vagina, short uterine cavity, distal vaginal extension, and bulky tumors in which the optimal dose distribution cannot be obtained by intracavitary brachytherapy (ICBT), interstitial brachytherapy (ISBT) is employed. Also in patients with bulky postoperative central pelvic recurrence, ISBT has proven to be effective [1-5]. With the advent of image-guided brachytherapy it has become possible to assess the dose volume histogram (DVH) in brachytherapy. Several studies have validated the D_{2cc} as a predictor of rectal and bladder toxicities for ICBT [6] or for ISBT [7]. D_{2cc} of the rectum and bladder have been introduced into daily clinical practice of gynecological image-guided brachytherapy. However in ICRU 38 vagina was not recognized as organ at risk

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during brachytherapy tough it is adjacent to target volume and radioactive sources [8].

The purpose of this study was to retrospectively analyze the incidence of vaginal morbidities after HDR-ISBT for gynecological cancers and to find clinical and dosimetric factors which affect the incidence of the vaginal morbidities.

Methods

The inclusion criteria of this single institutional retrospective study were patients with gynecological malignancies who were treated by HDR-ISBT with or without external beam radiation therapy (EBRT) with a follow-up length exceeding 6 months or more. Patients with distant metastasis outside of pelvis were excluded from current study. HDR-ISBT was applied for both primary and salvage intents. Patients with superficial vaginal disease with thickness less than 5 mm were treated with HDR-ICBT and did not treated by HDR-ISBT; therefore these patients were not included in this analysis. Also HDR-ISBT was not applied for those patients who had distant metastasis or for those patients with far advanced tumors which had not responded to EBRT performed before HDR-ISBT. These patients were treated with EBRT alone. One patient who succumbed to progressive cancer in 5.5 months after ISBT was also excluded in this analysis. The medical records of all patients with gynecological malignancies treated with HDR-ISBT at the National Cancer Center Hospital, Tokyo, Japan, between 2008 and 2011 were retrieved and 44 patients were included in this study.

In the patients without prior pelvic irradiation, pelvic EBRT was delivered before HDR-ISBT. The common EBRT portals were whole pelvic irradiation including gross tumor volume (GTV) with adequate margin as well as the pelvic lymph nodes basin up to the level of the common iliac (L4/5 junction). If the tumor involved the lower third of the vagina, or there were clinically palpable inguinal nodes, inguinal regions were also included in the EBRT portals. The initial 20-40 Gy was delivered to the whole pelvis with a 4-fields box technique and then pelvic irradiation was administered with a central shield being employed to reduce exposure of organs at risk (OAR). The total dose delivered to the pelvic side wall was up to 50 Gy in a conventional fractionation. In patients with a history of prior pelvic radiation therapy or in feeble elderly patients, no EBRT or smaller EBRT fields with a reduced total dose were employed. HDR-ISBT was basically performed after the central shield was inserted. However for those patients treated without EBRT, HDR-ISBT was applied as solitary radiotherapy modality. The detailed procedure of gynecological HDR-ISBT was described elsewhere [9]. In brief, transperineal needle applicator insertion was performed under either general or local anesthesia with the patients in lithotomy position and guided by trans-rectal ultrasound (TRUS) or CT which can be taken with the patients lying in lithotomy position with the applicators in place. For advanced large disease, a Syed-Neblett perineal template (Best Medical International, Inc., Springfield, VA) was used in order to sufficiently cover lateral disease extent. For rather localized small disease, with limited parametrial and/or paracolpial invasion, free-hand needle applicator insertion with or without a vaginal applicator was used with fewer needles inserted compared to the Syed-Neblett perineal template. Treatment planning was performed with brachytherapy planning system (Oncentra® Nucletron, Veenendaal, The Netherlands) using CT images taken by the large bore CT simulator (Aquilion LG®, Toshiba, Tokyo, Japan), which allows imaging of the patients in lithotomy position. Although different applicator was used throughout the patients, the calculation method applied was the same. The clinical target volume (CTV) was defined based on the CT image obtained after needle insertion, as well as physical examination immediately before needle insertion, the intra-operative TRUS image and the most recent MRI were also taken into account. Reference points were set on the surface of CTV and prescribed dose was delivered to those points. HDR-ISRT treatment plan was calculated initially by geometrical optimization or volume optimization and then manual graphical modification was followed to enclose the CTV by the prescription dose while minimizing high dose to OAR. The median HDR-ISBT dose was 24 Gy (range, 18-54 Gy), and median HDR-ISBT dose per fraction was 6 Gy (range, 4-6 Gy). HDR-ISBT was performed twice daily with each fraction 6 hours apart. HDR-ISBT was performed with MicroSelectron HDR (Nucletron, Veenendaal, The Netherlands) using Ir-192.

At the discretion of the attending physician, weekly CDDP 40 mg/m^2 was used in 10 patients concurrently with EBRT. In general, patients with bulky disease, good performance status and adequate organ function were selected for the candidate for the administration of concurrent chemoradiation. Patients were seen in follow up 1 week after HDR-ISBT for a skin check, then every 1-2 months for 2 years, every 3-4 months for 5 years, and every 6-12 months thereafter.

When adding doses of EBRT, HDR-ISBT, and HDR-ICBT, we used the equivalent dose in 2 Gy fractions (EQD_2) according to the LQ model [10,11]. For re-irradiated patients, prior central pelvic EBRT doses were also added to EQD_2 for OARs. For those who had prior HDR-ICBT without DVH parameters of OARs because of lack of three dimensional dose calculations, it was difficult to estimate EQD_2 for OARs. Therefore, prescribed dose for tumor in EQD_2 ($\alpha/\beta = 10$) was converted to EQD_2 for late responding tissue ($\alpha/\beta = 3$) and added

together. Time interval between prior RT and the current RT was not taken into consideration in this analysis.

Rectum and bladder were contoured as a whole organ. Vaginal wall was extracted with a thickness of 4 mm on all CT images according to the Vienna group [12]. As for rectum and bladder, dosimetric parameter of D_{2cc} was used because these values have been validated by several studies [6-8]. On the other hand, there is no validated parameter for vaginal dose; therefore $D_{0.5cc}$, D_{1cc}, D_{4cc}, D_{6cc}, and D_{8cc} were calculated along with D_{2cc} for vaginal wall dose volume parameters.

Late vaginal morbidities were retrospectively evaluated according to LENT-SOMA scales [13]. Because morbidity scores were evaluated retrospectively in this study, we focused on only vaginal ulcer which could be regarded as one of the severest symptoms and could be retrieved accurately from medical records.

Student's unpaired t-test was used to compare the continuous variables and Pearson's chi-square test to compare categorical variables. A p value of < 0.05 was considered as statistically significant. In addition, calculation of the area under the curve (AUC) of receiver operating characteristics (ROC) was used to determine the most predictive dosimetric parameter of vaginal ulcer. The predictive values of parameters were evaluated based on the AUC. The optimal threshold for each parameter was defined as the point yielding the minimal value for $(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$, which is the point on the ROC curve closest to the upper left-hand corner [14]. The obtained cutoff point was used for dividing patients into two groups and the incidences of vaginal ulcer were calculated by Kaplan-Meier method with the difference evaluated by log-lank test. All statistical analyses were performed using SPSS Statistics version 18.0 (SAS Institute, Tokyo, Japan).

This retrospective study was approved by the institutional review board of the National Cancer Center.

Results

There were 44 patients who met the eligibility criteria and 36 patients were alive at the time of the analysis (May 2012). The median follow-up length of living patients was 18.3 months (range, 7.6-39.5 months). The pretreatment characteristics of the 44 patients included in this study are summarized in Table 1. Median age was 56 years (range, 25-89 years). HDR-ISBT was applied as the primary therapy in 14 patients (31.8%) and as the salvage therapy in 30 patients (68.2%). Eight patients (18.2%) had previously received pelvic irradiation, in the form of EBRT and/or ICBT. Twenty four patients were treated with Syed-Neblett perineal template, 17 with free-hand with vaginal applicator and three with free-hand without vaginal applicator. Treatment details are summarized in Table 2. Ten patients underwent concurrent chemotherapy. In most cases HDR-ISBT dose per fraction was 6 Gy. Median total EQD_2 of CTV D_{90} was 67.7 Gy. Median EQD_2 of D_{2cc} for rectum and bladder was 60.8 Gy and 58.1 Gy, respectively. Median EQD_2 of $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} for vaginal wall were 210.7 Gy, 167.3 Gy, 131.5 Gy, 111.6 Gy, 100.0 Gy, and 83.2 Gy, respectively. Table 3 shows EQD_2 of rectum, bladder and vaginal wall for the patients with or without prior pelvic radiation therapy. For re-irradiation patients, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 91.1 Gy, 100.9 Gy, 260.3 Gy, 212.3 Gy, 170.1 Gy, 117.1 Gy, 105.2 Gy, and 94.7 Gy, respectively. For those without prior radiation therapy, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 56.3 Gy, 54.3 Gy, 147.4 Gy, 126.2 Gy, 108.0 Gy, 103.5 Gy, 94.7 Gy, and 80.7 Gy, respectively (Table 3). In EQD_2 of D_{2cc} for rectum, bladder and vaginal wall the difference was statistically significant ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively).

As for late morbidities of vagina, five patients experienced vaginal ulcer after HDR-ISBT. All of vaginal ulcer occurred within two years after completion of the HDR-ISBT. Patient characteristics and objective/management

Table 1 Patients characteristics (n = 44)

		Patients (n)
Median age (years, range)		56 (25-89)
Primary site	Cervix	24 (54.6%)
	Vagina	12 (27.3%)
	Corpus	5 (11.3%)
	Ovary	2 (4.5%)
	Vulva	1 (2.3%)
Primary therapy		14 (31.8%)
	Cervical cancer	4 (9.1%)
	Vaginal cancer	10 (22.7%)
Salvage therapy		30 (68.2%)
	Post ope regidual tumor	5 (11.4%)
	Post ope recurrent tumor	21 (47.7%)
	Post RT recurrent tumor	4 (9.1%)
Histology	Scc	25 (56.8%)
	Adeno	16 (36.4%)
	Others	3 (6.8%)
Prior pelvic RT*	Yes	8 (18.2%)
	No	36 (81.8%)
Median tumor size (cm, range)		3.6 (1.0-8.0)
Pelvic LN† metastais	Yes	11 (25%)
	No	33 (75%)

*RT radiation therapy.
†LN lymph node.

Table 2 Treatment details (n = 44)

	Median range
Central pelvic dose of EBRT* (Gy)	30 (0-50)
No. of needles used in HDR-ISBT†	15 (5-29)
HDR-ISBT† fractions	4 (3-9)
HDR-ISBT† dose per fraction (Gy)	6 (4-6)
CTV†† (ml)	35.1 (2.4-142.1)
CTV†† D_{90} in EQD_2‖ (Gy)	67.7 (48.8-94.2)
Rectum D_{2cc}¶ in EQD_2‖ (Gy)	60.8 (30.5-114.3)
Bladder D_{2cc}¶ in EQD_2‖ (Gy)	58.1 (7.3-120.3)
Vaginal wall $D_{0.5cc}$¶ in EQD_2‖ (Gy)	210.7 (51.5-468.1)
Vaginal wall D_{1cc}¶ in EQD_2‖ (Gy)	167.3 (49.9-352.1)
Vaginal wall D_{2cc}¶ in EQD_2‖ (Gy)	131.5 (43.7-294.4)
Vaginal wall D_{4cc}¶ in EQD_2‖ (Gy)	111.6 (34.0-200.8)
Vaginal wall D_{6cc}¶ in EQD_2‖ (Gy)	100.0 (20.4-173.7)
Vaginal wall D_{8cc}¶ in EQD_2‖ (Gy)	83.2 (10.3-144.4)
Concurrent chemotherapy	
Yes	10 patients
No	34 patients

*EBRT: external beam radiation therapy.
†HDR-ISBT: high-dose-rate interstitial brachytherapy.
††CTV: clinical target volume.
‖EQD2: equivalent dose in 2 Gy fractions.
¶D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6 and 8 cm3 of tissue.

Table 3 DVH parameters for bladder and vaginal wall with or withour prior radiation therapy

	Prior pelvic RT‡ (+) (n = 8)	Prior pelvic RT‡ (-) (n = 36)	*p* value
eMedian rectum D_{2cc}† (EQD_2*, Gy, range)	91.1 (71.0-114.3)	56.3 (30.5-82.7)	< 0.001*
Median bladder D_{2cc}† (EQD2*, Gy, range)	100.9 (69.7-120.3)	54.3 (7.3-82.7)	< 0.001*
Median vaginal wall $D_{0.5cc}$† (EQD2*, Gy, range)	260.3 (59.9-349.3)	147.4 (47.9-267.3)	0.109
Median vaginal wall D_{1cc}† (EQD2*, Gy, range)	212.3 (58.2-277.5)	126.2(33.6-182.7)	0.013
Median vaginal wall D_{2cc}† (EQD2*, Gy, range)	170.1 (56.6-247.5)	108.0 (31.7-150.9)	0.001*
Median vaginal wall D_{4cc}† (EQD2*, Gy, range)	117.1 (34.0-200.8)	103.5 (39.1-139.4)	0.139
Median vaginal wall D_{6cc}† (EQD2*, Gy, range)	105.2 (33.0-173.7)	94.7 (20.4-138.7)	0.097
Median vaginal wall D_{8cc}† (EQD2*, Gy, range)	94.7 (32.4-144.4)	80.7 (10.3-130.4)	0.105

‡RT: radiation therapy.
*EQD2: equivalent dose in 2 Gy fractions.
†D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.

scores of vaginal ulcer according to LENT-SOMA are summarized in Table 4. Two patients had superficial and > 1 cm^2 vaginal ulcer and three had vaginal fistula (two vesicovaginal fistulae and one vesicovaginorectal fistula). Three out of the five patients had prior pelvic irradiation and the interval between prior pelvic irradiation and secondary pelvic irradiation was 15, 27, and 40 months, respectively. All of the three patients with vaginal fistula received hyperbaric oxygen therapy without success. Two underwent surgical intervention (one total cystectomy for massive hematuria and one nephrostomy) for their vesicovaginal fistula, while one was followed up conservatively with a persistent vesicovaginal fistula. The other two patients with grade 2 vaginal ulcer were treated conservatively. The overall 2-year actuarial incidence of vaginal ulcer was 11.4%; 37.5% for re-irradiation patients and 5.6% for those without prior radiation therapy (Figure 1a). Comparison of dose-volume parameters of the vaginal wall is shown in Table 5 for the patient with and without vaginal ulcer. It was shown that the incidence of vaginal ulcer in the patients with prior pelvic irradiation was statistically higher than that of the patients without prior pelvic irradiation ($p = 0.035$). It was also shown that the mean EQD_2 of vaginal wall D_{2cc} of patients with or without vaginal ulcer was statistically different ($p = 0.025$). There was no relationship between administration of concurrent chemotherapy and manifestation of vaginal ulcer ($p = 0.256$), number of needles used in HDR-ISBT ($p = 0.293$) nor bladder D_{2cc} EQD_2 ($p = 0.091$). The ROC analysis revealed that vaginal wall D_{2cc} was the best dosimetric parameter predicting the incidence of vaginal ulcer and the cutoff value of 145 Gy in vaginal wall D_{2cc} provided the lowest p value in log-rank test (Table 6). Figure 1b shows Kaplan-Meyer curve for the incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2. The 2-year incidence rates of vaginal ulcer in the patients with vaginal wall D_{2cc} equal to or less than 145 Gy in EQD_2 and over 145 Gy were 3.7% and 23.5%, respectively, with a statistically significant difference ($p = 0.026$).

Discussion

Although the Manchester method of ICBT for the cervical cancer was developed to avoid the occurrence of radiation induced vaginal ulcer and necrosis, vaginal ulcer is now very rarely encountered because vaginal wall is relatively radioresistant and typical ICBT delivers radiation dose less than the tolerance of the relatively radioresistant vaginal wall. In a retrospective study of cervical cancer patients using EBRT and the film based low-dose rate (LDR) brachytherapy, Samuel et al. showed that vaginal tolerance dose was above 150 Gy [15]. In recent advancement of image guided brachytherapy (IGBT), rectum and bladder doses were recommended to be

Table 4 Patient characteristics who developed vaginal ulcer

Patient no.	Age at HDR-ISBT*	Primary site	Prior pelvic RT	Interval between prior RT and HDR-ISBT* (mo)	HDR-ISBT* with/without EBRT††	Total vaginal wall $D_{0.5cc}$#/D_{1cc}#/D_{2cc}# in EQD_2## (Gy)	LENT SOMA¶ objective score	LENT SOMA¶ management score
1	40	Cervix	WPRT† 45 Gy/25fr + EBRT†† boost 15 Gy/5fr	27	HDR-ISBT* 36 Gy/9fr	272.1/202.6/169.1	4	4
2	51	Cervix	None	None	WPRT† 30 Gy/15fr + CS‖ 20 Gy/10fr + HDR-ISBT* 24 Gy/4fr	215.2/171.8/145.4	4	3
3	64	Corpus	None	None	WPRT† 30 Gy/15fr + HDR-ISBT* 30 Gy/5fr	196.6/141.5/109.1	2	1
4	64	Cervix	WPRT† 40 Gy/20fr + CS 10 Gy/5 + HDR-ICBT 18 Gy/3fr	40	HDR-ISBT* 48 Gy/8fr	465.4/352.1/294.4	2	1
5	67	Cervix	WPRT† 50 Gy/50fr + HDR-ICBT 12 Gy/3fr	15	HDR-ISBT* 42 Gy/7fr	234.0/211.1/193.5	4	3

*HDR-ISBT: high-dose-rate interstitial brachytherapy.
†WPRT: whole pelvis radiation therapy.
††EBRT: external beam radiation therapy.
‖CS: radiation therapy with center shielding.
¶LENT-SOMA: Late Effects of Normal Tissues - Subjective, Objective, Management, Analytic.
#D0.5cc, D1cc, D2cc: most exposed 0.5, 1 and 2 cm3 of tissue.
##EQD2: equivalent dose in 2 Gy fractions.

reported in the treatment of ICBT for cervical cancer but vagina was not mentioned as OAR [9]. In the GEC-ESTRO working group (II) or American Brachytherapy Society guidelines, vagina was taken into consideration for OAR but it was stated that the vaginal dose volume parameters still need to be defined [16,17]. Dimopoulos et al. reported clinical result of primary vaginal cancer treated with IGBT and they experienced two vaginal fistulae and one periurethral necrosis. However they did not specify DVH parameters of vaginal wall with vaginal complication [18]. Lee et al. reported in detail the toxicity analysis of CT based HDR-ISBT for gynecologic malignancies. They reported that D_{2cc} for the rectum was a reliable predictor of late rectal complication; however because of limited number of events it was not able to explore the DHV parameters for vaginal complication [5]. Recently, Vienna group tried to find out DVH parameters that correlate with vaginal late morbidities but vaginal D_{2cc} did not relate with the vaginal morbidities [12]. The calculation method was the same as

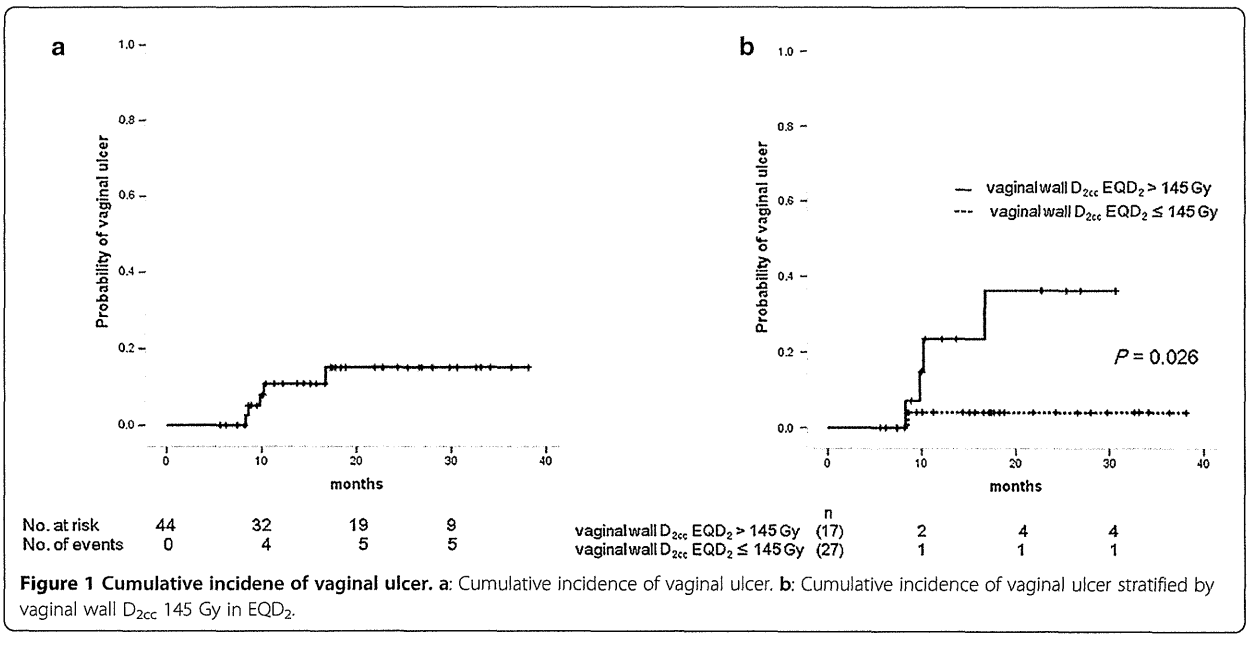

Figure 1 Cumulative incidene of vaginal ulcer. a: Cumulative incidence of vaginal ulcer. **b**: Cumulative incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2.