Table 5 Clinical predictors of vaginal ulcer

Characteristic		Vaginal ulcer (+) (n = 5)	Vaginal ulcer (-) (n = 39)	p value
Prior pelvic RT*		3	5	
	Yes	2	34	0.035*
	No			
Concurrent chemotherapy				
	Yes	0	10	0.256
	No	5	29	
Median number of needles used in HDR-ISBT[f] (range)		14 (10-24)	15 (5-29)	0.293
Median CTV[†] (ml, range)		54.7 (17.7-114.0)	34.7 (2.4-142.1)	0.271
Median rectum D_{2cc}[††] (EQD_2[‖], Gy, range)		84.2 (34.0-100.7)	57.9 (30.5-114.3)	0.118
Median bladder D_{2cc}[††] (EQD_2[‖], Gy, range)		69.3 (37.4-113.5)	57.7 (7.3-120.3)	0.091
Median vaginal wall $D_{0.5cc}$[††] (EQD_2[‖], Gy, range)		206.4 (106.6-349.3)	149.4 (47.9-310.1)	0.243
Median vaginal wall D_{1cc}[††] (EQD_2[‖], Gy, range)		169.1 (91.6-277.5)	127.9 (33.6-220.8)	0.096
Median vaginal wall D_{2cc}[††] (EQD_2[‖], Gy, range)		152.5 (71.1-247.5)	109.0 (31.7-201.9)	0.025*
Median vaginal wall D_{4cc}[††] (EQD_2[‖], Gy, range)		115.5 (83.8-200.8)	110.6 (34.0-153.2)	0.152
Median vaginal wall D_{6cc}[††] (EQD_2[‖], Gy, range)		102.5 (60.4-173.7)	99.5 (20.4-146.3)	0.266
Median vaginal wall D_{8cc}[††] (EQD_2[‖], Gy, range)		82.0 (47.6-144.4)	84.3 (10.3-140.3)	0.511

*RT: radiation therapy.
[f]HDR-ISBT.
[†]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.

the current study, which was composed of EBRT and ICBT/ISBT and normalized to 2 Gy per fraction (EQD_2) using the linear-quadratic model with α/β of 3 Gy for the vaginal morbidities [10-12,16]. The difference between Vienna group and the current study was that there were more patients with severe vaginal morbidities in the current study, presumably because there were more patients who received re-irradiation and current study excluded the patients treated with HDR-ICBT. HDR-ISBT delivers higher dose to the vaginal wall than HDR-ICBT because the multiple needle applicators directly contact vaginal wall. According to the current results, after vaginal wall $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} having been compared, vaginal wall D_{2cc} was found to be the most relevant DVH parameter predicting the incidence of vaginal ulcer. ROC analysis also showed that vaginal wall D_{2cc} of 145 Gy in EQD_2 can be used as clinical cutoff dose predicting vaginal ulcer. This figure is quite similar to the vaginal tolerance dose of 150 Gy derived from a retrospective study of LDR brachytherapy which was previously mentioned [15]. The current report is the first one concerning about vaginal DVH parameter and complication using modern era of three-dimensional image-guided brachytherapy. It was also found in this study that the history of prior pelvic irradiation was another significant predictive factor for vaginal ulcer (Table 5). Lee et al. reported a patient with colovaginal fistula with previous EBRT [5,7]. As shown in Table 3,

Table 6 Dosimetric predictors for the development of vaginal ulcer

Parameter	ROC[†] AUC*	Cutoff[¶]	2-y incidence ofvaginal ulcer (%)	P value[#]
Vaginal wall $D_{0.5cc}$[‖] (EQD_2[††])	0.667	≤195 Gy	0.0	0.058
		>195 Gy	18.5	
Vaginal wall D_{1cc}[‖] (EQD_2[††])	0.682	≤171 Gy	4.2	0.091
		>171 Gy	20.0	
Vaginal wall D_{2cc}[‖] ($EQD2$[††])	0.733	≤145 Gy	3.7	0.026*
		>145 Gy	23.5	
Vaginal wall D_{4cc}[‖] (EQD_2[††])	0.618	≤83 Gy	0.0	0.119
		>83 Gy	15.6	
Vaginal wall D_{6cc}[‖] (EQD_2[††])	0.569	≤86 Gy	5.6	0.323
		>86 Gy	15.4	
Vaginal wall D_{8cc}[‖] (EQD_2[††])	0.559	≤75 Gy	5.6	0.323
		>75 Gy	15.4	

*AUC: area under the curve.
[†]ROC: receiver operator characteristic.
[††]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.
[¶]Cutoff refers to the most predictive value from the AUC of ROC curve.
[#] Univariate analysis by log-rank test.

both rectum and bladder D_{2cc} was significantly higher in patients with prior pelvic irradiation than those without prior pelvic irradiation. However both rectum and bladder D_{2cc} was not in itself a significant prognostic factor for vaginal ulcer and could not be used as a surrogate indicator (Table 5).

There were several limitations in this study. Contouring of the vagina was not based on MRI but CT, which is inferior to MRI in tissue contrast. However because 41 out of 44 patients were inserted either cylinder or mold into their vagina, contouring of vagina was considered to be precise. The time interval between the prior pelvic RT and HDR-ISBT was not taken into consideration for the calculation of the total dose for OARs. Additionally, this study was a retrospective study with small number of patients with heterogeneous tumor origin, heterogeneous treatment applied, small number of events, and with short follow-up period. Therefore we should be cautious about the results of the current study. However even tumor origin differed greatly in current cohorts of study, it is considered to be feasible because the main concern in current study was focused on only the vaginal toxicity.

It should be stressed that with the introduction of HDR-ISBT in gynecological malignancies and increment of vaginal dose, vaginal tolerance dose must be taken into consideration. Further discussion and validation of vaginal DVH parameters in image-guided brachytherapy in a multicenter prospective study is needed.

Conclusions

The DVH parameters for vagina are essential for treatment planning and optimization in image based HDR-ISBT in gynecological malignancies. Vaginal wall D_{2cc} in EQD_2 should be monitored and be kept under 145 Gy in order to avoid vaginal ulcer. Also in patients with prior pelvic irradiation, vaginal wall dose including the prior radiation dose should be kept lower than 145 Gy.

Consent

Written informed consent was obtained from the patient for the publication of this report and any accompanying images.

Abbreviations

HDR-ISBT: High-dose rate interstitial brachytherapy; EQD_2: Dose in equivalent in 2 Gy fractions; ICBT: Intracavitary brachytherapy; ISBT: Interstitial brachytherapy; DVH: Dose volume histogram; EBRT: External beam radiation therapy; GTV: Gross tumor volume; CTV: Clinical target volume; OAR: Organ at risk; AUC: Area under the curve; ROC: Receiver operating characteristics; IGBT: Image guided brachytherapy; PDR: Pulsed dose rate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TK, MS, RY, KH, MK, SS, KT, KY, KI, MM, and YI performed the treatment. NM and JI analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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RESEARCH ARTICLE Open Access

CT based three dimensional dose-volume evaluations for high-dose rate intracavitary brachytherapy for cervical cancer

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Abstract

Background: In this study, high risk clinical target volumes (HR-CTVs) according to GEC-ESTRO guideline were contoured retrospectively based on CT images taken at the time of high-dose rate intracavitary brachytherapy (HDR-ICBT) and correlation between clinical outcome and dose of HR-CTV were analyzed.

Methods: Our study population consists of 51 patients with cervical cancer (Stages IB-IVA) treated with 50 Gy external beam radiotherapy (EBRT) using central shield combined with 2–5 times of 6 Gy HDR-ICBT with or without weekly cisplatin. Dose calculation was based on Manchester system and prescribed dose of 6 Gy were delivered for point A. CT images taken at the time of each HDR-ICBT were reviewed and HR-CTVs were contoured. Doses were converted to the equivalent dose in 2 Gy (EQD_2) by applying the linear quadratic model ($\alpha/\beta = 10$ Gy).

Results: Three-year overall survival, Progression-free survival, and local control rate was 82.4%, 85.3% and 91.7%, respectively. Median cumulative dose of HR-CTV D_{90} was 65.0 Gy (52.7-101.7 Gy). Median length from tandem to the most lateral edge of HR-CTV at the first ICBT was 29.2 mm (range, 18.0-51.9 mm). On univariate analysis, both LCR and PFS was significantly favorable in those patients D_{90} for HR-CTV was 60 Gy or greater ($p = 0.001$ and 0.03, respectively). PFS was significantly favorable in those patients maximum length from tandem to edge of HR-CTV at first ICBT was shorter than 3.5 cm ($p = 0.042$).

Conclusion: Volume-dose showed a relationship to the clinical outcome in CT based brachytherapy for cervical carcinoma.

Keywords: Brachytherapy, Image-based gynecological brachytherapy, Cervical cancer, IGBT, CT-based gynecological brachytherapy

Background

Standard therapy for patients with locally advanced cervical cancer is combination of external beam radiotherapy (EBRT) and brachytherapy with concurrent chemotherapy [1-5]. Intracavitary brachytherapy employing intrauterine (tandem) and vaginal (ovoid) sources based on Manchester principles, has been the standard for many decades [6,7]. Manchester system is point-based (i.e. two-dimensional) and uses orthogonal x-ray images for calculation and prescription of treatment doses. This concept neglects each tumor size or shape because prescribed dose is delivered to a fixed reference points. Therefore while excellent long-term tumor control rates can be obtained for patients with small tumors, for larger tumors relapse rate are high [8,9]. Over the decades, GEC-ESTRO [10,11] and ABS [12] proposed the concept of 3D image-based brachytherapy (IGBT) for the cervical cancer. Recently improved clinical outcomes are reported using IGBT for the advanced cervical carcinomas [13-19]. GEC-ESTRO working group recommend using MRI for determining high-risk clinical target volume (HR-CTV) and intermediate-risk CTV (IR-CTV) because MRI is superior to CT for delineating the normal anatomy of the female pelvis and for identifying cervical carcinoma extension [19-22]. However, practically majority

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of institutions do not have access to an MRI unit every time of brachytherapy treatment. In many circumstances CT scanners are often more widely available than MRI, therefore Viswanathan et al. developed guidelines for standard contouring of HR-CTV based on CT images [23]. From 2008 we introduced CT imaging in gynecological brachytherapy but continued to use Manchester system for dose calculation. In this study, we analyzed correlations between clinical outcome and dose of HR-CTV contoured based on CT images.

Methods

Patients included in current study are females with cervical carcinoma treated by primary radiation therapy including brachytherapy with or without concurrent chemotherapy from April 2008 to December 2010. As mentioned above, our department introduced CT imaging in the process of high-dose rate intracavitary brachytherapy (HDR-ICBT) for cervical cancer from 2008. Sixty two patients were identified who had CT image after insertion of brachytherapy applicator and 9 patients were excluded because of having distant disease beyond pelvis and another 2 patients were excluded because they were treated by combination of ICBT and interstitial brachytherapy (ISBT). Therefore current study consisted of 51 patients. All patients underwent pelvic examination, cystoscopy, pyeloureterography, chest X-ray/CT, pelvic CT/magnetic resonance image (MRI), and blood test. Maximum tumor diameters were measured based on the CT/MRI findings. All biopsy specimens were diagnosed in Department of Pathology of our hospital.

Treatment

Principles of management of the cervical cancer in this institute were described elsewhere [24]. The treatment policy for locally advanced cervical cancer is concurrent chemoradiation therapy (cCRT) with chemotherapy regimen of weekly cisplatin (40 mg/m^2/week) or cisplatin (50 mg/m^2/3 weeks) plus oral S-1 (80–120 mg/body/day). Concurrent chemoradiotherapy was not performed in the patients with insufficient renal function (serum creatinine > 1.5 mg/dl) and aged over 75 years. Supportive treatments such as blood transfusions were encouraged during radiotherapy.

Radiotherapy

EBRT was delivered by 3D conformal technique with linear accelerator (Clinac iX, Varian Medical System, Palo Alto, CA) using 15 MV photon beam. Treatment planning was based on CT images of 3 mm slice thickness taken by Aquilion LB CT scanner (Toshiba Medical Systems, Japan). The common EBRT portals included whole uterus, as well as parametrium, upper part of vagina down to the level of lower border of obturator foramens, and the draining pelvic lymph nodes up to the level of the common iliac (L4/5 junction). The nodal CTV included internal (obturator and hypogastric), external, and common iliac lymph nodes as well as presacral lymph nodes down to the level of S3. If the primary lesion involved lower third of vagina or there were clinically palpable metastatic inguinal nodes, inguinal regions were also included in EBRT fields. The initial 20–40 Gy was delivered to the whole pelvis with a 4-field box and then pelvic irradiation with a 4 cm-width of central shield (CS) being ensued reducing organ at risk (OAR) exposure. The initiation of CS was depend upon tumor shrinkage. Every week tumor response was accessed by attending radiation oncologist by physical examination. For early responding tumor width of which was smaller than 4 cm after having received 20 Gy of EBRT, CS was initiated. For late responding tumor width of which was larger than 4 cm at 20 Gy, EBRT was continued until tumor width became smaller than 4 cm. For tumors in which response of radiation was unfavorable, CS did not introduced. Total pelvic side wall dose was 50 Gy in 25 fractions. After the CS was inserted, HDR-ICBT was performed in 1–2 sessions/week, but EBRT and HDR-ICBT were not carried out on the same day. All brachytherapy was carried out by ^{192}Ir remote after loading system (RALS, MicroSelectron HDR™, Nucletron, Veennendaal, The Netherlands). ICBT with tandem + ovoid applicators without shielding was performed with a prescribed dose of 6 Gy in point A using Manchester method. A tandem-cylinder was used in the cases with a vaginal involvement exceeding more than one-third of total vaginal length. At each brachytherapy session, CT image of 3 mm slice thickness was taken by a large bore CT simulator (Aquilion™, Toshiba, Tokyo, Japan) situated in operating room with the patient lying in lithotomy position with the applicators in place. Before the acquisition of CT, bladder was filled with 100 ml of saline. Emptiness of rectum was checked at the time of gynecological examination before insertion of the applicators. For dose calculation of ICBT, Oncentra® (Nucletron, Veennendaal, The Netherlands) was used. HR-CTV was determined based on CT images according to Viswanathan's contouring guidelines [23]. Rectum and bladder were contoured as OARs. Dose constraints for OARs were determined as followed; D_{2cc} bladder < 90 Gy EQD_2, D_{2cc} rectum < 75 Gy EQD_2. In order to fulfill these dose constraints for OARs, tumors with insufficient response after EBRT and required 50 Gy of EBRT without CS generally could only afford two times of brachytherapy sessions while tumors with sufficient response and started CS only after 20 Gy of EBRT could undergo four or even five times of brachytherapy sessions. The workload with using CT-based IGBT required only additional several minutes for contouring targets and OARs compared with conventional X-ray based 2D planning.

Follow-up

All patients were evaluated weekly for toxicity during radiotherapy through physical examination and blood

tests. CT and/or MRI scans and cytology were performed 1–3 months after radiotherapy, and physical examination and blood tests were performed regularly every 1–6 months.

Statistical analysis

Overall survival rate was estimated from the start of radiation therapy to the date of death or of the last follow-up. Progression-free survival rate was estimated to the date of any disease relapse considered as an event. Patients without relapse who died of another disease or still alive were censored at the time of death or last follow-up. Local control rate which includes central and parametrium relapses was considered as an event, and censored at the time of death, non-local relapse, or last follow-up. Overall survival, Progression-free survival, and local control rate were calculated by the Kaplan-Meier method.

For adding dose of EBRT and HDR-ICBT, the equivalent dose in 2 Gy fractions (EQD_2) [11] according to LQ model [25] was calculated by the following formula:

$$EQD_2 = \frac{Nd\left(1+\frac{d}{\alpha/\beta}\right)}{1+\frac{2}{\alpha/\beta}}$$

The parameter N indicates the number of fractions and d the dose per fraction. For calculating tumor doses, α/β was assumed as 10 Gy. Because after insertion of CS most of the primary disease did not receive EBRT, EQD_2 of EBRT before the initiation of CS was added to the EQD_2 of HDR-ICBT. As calculation of HDR-ICBT was based on CT taken by each brachytherapy session, EQD_2 at every fraction was calculated and added together.

The survival curves were compared by the log-rank test. For univariate analysis, all of the variables were dichotomized at the median. Statistical significance was set to less than 0.05 as usual. All of the 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

Among 51 patients included in this study, 42 patients were alive at the time of the analysis and 39 were alive without disease recurrence (December 2012). The pretreatment characteristics of the 51 patients are summarized in Table 1. Treatment details were summarized in Table 2. Among 30 patients who received concurrent chemotherapy, 9 patients received cisplatin plus S-1. The median value of EQD_2 for FIGO I/II/III/IVA was 64.55 Gy, 64.97 Gy, 64.68 Gy, and 63.35 Gy, respectively. The median follow-up length of living entire patients was 39.2 months (range, 24.3-52.0 months). Three-year Overall survival, Progression-free survival and local control rate were 82.4%, 85.3% and 91.7%, respectively (Figure 1). At the time of analysis 39 patients were alive without disease recurrence, while 5 patients died because of cancer and 4 due to other reasons without any evidence of cervical cancer. Eight out of 51 patients (15.7%) experienced persistent disease or disease recurrence after definitive radiotherapy (one, two, three, and two patients in FIGO I/II/III/IVA, respectively). Two patients recurred at only local site, 2 both local and distance simultaneously, and 4 distant only. No one experienced regional lymph node recurrence. Among seven FIGO stage IVA patients, one patient experienced local recurrence and eventually died of disease, one experienced single lung metastasis which was successfully salvaged by six cycles of carboplatin and paclitaxel followed by stereotactic radiation therapy for lung metastasis, and one elderly patient died from chronic kidney dysfunction without any evidence of disease recurrence. Figure 2 shows example of patient who experienced local recurrence. Tumor extended to pelvic wall at diagnosis (Figure 2a). The right lateral part of HR-CTV was not covered by isodose line of 60 Gy equivalent dose in 2 Gy per fraction (EQD_2, Figure 2b). In axial MR image 3 months after completion of treatment (Figure 2c), the persistent disease was found in the area not sufficiently treated by brachytherapy. On univariate analysis, both local control rate and Progression-free survival was significantly favorable in those patients with D_{90} for HR-CTV equal to or greater than 60 Gy (Figure 3; $p = 0.001$ and 0.03, respectively). The number of patients with HR-CTV $D_{90} < 60$ Gy and ≥ 60 Gy was 12 and 39, respectively. Median volume of

Table 1 Patients characteristics (n = 51)

Characteristics		No. of patients
Age	Median (range)	62 (28-90)
FIGO stage	I/II/III/IVA	10/15/19/7
Vaginal invasion	Yes	19
	No	32
Parametrium invasion	Yes	33
	No	18
Corpus invasion	Yes	15
	No	36
Pyometra	Yes	6
	No	45
Pelvic LN metastasis	Yes	11
	No	40
Pathology	Scc	48
	Adeno	3
Initial tumor size (cm)		4.5 (1.8-7.7)
Pre treatment Scc (mg/dl)		7.0 (0.9-94.2)

LN lymph node.

Table 2 Treatment details

EBRT* central pelvic dose (Gy)	Median (range)	30 (20-50)
HDR-ICBT† dose for point A	Median (range)	24 (12-30)
Applicor type	Tandem + ovoid	42
	Tandem + cylinder	9
Concurrent chemotheraphy	Yes	30
	No	21
TTT†† (days)	Median (range)	42 (36-67)
Volume of HR-CTV at first ICBT (ml)	Median (range)	23.3 (8.3-100.8)
Maximum diameter of HR-CTV at first ICBT (mm)	Median (range)	46.9 (32.2-77.5)
Maximum length from tandem to edge of HR-CTV at first ICBT (mm)	Median (range)	29.2 (18.0-51.9)
$EQD_2^{\S}$ of point A	Median (range)	62 (52-72.3)
$EQD_2^{\S}$ of HR-CTV D_{90}^{**}	Media (range)	65.0 (52.7-101.7)

*EBRT: external beam radiation therapy.
†HDR-ICBT: high-dose rate intracavitary brachytherapy.
††TTT: total treatment time.
§ EDQ_2: equivalent dose in 2 Gy fractions.
**HR-CTV D_{90}: dose covering 90% of the HR-CTV.

HR-CTV at the first application of brachytherapy in each group was 31.8 ml and 21.1 ml, respectively and patients with HR-CTV $D_{90} < 60$ Gy had statistically larger volume compared with that of patients with HR-CTV $D_{90} \geq 60$ Gy ($p = 0.022$). Three-year local control rate and Progression-free survival for those whose HR-CTV $D_{90} < 60$ Gy was 72.9% and 64.3% whereas that of patients with HR-CTV $D_{90} \geq 60$ Gy was 97.3% and 91.5%, respectively. Progression-free survival was significantly favorable in those patients when the maximum length from tandem to the margin of HR-CTV at first ICBT was shorter than 3.5 cm ($p = 0.042$).

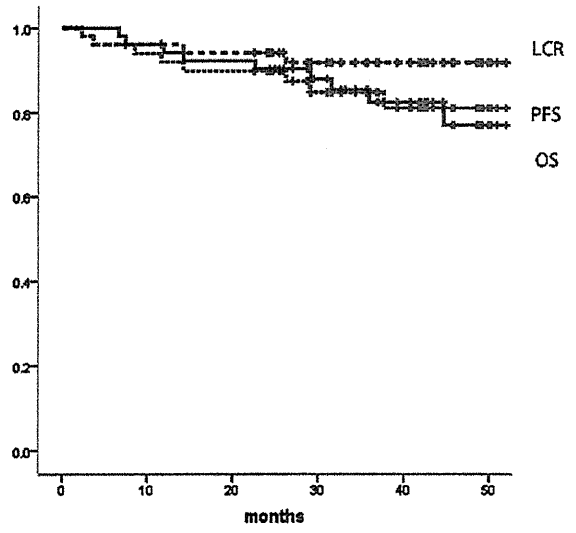

Figure 1 Kaplan-Meyer curves of local control rate (LCR), progression survival (PFS), and overall survival (OS).

Treatment related toxicities

One patient experienced sigmoid colon perforation 1 month after completion of radiotherapy which required colostomy. Because cumulative dose for sigmoid colon D_{2cc} was only 43.8 Gy (EQD_2, $\alpha/\beta = 3$ Gy) and development of the perforation was rather too early, it was implausible that radiation played a major role developing this severe morbidity. Two patients developed grade 2 proctitis and none experienced greater than grade 2 cystitis or vaginitis.

Discussion

In the current study, definitive radiotherapy using traditional Manchester method with or without concurrent chemotherapy for cervical carcinoma resulted in favorable local control with only 4 local recurrences (7.8%).

Since the introduction of the concept of IGBT [10-12], several improved clinical results have been reported [13-18]. It is recommended in GEC-ESTRO working group that MRI should be used to determine IR-CTV and HR-CTV because of its superiority of tissue discrimination over CT image [20-22,25]. However, it is even now hard for most of brachytherapy suits to prepare MRI instruments for the use of every brachytherapy procedure for cervical cancer. As an alternative and practical solution, Viswanathan et al. proposed a guideline to contour HR-CTV based on CT images [23]. Current study was to the best of our knowledge first report which validated this CT based HR-CTV contouring guideline in clinical practice. Schmid et al. reported interesting study concerning the feasibility of transrectal ultrasonography for identifying HR-CTV in comparison with MRI [26]. However authors still believe utilizing CT for brachytherapy is the most realistic solution for

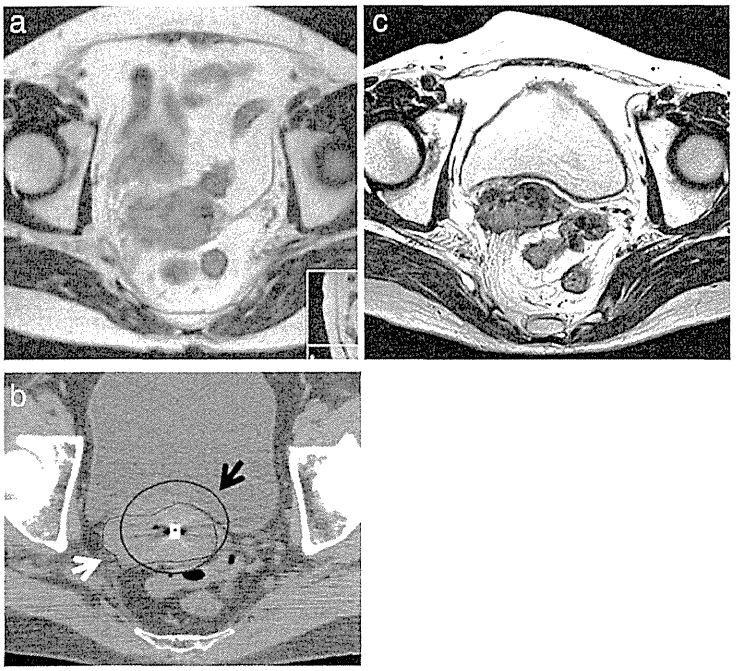

Figure 2 Representative images of patient who experienced local relapse. a. Axial MR T2 weighted image before treatment. Tumor extends to right pelvic wall. **b**. Axial CT image at the first session of intracavitary brachytherapy (ICBT). Tumor still extends to right pelvic wall after 40 Gy of whole pelvic EBRT. Black arrow represents isodose line of 60 Gy (EQD_2) and white arrow HR-CTV at the time of brachytherapy. **c**. Axial MR T2 weighted image 3 months after completion of chemoradiotherapy. Persistent disease was found in right parametrium.

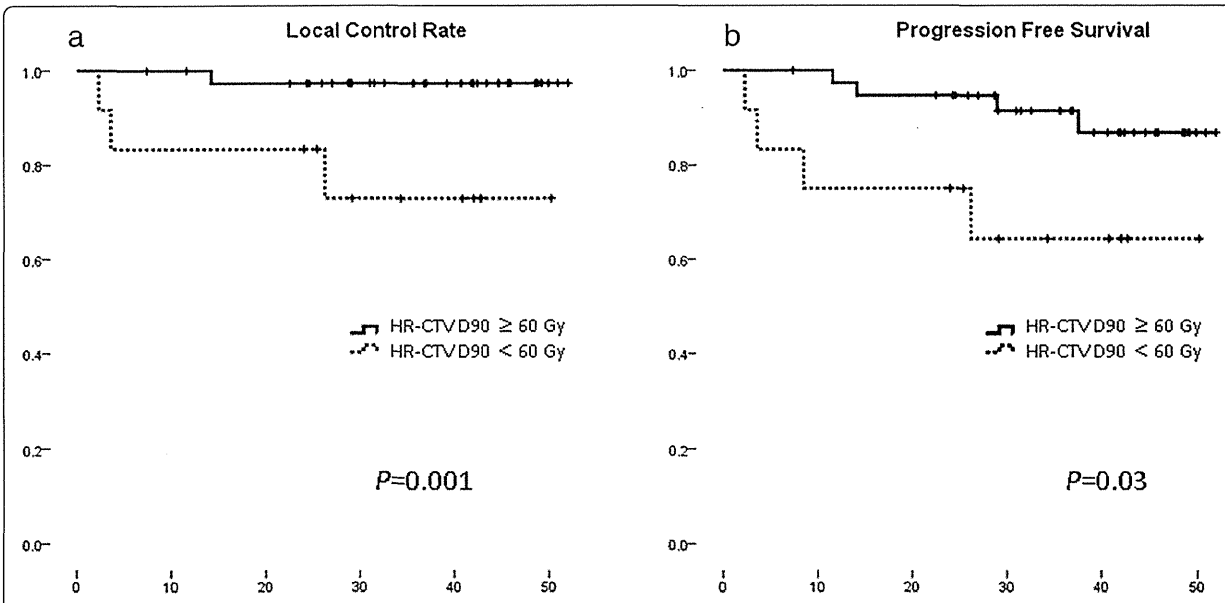

Figure 3 Local control rate (LCR) and progression free survival (PFS) curve stratified by HR-CTV D90 60 Gy (EQD2). a. Local control rate (LCR) stratified by HR-CTV D_{90} 60 Gy (EQD_2). **b**. Progression free survival (PFS) stratified by HR-CTV D_{90} 60 Gy (EQD_2).

the future evolution of image-guided brachytherapy for cervical cancer because of its prevalence and reproducibility.

Dimopoulos et al. analyzed the relationships between dose-volume histogram (DVH) and local control using MRI-based IGBT for cervical cancer and found out that the D_{90} for HR-CTV greater than 87 Gy resulted in excellent local control [13]. In current study, the cut-off value of D_{90} was 60 Gy and it was much lower than what Dimopoulos et al. pointed out. It has been known that Japanese centers use lower cumulative dose schedules with shorter overall treatment time (OTT) than those of US and Europe [27,28]. Recently Toita et al. showed the efficacy of Japanese schedule in a series of multicenter prospective trials in which Stage I and II with small (<4 cm) tumor diameter can be effectively treated by BED 62 Gy_{10} (JAROG0401/JROSG04-2) [29] and Stage III/IVA by BED 62–65 Gy_{10} at point A (JCOG1066) [30]. Therefore it is reasonable that in current study the cut-off value is much lower than Vienna group. In addition 60 Gy could be used as a target dose for HR-CTV D_{90} in institutions which perform IGBT with Japanese schedule. However further evidence must be accumulated in order to validate the value of HR-CTV $D_{90} \geq 60$ Gy in Japanese schedule.

In current study it was revealed that PFS was significantly favorable if the maximum length from tandem to the margin of HR-CTV at the first ICBT was shorter than 3.5 cm. Therefore if the maximum distance between uterine cavity and margin of HR-CTV is longer than 3.5 cm at the first session of brachytherapy, application of image-guided brachytherapy or combined intracavitary/interstitial brachytherapy [16,31-33] would improve clinical results.

From current study, it was demonstrated that favorable local control could be achieved for tumors with HR-CTV $D_{90} \geq 60$ Gy using conventional Manchester method. However for tumors with delayed response after EBRT and HR-CTV D_{90} could only be under 60 Gy by Manchester method, further treatment improvement is warranted. In this context, maximum length from tandem to the rim of HR-CTV ≥ 3.5 cm could be used as a cut-off point where ISBT would play an important role. Currently in our institution tumors of which maximum length from tandem to the rim of HR-CTV is longer than 3.5 cm at the time of brachytherapy are treated by the combination of ICBT and ISBT or ISBT alone. Improvement of clinical results after the introduction of the combination of ICBT and ISBT compared with conventional technique will be reported elsewhere.

This study has several limitations. This is a result from single retrospective study with a limited follow-up period and HR-CTV was determined based on CT images rather than MR images. Viswanathan et al. compared CT based and MRI based CTV and concluded that the width of CT based CTV was larger than that of MRI [23]. Therefore HR-CTV contoured based on CT in this study may overestimate the tumor volume in lateral direction. This may be part of the reason of lower cut-off value of HR-CTV D_{90} in this study. However it will take long before MRI will be available in majority of brachytherapy suit. At present as current standard for IGBT is based on MRI, IGBT is not so popular after its introduction in the treatment of cervical cancer brachytherapy because MRI itself is not prevalent yet. Therefore it is worth accumulating evidence that IGBT based on CT image could also achieve favorable clinical results if used properly.

Conclusions

Dose-volume relationship was found in CT-based intracavitary brachytherapy for cervical carcinoma in Japanese schedule. Further improvement could be expected for cervical cancers with insufficient response after EBRT. For such tumor, ISBT would play an important role and should be investigated.

Abbreviations

EBRT: External beam radiotherapy; HR-CTV: High-risk clinical target volume; IR-CTV: Intermediate-risk clinical target volume; HDR-ICBT: High-dose rate intracavitary brachytherapy; ISBT: Interstitial brachytherapy; cCRT: Concurrent chemoradiation therapy; CS: Central shield; OAR: Organ at risk; EQD_2: The equivalent dose in 2 Gy fractions.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NM, AW, SN, HO, and JI have made substantial contributions to conception and design. NM and JI have been involved in drafting the manuscript or revising it critically for important intellectual content. MM, MS, KI, YI, and TK participated in acquisition and interpretation of data. All authors read and approved the final manuscript.

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A Dosimetric Analysis of Intensity Modulated Radiation Therapy with Bone Marrow Sparing for Cervical Cancer

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Abstract. *Background/Aim: The purpose of this study was to compare intensity-modulated radiation therapy (IMRT) plan with (Bone Marrow Sparing (BMS) – IMRT) or without (normal-IMRT) an intention of avoiding bone marrow in order to minimize treatment related toxicity. Materials and Methods: Computed tomography (CT) images of 10 consecutive postoperative cervical cancer patients were used. All patients were already treated by normal-IMRT. BMS-IMRTs were created for this study and dose volume histogram parameters were compared. Results: Both planning target volume (PTV) D95% and D97% were statistically lower in BMS-IMRT than normal-IMRT, however, the difference was lower than 3%. There were statistically no difference between BMS-IMRT and normal-IMRT in the mean value of rectum V_{30Gy}, V_{50Gy}; bladder V_{45Gy}, V_{50Gy}; Bowel V_{35Gy}, and V_{50Gy}. Both in whole pelvic bone (WPB) and inner cavity of pelvic bone (ICPB), the mean value of V_{10Gy}, V_{30Gy}, and V_{40Gy} of BMS-IMRT were statistically lower than that of normal-IMRT. Conclusion: Both lower and higher dose for WPB as well as ICPB were effectively lowered by BMS-IMRT.*

Postoperative radiation therapy is an established treatment for intermediate-risk and high-risk cervical patients (1, 2) as well as endometrial cancer patients (3, 4). Conventional radiation techniques for whole-pelvic radiation therapy (WPRT) involve 4 static photon fields. These techniques expose most of the contents of the true pelvis including small bowel equal to the prescribed dose as target volume. The highly conformal technique of intensity-modulated radiation therapy (IMRT) has a potential of delivering radiation dose while avoiding surrounding normal tissues and its advantage has been proven in several anatomical sites such as head and neck cancer (5, 6) or prostate cancer (7, 8). However the profit of using IMRT in the field of gynecological cancer is controversial (9-11). The Radiation Therapy Oncology Group (RTOG) conducted a multi-institutional prospective phase II trial (RTOG 0418) using IMRT for postoperative endometrial and cervical patients in order to determine if IMRT can be performed in multi-institution settings and to test the hypothesis that IMRT can reduce short-term bowel injury and recently positive preliminary results were presented (12, 13). Although in the RTOG 0418 bone marrow sparing was not included in its protocol, weekly cisplatin was administered successfully concurrent with radiation therapy. As much as 83% of patients received 5 or more cycles of weekly cisplatin and 90% at least 4 cycles (13). Compared to results from other series with conventional technique radiation therapy which also used concurrent cisplatin, this result was favorable, although it is difficult to make any direct comparisons (2, 14). Therefore it is supposed that if bone marrow sparing was intended from the beginning, bone marrow protection would lead to better results. The aim of this study is to investigate the difference of dose volume histogram (DVH) parameters between IMRT plan with or without bone marrow sparing intention using computed tomography (CT) images of posthysterectomy cervical cancer patients.

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Key Words: Cervical cancer, radical hysterectomy, postoperative radiation therapy, IMRT, bone marrow sparing.

Materials and Methods

Patients. Ten consecutive patients constitute this retrospective planning study. These subjects underwent radical hysterectomy and

pelvic lymphadenectomy and postoperative radiation therapy for early-stage high-risk cervical carcinoma between May 2012 and June 2013. Eligibility criteria for postoperative adjuvant radiation therapy were: (i) pelvic lymph node metastasis, (ii) parametrial invasion, and (iii) a positive surgical margin.

Radiation therapy. Before simulation CT was taken, a customized immobilization device was fabricated to minimize variability in the daily setup error. Fiducial markers were inserted into the vaginal cuff to visualize it on CT images. CT scans under full and empty bladder were taken in order to account for the motion of vagina influenced by the content of bladder. CT scans of 2 mm slice thickness were taken by Aquilion LB CT scanner (TOSHIBA Medical Systems, Tochigi, Japan). The clinical target volume (CTV) was contoured on the individual axial CT slices of each patient. The overall CTV includes both the vaginal cuff/paracolpium CTV and the nodal CTV. The vaginal cuff/paracolpium CTV was contoured in a manner similar to the Radiation Therapy Oncology Group (RTOG) (15); Cranial margin: 0.5 cm cranial from the upper part of vaginal cuff metallic marker. Anterior margin: posterior border of bladder or retropubic fat pad. Posterior margin: Anterior part of mesorectal fascia or anterior wall of rectum. Lateral margin: medial edge of internal obturator muscle, piriformis muscle, coccygeus muscle and ishial ramus. Caudal margin: 3 cm below from the upper part of vaginal cuff metallic marker. The nodal CTV included lymph nodes that drain the involved site and adjacent perinodal soft tissue. This included the internal (obturator and hypogastric), external, and common iliac lymph nodes; presacral lymph nodes and soft tissues also included down to the level of S3. The upper limit of the nodal CTV was L4/5 interspace. If a common iliac lymph node metastasis was found pathologically, the nodal CTV was extended at the level of L2/3 interspace. The nodal CTV was based on the Japan Clinical Oncology Group Gynecologic Cancer Study Group (JCOG-GCSG) consensus guidelines for the delineation of CTV for pelvic lymph nodes (16). We used the JCOG-GCSG guideline for reference aiding nodal CTV because it included adipose connective tissue between the iliopsoas muscles and the lateral surface of the vertebral body which was not included in RTOG guideline. This area was also included in an atlas of Taylor *et al.* (17, 18). The CTV was expanded by 7 mm to create the planning target volume (PTV). For normal structures, the small bowel (contoured as a peritoneal space), rectum and bladder (both contoured as a whole organ) and femoral head were routinely contoured according to RTOG normal tissue contouring guideline (19). For the purpose of this study, both pelvic bone and pelvic bone marrow were also contoured. Mahantshetty *et al.* demonstrated that inner cavity of pelvic bone (ICPB) was a better surrogate of active bone marrow than whole pelvic bone (WPB) (20); therefore, both WPB and ICPB were prepared in this study. In order to extract WPB from CT images, bone auto-contouring was performed by including tissue with density of higher than 100 on each slice with 'fill' function activated. Before extracting ICPB, a tentative structure was created using auto-contouring function which includes tissue with density between -100 to 200. This structure includes ICPB as well as extra-bone soft tissue. Therefore, the overlap volume of WPB and the tentative structure was created and this structure became the ICPB. Figure 1 shows representative axial figure of the contour for ICPB and WPB. In this figure, WPB was contoured in pink line while ICPB in green.

Prescription dose was 50 Gy in 25 fractions. The planned goals were to provide a homogenous PTV dose while minimizing the dose delivered to the small bowel, bladder and rectum. Typical input parameters for normal-IMRT plans were as follows: PTV mean dose ranges from 100 to 105% while no more than 2% of the volume of PTV to receive a dose that is 60 Gy or greater; no more than 40% of the volume of the small bowel to receive a dose that is greater than 40 Gy and no more volume of small bowel greater than 1 cc to receive more than 55 Gy; no more than 40% of the volume of the rectum to receive more than 50 Gy and no volume within the rectum receives dose that is 55 Gy or greater; no more than 50% of the volume of the bladder to receive more than 45 Gy and no volume within the bladder receives dose that is 55 Gy or greater; and no more than 20% of the volume of the femoral head to receive more than 30 Gy. Dose constraints of BMS-IMRT planning were as follows: the same dose constraints for PTV and organ at risk (OAR) were used. For the use of DVH-based optimization, virtual structures were created; WPB-PTV and ICPB-PTV which were overlap structure of WPB and PTV, and ICPB and PTV, respectively. The general priority for each structure was presented as below: 70, 90, 65, 50, 65, and 65 for PTV, small bowel, rectum, bladder, WPB-PTV, and ICPB-PTV, respectively.

The way of delivering IMRT was by Volumetric Modulated Arc Therapy (VMAT) using 2 arcs via a computer-controlled auto sequence multileaf collimator on a linear accelerator (Clinac iX, Varian Medical System, Palo Alto, CA, USA) using a 15 MV photon beam. Dose calculation with a calculation grid of 1.0 mm was done and the calculation algorism was Acuros (Link or supplier). The radiotherapy was planned using the Eclipse Planning System (version 10.0, Varian Medical System, Palo Alto, CA, USA).

No chemotherapy was used concurrently with radiation therapy in our institution for early stage high-risk post-hysterectomy cervical cancer patients. DVH parameters between normal-IMRT and BMS-IMRT were compared in this study. The difference of the mean value of each parameter was analyzed by paired *t*-test and *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed using the SPSS™ version 18.0 (SAS Institute, Tokyo, Japan). This retrospective study was approved by the local Institutional Review Board.

Results

Since it was not long before IMRT was introduced in the treatment for postoperative cervical cancer patients in our institution, only 10 patients were included in the current study. Table I shows the patients' characteristics. Median age was 39 (range 25-66) years. Sixty percent of patients were staged as IB1 and there was no IIA patient. Half of the patients had squamous cell carcinoma. Seventy and ninety percent of patients had parametrium invasion and pelvic lymph node metastasis, respectively. All the patients were diagnosed as surgical margin negative.

Figure 2a and Table II shows boxplots and actual numbers of DVH parameters for PTV. Although in PTV $D_{95\%}$ and $D_{97\%}$ the mean value of BMS-IMRT were statistically lower than that of normal-IMRT, the differences were smaller than 3% and the influence of this very minute difference on clinical result is unknown. On the other hand, the mean value of PTV median in BSM-IMRT was statistically higher than that of normal-IMRT. There was no statistical difference in PTV D_{max}

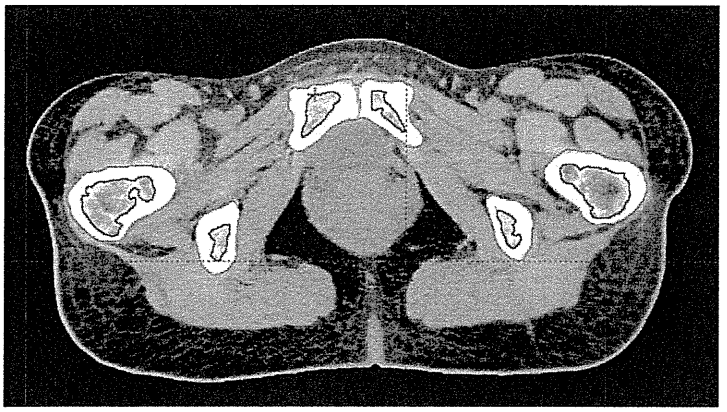

Figure 1. *An axial figure showing contour for inner cavity of pelvic bone (green line) and whole pelvic bone (pink line).*

Table I. *Patients' characteristics (n=10).*

Median age (range)	39 (25-66)
Clinical stage	
IB1	6
IB2	2
IIA	0
IIB	2
Histology	
Scc	5
Adeno	3
Adenosquamous	2
Parametrium invasion	
Yes	7
No	3
Pelvic LN metastasis	
Yes	9
No	1
Median Number of pelvic LN metastasis (range)	2 (0-8)
Surgical margin	
Positive	0
Negative	10
Median tumor size (cm, range)	4.1 (1-5.4)

Table II. *The mean value of DVH parameters for PTV.*

	Normal-IMRT		BMS-IMRT		
	mean (%)	SD†	mean (%)	SD†	*p*-Value
PTV D_{max}	109.7	3.7	110.8	1.4	0.258
PTV $D_{95\%}$	94.6	2.3	92.5	1.9	*<0.01
PTV $D_{97\%}$	93.4	2.5	90.8	2.6	*0.05
PTV median	100.4	0.4	100.8	0.5	*0.05

†SD: standard deviation, PTV: planning target volume, DVH: dose volume histogram.

between both plans. Therefore it was supposed that these small differences would not bring about any clinical relevant differences. For comparison, another plan was created in which the PTV coverage was prioritized to be of the same degree as normal-IMRT. In these plans, favorable sparing of bone marrow did not occur (data not shown).

Figure 2b shows boxplots of DVH parameters for OARs. There were no statistical difference between normal-IMRT and BMS-IMRMT in the mean value of rectum V_{30Gy}, V_{40Gy}, V_{50Gy}, bladder V_{45Gy}, V_{50Gy}, Bowel V_{35Gy}, V_{40Gy}, V_{45Gy}, and V_{50Gy}. There was a statistically significant difference between the mean value of bladder V_{35Gy} and femoral head V_{30Gy}. Although a statistical significance was found between normal- and BMS-IMRT in bladder V_{35Gy}, as shown in the Figure 2 the difference was quite small; therefore it was not known whether this small difference would be connected to clinically evident difference. The dose of femoral head was effectively lowered in BMS-IMRT than that of normal-IMRT. It was supposed that this difference was caused because the femoral head was included in the structure of pelvic bone.

Figure 3a and 3b show boxplots of DVH parameters for WPB and ICPB. Both in WPB and ICPB, the mean value of V_{10Gy}, V_{20Gy}, V_{30Gy}, and V_{40Gy} of BMS-IMRT were statistically lower than that of normal-IMRT. Figure 4 shows an example of dose distribution of BMS-IMRT and normal-IMRT. In this Figure, the area receiving a dose of 40 Gy or higher was colored. Visually, it was clear that the spinal body was effectively avoided in BMS-IMRT compared with normal-IMRT. Because the 15 MV photon beam is not used in the majority of institutions in the United States, another BMS-IMRT plan was created in 3 representative patients using a 6 MV photon beam and the DVH was compared.

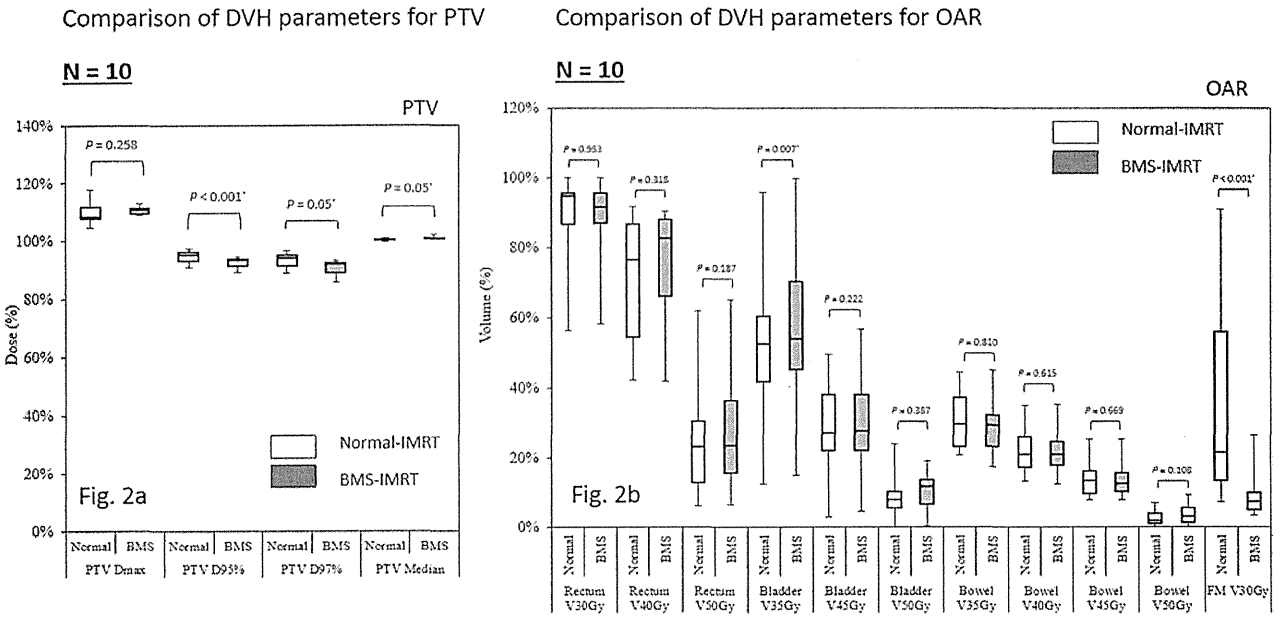

Figure 2. *Boxplots of dose volume histogram (DVH) parameters for planning target volume (PTV) (a) and organ at risks (OARs) (b).Note: spell out Femoral head (FM).*

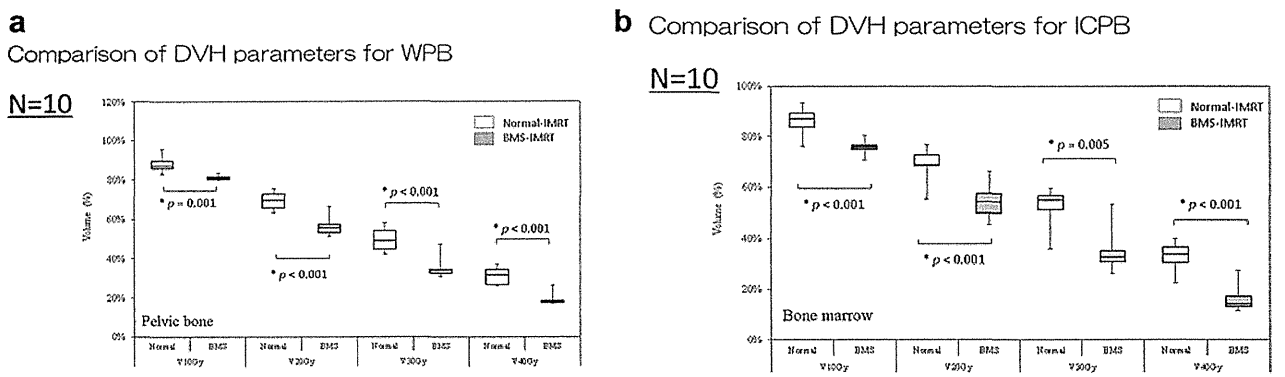

Figure 3. *Boxplots of dose volume histogram (DVH) parameters for whole pelvic bone (WPB) (a) and inner cavity of pelvic bone (ICPB) (b).*

Table III summarizes the difference of DVH parameters between the 6 MV and 15 MV photon beams. The same degree of BMS and OAR sparing was achieved by using the 6 MV photon beam, while the monitor unit of this beam was higher because of its lower photon energy.

Discussion

It was demonstrated that early-stage cervical cancer patients who underwent radical hysterectomy and exhibited high-risk feature(s) could benefit postoperative concurrent chemoradiotherapy by a multicenter prospective phase III randomized clinical trial (2). It was also shown in this study that it was important to deliver as many chemotherapy cycles as possible. In the RTOG 0418 trial (12), postoperative endometrial and cervical cancer patients were entered and treated by postoperative radiation therapy by IMRT. In the present study, cervical cancer patients were treated by concurrent chemoradiotherapy using weekly

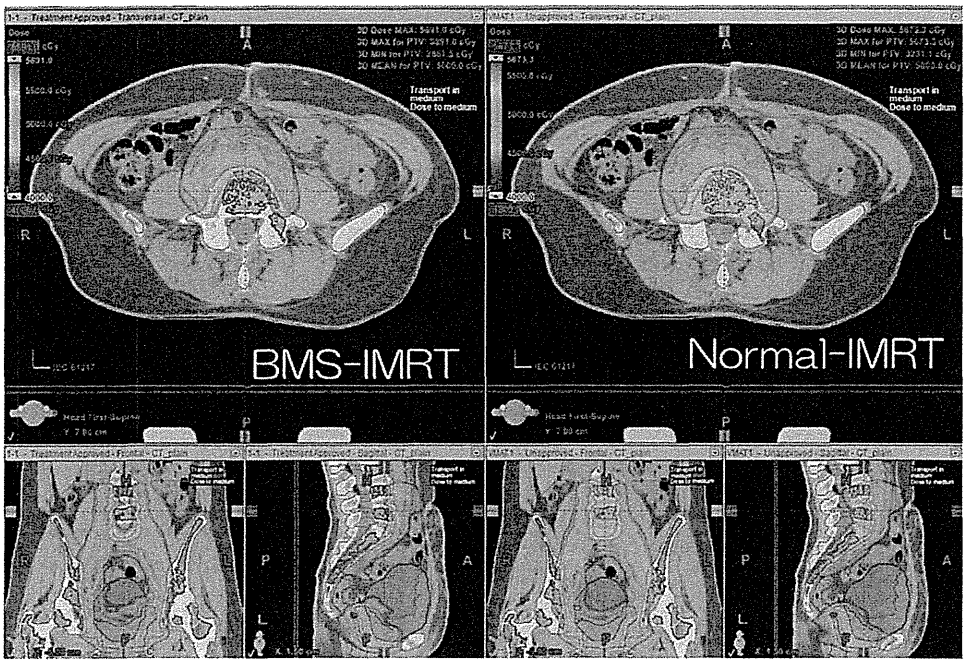

Figure 4. *An example of dose distribution of bone marrow sparing (BMS)–IMRT and normal-IMRT. The area which received 40 Gy or more was colored.*

cisplatin 40 mg/m^2. Although bone marrow sparing was not intended in the protocol of RTOG 0418, favorable hematological profiles were shown; 83% received 5 or more cycles of cisplatin and 90% received at least 4 cycles of cisplatin (13). The RTOG 0418 data demonstrated that pelvic bone V_{40Gy} >37% was associated with grade 2 or higher hematologic toxicity (13). On the other hand, Rose *et al.* reported that patients with V10Gy ≥95% as well as V_{20Gy} ≥76% were more likely to experience grade ≥3 leukopenia (21). Mell *et al.* also showed from a small sized retrospective study that bone marrow V_{10Gy} was a strong predictor for grade 2 or worse leukopenia (22). Albuquerque *et al.* reported the importance of the volume of bone receiving 20 Gy (23). Therefore V_{10Gy}, V_{20Gy}, V_{30Gy} and V_{40Gy} were extracted and compared in the current study since controversies still remain over whether lower or higher dose on bone marrow affects its function. Mahantshetty *et al.* demonstrated that ICPB was a better surrogate of active bone marrow than whole pelvic bone WPB (20); therefore, both WPB and ICPB were examined in this study.

In the current study, it was clearly demonstrated that both lower and higher dose for WPB as well as ICPB were significantly lower in the BMS-IMRT plan compared with the normal-IMRT plan without changing the coverage of target volume and other organs at risk. Therefore, it is important to include bone marrow structures into dose constraint structures for post-hysterectomy radiation therapy by IMRT especially with concurrent use of chemotherapy. Our institution uses a 15 MV photon beam for irradiating the pelvic region because a high energy photon beam has an advantage of delivering photons to deep seated organs with

Table III. *Comparison of dose volume histogram parameters of bone marrow sparing intensity-modified radiation therapy plans between 6 MV and 15 MV photon beams.*

	6X-15X (%)		6X-15X (%)
PTV D_{max}	0.80	Rectum V_{30Gy}	–2.07
PTV $D_{95\%}$	0.07	Rectum V_{40Gy}	–0.33
PTV $D_{97\%}$	0.10	Rectum V_{50Gy}	2.17
PTV Median	–0.01	Bladder V_{35Gy}	0.97
PB V_{10Gy}	0.53	Bladder V_{45Gy}	0.53
PB V_{20Gy}	0.60	Bladder V_{50Gy}	1.67
PB V_{30Gy}	0.10	Bowel V_{35Gy}	0.73
PB V_{40Gy}	0.23	Bowel V_{40Gy}	0.50
BM V_{10Gy}	0.37	Bowel V_{45Gy}	0.70
BM V_{20Gy}	–0.57	Bowel V_{50Gy}	0.83
BM V_{30Gy}	–0.03	FM V_{30Gy}	0.80
BM V_{40Gy}	0.50	MU	93 (MU)

PTV: planning target volume, PB: pelvic bone, BM, bone marrow, FM: femoral head, MU: monitor unit.

less attenuation; however, the 15 MV photon beam has a concern about creating neutrons along with photons. Therefore, another BMS-IMRT plan was created using a 6 MV photon beam. Since the same trend was obtained by using the 6 MV photon, BMS-IMRT may protect the patient's bone marrow function in institutions where such a beam is used for cervical cancer.

Among the limitations of this work it has to be noted that it was a single-institution retrospective study with small number of patients that analyzed only DVH parameters virtually based on CTs taken before radiotherapy. Also, it is important to maintain the patient's anatomical relationship as planning CT, namely monitoring the filling of bladder and the emptiness of rectum because otherwise intended OAR sparing will not be achieved. Since this study was represents a "plan-to-be" approach, we have to verify the efficacy of BMS-IMRT in a clinical setting. Recently RTOG 1203 was launched in order to validate whether IMRT could decrease acute gastrointestinal toxicity compared with conventional radiotherapy with bone marrow sparing included in the protocol. Therefore, important information about the influence of IMRT over bone marrow will be available soon.

Whether WPB or ICPB should be more appropriate surrogate structures for bone marrow function(s) will be confirmed by future prospective studies. Also, the appropriate doses for bone marrow's protection need to be further investigated in the near future.

Conclusion

Both lower and higher dose for WPB as well as ICPB were effectively decreased by IMRT with an intention of avoiding damage of bone marrow structure without compromising the coverage of target volume and other organs at risk.

Conflicts of Interest

There is no conflict of interest to be declared.

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Original Article

Mucinous breast carcinoma with a lobular neoplasia component: A subset with aberrant expression of cell adhesion and polarity molecules and lack of neuroendocrine differentiation

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We investigated whether some mucinous carcinomas (MUCs) are associated with lobular neoplasia (LN) components, and if so, whether this subset has any distinct biological properties. MUC specimens from 41 patients were stratified into pure and mixed types. The LN components adjacent to MUC lesions were examined histopathologically. We also tested immunohistochemically for E-cadherin, β-catenin, and the neuroendocrine markers chromogranin A and synaptophysin; and compared results between MUCs with and without LN. Of 41 patients with MUC, LN was detected in 12 patients (29%); LN alone was the noninvasive component in 8 patients (20%). Decreased E-cadherin and β-catenin expression in the MUC component was detected in 2 (17%) and 7 (58%) cases, respectively, of MUC with LN, compared with 0% ($P = 0.080$) and 21% ($P = 0.018$) in MUCs without LN. Neuroendocrine factors were frequently detected in MUCs with LN (42%) and without LN (52%), but tended to be less frequent in MUCs with only LN components (25%) than in other MUCs (55%; $P = 0.133$). MUCs associated with LN components appear to be a biologically characteristic subset that frequently shows decreased cell–cell adhesion, cell polarity molecules and lack of neuroendocrine differentiation.

Key words: breast, E-cadherin, lobular neoplasia, mucinous carcinoma, neuroendocrine differentiation, β-catenin

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Mucinous carcinoma (MUC) comprises 1–6% of all breast cancers.[1–4] Up to 75% of MUCs are associated with the component of intraductal carcinoma, and it is generally believed that MUC of the breast originates from ductal carcinoma *in situ* (DCIS), usually of the common or endocrine (or solid-papillary) subtype.[5]

Neuroendocrine differentiation in MUC was reported in 1980 by Capella *et al.*, who used structural and cytological features to classify cases of MUC as type A (paucicellular), type B (hypercellular), and type AB (the intermediate form).[6] Type B MUC frequently shows neuroendocrine differentiation but type A MUC does not.[7] Neuroendocrine differentiation in MUC is immunohistochemically identified by the expression of chromogranin A and/or synaptophysin.[8] However, not many studies have been performed on the morphologic characteristics and differentiation of type A MUC.

Lobular neoplasia (LN) is composed of lobular carcinoma *in situ* (LCIS) and atypical lobular hyperplasia (ALH). It is thought that LN constitutes a risk factor for concurrent ipsilateral and/or contralateral breast cancers and a non-obligate precursor for the development of invasive carcinoma in either breast.[9] LN is found in 0.3–3.9% of otherwise benign breast biopsies.[10–15] Since we have experienced some cases of MUC accompanied by an extensive LN component, we speculated that a subset of MUC occurs in association with LN and may show unique biological properties. We were particularly interested in the percentage of MUC with an LN component and in its association with aberrant cell adhesion, cell polarity molecules, and neuroendocrine differentiation. In this study, we included cases of MUC with an LN component and characterized their biological characteristics by performing immunohistochemical analysis of the expression of E-cadherin,

β-catenin, and the neuroendocrine markers chromogranin A and synaptophysin and comparing the results with those for MUC without LN.

MATERIALS AND METHODS

The subjects comprised 41 consecutive patients who had MUC that had been surgically resected at National Cancer Center Hospital, Tokyo, between 2009 and 2011. Clinical and pathological T and N factors were registered according to the Cancer Staging Manual of the American Joint Committee on Cancer (AJCC), 7th edition. The cut-off value for ER and PR positivity was 10% positive cells, regardless of intensity. HER2 positivity was defined as an HER2 score of 3+ (>30% strong membrane immunoreaction-positive cells) or an HER2 gene/centromere 17 copy ratio of ≥2.0, as assessed by fluorescence *in situ* hybridization.

Mucinous carcinoma was also histopathologically stratified into pure and mixed types, with the former composed of >90% MUC in the invasive carcinoma component, and the latter being mixed with 50–90% MUC with 10–49% conventional invasive ductal carcinoma in the invasive carcinoma component.[5] The presence of the LN components LCIS or ALH adjacent to the MUC lesion was examined histopathologically. Simple lobular hyperplasia was not taken into account. The MUC component was also subclassified into type A (hypocellular) and type B (hypercellular) based on the cellularity and extracellular mucin status according to Capella's criteria.[6]

In the MUC and LN components, E-cadherin (1:100, mouse monoclonal, clone NCH-38, Dako, Glostrup, Denmark), β-catenin (1:500, mouse monoclonal, 14/β-catenin, BD Biosciences, San Jose, CA, USA), chromogranin A (1:100, rabbit polyclonal, A0430, Dako), and synaptophysin (ready-to-use, mouse monoclonal, 27G12, Nichirei, Tokyo) expression was examined by immunohistochemistry. Antigen retrieval was performed in TRS buffer (Dako) for E-cadherin, citrate buffer for β-catenin and chromogranin A, and TRS, pH 9.0 (Dako), for synaptophysin. Immunohistochemistry was performed with the Envision method and a Dako Autostainer.

This study was conducted under internal review board approval with written informed consent obtained from the patients.

Statistical analysis

The Mann–Whitney test was used to compare age between MUCs with and without the LN component, while the χ^2 test or Fisher's exact test was used to compare other variables. Differences were considered significant at *P*-values less than 0.05. SPSS statistical software (version 19, IBM SPSS Statistics, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Lobular neoplasia was detected in the background of MUC in twelve cases (29%), varying from 1 to 85 mm in diameter: eight with LN only and four with both LN and DCIS components. DCIS alone was detected in the background of MUC in 19 cases, but the noninvasive carcinoma component was not observed in the other 10 cases. Extensive intraductal spread (EIC[+]) was seen in 14 cases, with the DCIS component alone noted in nine, both DCIS and LN components in four, and LN component alone in one case. These LN and DCIS components were located in continuity with or around the main MUC lesion on the representative cut surface of the tumor. Therefore, we considered that there is a possibility that these LN and/or DCIS components might be precursors for the MUC component, although genetic studies are needed to draw a conclusion.

The clinicopathological characteristics of the 41 patients are listed in Table 1. The median age was 62.5 years (range, 35–81 years). There were no significant differences in clinicopathological characteristics between the 12 patients with MUC with the LN component and the 29 patients without, with regard to pure/mixed type, A/B type, and nuclear grade. In three cases of mixed type MUC with LN, the invasive component other than MUC did not contain invasive lobular carcinoma. The percentage of axillary lymph node metastases tended to be larger in the former group: three (25%) of 12 patients with MUC with the LN component showed histological lymph node metastases, whereas only three (10%) of 29 patients with MUC without the LN component showed histological lymph node metastases. All MUCs, both with LN and without LN, expressed ER/PgR and lacked HER2 overexpression, except in one case.

Figures 1 and 2 show photomicrographs of representative cases of MUC with the LN component.

Reduction and disappearance of membrane immunoreactivity in >50% of constituent tumor cells was judged abnormal for E-cadherin and β-catenin expression. Only two cases showed a decrease in E-cadherin expression; in the other 39 cases, E-cadherin was diffusely expressed and <5% of cells showed a decrease in expression. Likewise, 13 of 41 MUCs showed a significant decrease in β-catenin expression; in the other 28 cases, β-catenin was expressed diffusely and <5% of cells showed a decrease in expression. However, for both E-cadherin and β-catenin, it was unclear whether the decreased expression in <5% of cells was a focal decrease or uneven staining due to a technical artifact. Therefore, we set the cut-off value at 50% for both E-cadherin and β-catenin.

Table 1 Correlations of clinicopathological parameters of mucinous carcinoma with/without lobular neoplasia component

	Number of cases (%)			
		Non-invasive carcinoma component		
Parameter	Total	With LN (n = 12)	Without LN (n = 29)	P
Age				
Average (range)	62.5 (35–81)	62.6 (42–78)	62.5 (35–81)	0.774
<50	9	1 (9)	8 (28)	0.124
≥50	32	11 (91)	21 (72)	
Histological type				
Pure type	28	9 (75)	19 (66)	0.553
Mixed type	13	3 (25)	10 (34)	
Morphological type				
Type A	19	5 (42)	14 (48)	0.699
Type B	22	7 (58)	15 (52)	
cT-factor				
T1	26	8 (67)	18 (62)	0.505
T2	12	4 (33)	8 (28)	
T3	3	0 (0)	3 (10)	
pT-factor				
T1	28	9 (75)	19 (66)	0.729
T2	12	3 (25)	9 (31)	
T3	1	0 (0)	1 (3)	
LVI				
Negative	35	11 (91)	24 (83)	0.463
Positive	6	1 (9)	5 (17)	
Nuclear grade				
1	33	10 (83)	23 (79)	0.767
2	8	2 (17)	6 (21)	
3	0	0 (0)	0 (0)	
Hormone receptor				
Positive	41	12 (100)	29 (100)	
Negative	0	0 (0)	0 (0)	—
HER2				
Positive	1	1 (9)	0 (0)	0.116
Negative	40	11 (91)	29 (100)	
Lymph node metastasis				
Positive	6	3 (25)	3 (10)	0.088
Negative	31	6 (50)	25 (86)	
Unknown	4	3 (25)	1 (4)	

LVI, Lymphovascular invasion.

Immunohistochemical analysis showed that the membrane expression of E-cadherin in the MUC component decreased in two (17%) MUCs with an LN component but not in MUCs without LN (P = 0.080; Table 2). Likewise, a decrease in the membrane expression of β-catenin in the MUC component was detected in seven (58%) MUCs with the LN component and in six (21%) MUCs without LN (P = 0.018; Table 2).

A decrease in the membrane expression of E-cadherin and β-catenin in the MUC component was detected in two (25%) and seven (87%) of eight MUCs with the LN component only, while the decrease was detected in zero (0%) and six (18%) of 33 MUCs with both LN and DCIS components or without the LN component (P = 0.034 and 0.00051; Table 3). In Figures 3 and 4, two cases of MUC showing different expression patterns of E-cadherin and β-catenin are presented.

Chromogranin A and/or synaptophysin positivity in the MUC component was >50% in 14 cases, >20–50% in zero cases, >10–20% in six cases, >0–10% in seven cases, and negative in 14 cases. Because chromogranin A- or synaptophysin-positive cells were easily identified, the expression of either chromogranin A or synaptophysin in 10% or more of tumor cells was judged as the neuroendocrine immunophenotype. Positive results were obtained for neuroendocrine marker expression in 20 (49%) of 41 MUCs, and this expression was observed in 42% (5 of 12) of MUC with LNs and 52% (15 of 29) of MUCs without LNs (Table 2).

Among the eight MUCs with only the LN component, the neuroendocrine immunophenotype was detected in two (25%). This tended to be lower than the rate of the neuroendocrine immunophenotype in MUCs with both LN and DCIS components or without the LN component (18 of 33 [55%]),