Prognostic Value of Severe Lymphopenia During Pelvic Concurrent Chemoradiotherapy in Cervical Cancer

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Abstract. *Aim: To investigate whether common terminology criteria for adverse events (CTCAE) grade 4 lymphopenia (<200 cells/μl) during concurrent chemoradiotherapy (CCRT) is relevant to poor survival. Patients and Methods: We analyzed 124 patients with newly diagnosed Federation of Gynecology and Obstetrics (FIGO) stage I-III cervical cancer who received weekly cisplatin-based CCRT and brachytherapy using Kaplan-Meier curves and the Cox proportional hazard models. Results: Grade 4 lymphopenia significantly predicted disease-specific survival (DSS) and progression-free survival (PFS) (adjusted hazard ratio (95% confidence interval (CI))=3.6 (1.37-9.44), p=0.009 and 3.28 (1.27-8.48), p=0.014, respectively). The 5-year DSS and 3-year PFS were significantly higher among patients with grade 2-3 lymphopenia (≥200 cells/μl) than among those with grade 4 lymphopenia (84.8% vs. 50.4%, p<0.001, and 80.7% vs. 50%, p=0.002, respectively). Conclusion: Severe lymphopenia during CCRT could predict poor survival.*

Decreased tumor-infiltrating lymphocytes (TILs) are known to be a poor prognostic factor for various cancers (1). In addition, peripheral blood lymphopenia prior to treatment was also associated with poor survival in advanced cancers (2). This observation implied that lymphopenia by cancer immunoediting might result in cancer progression (3).

In this regard, concurrent chemoradiotherapy (CCRT) for cervical cancer as a primary modality might have both a positive effect to sustain circulating lymphocytes by controlling tumor and a negative effect to decrease them by irradiation of peripheral blood vessels included in the pelvic CCRT field. Therefore, lymphopenia during CCRT might be a representative factor to explain progressive cancer, poor treatment response and CCRT-induced immunosuppression, that might result in poor survival. This assumption could be supported by a previous study indicating that lymphopenia, more severe than common terminology criteria for adverse events (CTCAE) grade 2 (500 to <800 cells/μl) at the end of pelvic radiation therapy (RT), occurred in more than 70% of patients, while more severe lymphopenia reflected lack of tumor regression in gynecological neoplasms (4). In addition, an absolute lymphocyte count during CCRT below 200–300 cells/μl in other sites, such as lung and head and neck for treatment of limited-stage small cell lung and nasopharyngeal cancer, also could predict progression-free survival (PFS) and disease-specific survival (DSS) (5, 6).

Taken together, CTCAE grade 4 lymphopenia (<200 cells/μl) during pelvic CCRT might be relevant to poor treatment outcome of cervical cancer. Therefore, we investigated whether absolute lymphocyte count (ALC) below 200 cells/μl in International Federation of Gynecology and Obstetrics (FIGO) stage I-III cervical cancer treated with CCRT, followed by high-dose intracavitary brachytherapy (HDR BT), could be associated with PFS and DSS.

Materials and Methods

This study was reviewed and approved by the Ajou University Hospital Institutional Review Board (AJIRB-MED-MDB-15-125). We selected 124 patients with newly diagnosed FIGO stage I-III cervical cancer who were treated with weekly cisplatin-based CCRT followed by high dose rate intracavitary brachytherapy (HDR BT) at Ajou University Hospital from April 2001 to May 2012. The following patients were excluded: 28 patients who were treated with a point A dose of less than 64 Gy, normalized to an equivalent dose in 2 Gy using an α/β ratio of 10 Gy (EQD2) or a HDR BT less than three fractionations, 2 patients who were followed for less than 12 months and 16 patients who had no available ALC in the fourth or fifth week during pelvic CCRT. All patients in whom disease was confirmed histologically by biopsy underwent physical examinations, baseline laboratory tests, chest X-rays and computed tomography (CT) or magnetic resonance imaging (MRI) to evaluate pelvic or para-aortic lymph node (PALN) involvement. Patients suspected of having rectal or bladder invasion underwent cystoscopy and

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sigmoidoscopy. The patients were followed every 1-3 months for the first 3 years and every 6 months thereafter. Primary cervical tumors, regional lymph nodes and distant metastases were evaluated with pelvic exams, Pap smears, tumor markers, such as squamous cell carcinoma antigen (SCC Ag) and Cyfra 21-1, and CT or positron emission tomography (PET) CT. The median follow-up period was 63 months (range=14-163 months) and the median follow-up of censored patients was 87 months (first quartile=54 months, third quartile=107 months, range=15-163 months). The end-points were DSS and PFS. Patients who experienced disease progression were treated with chemotherapy (CTx), RT or conservative care.

External-beam RT (EBRT) was delivered using 10-15 MV photons to the whole pelvis or PALNs. The RT dose to the whole pelvis was 45 Gy delivered in 25 fractions, followed by 4-10 Gy in 2-5 fractions to the low pelvis and a midline block (MLB) was applied after 36-53 Gy (central EBRT dose), depending on the reduction of the tumor size assessed by physical exam or/and pelvic MRI. Six patients did not use an MLB. Thirty-eight patients with bulky tumors received pelvic CCRT consisting of 15-18 Gy delivered in 10-12 fractions twice a day for 5 or 6 days after 18 Gy in 10 fractions for two weeks, followed by RT consisting of 9-10.8 Gy in 5-6 fractions (partial BID) to shorten the overall treatment time (OT), defined as the time between the start and end of RT (7). Pelvic RT dose scheme of partial BID was 45 Gy (or 45.6 Gy) in 27 fractions. The patients underwent HDR BT (Iridium-192; Microselectron, Nucletron, Veenendaal, Netherlands) applied with a median point A dose of 28 Gy (range= 12-30 Gy, 4 or 5 Gy per fraction, biweekly). In 14 of 79 patients with pelvic lymph node metastasis, including bulky tumors or high pre-treatment SCC Ag levels, the PALN field was irradiated with 37.8-44.2 Gy delivered in 21-26 fractions (8, 9). Weekly concurrent cisplatin (30-70 mg/m^2) was administered for a total of 4-6 cycles during RT in all patients.

The total dose (TD) was the sum of EBRT EQD2 at the central pelvis and HDR BT EQD2 at A point. The HDR BT ratio was defined as the ratio of HDR BT dose to TD. The patients underwent assessments of hemoglobin (Hb), SCC Ag and ALC before the initial treatment (baseline ALC). The complete blood count (CBC) was assessed once a week for five weeks when patients underwent pelvic CCRT. We defined the "min ALC" as the minimum ALC during pelvic CCRT. The lymphocyte toxicity grading of CTCAE version 4.03 was evaluated by min ALC as grade 2 (500≤ min ALC <800 cells/μl), grade 3 (200≤ min ALC <500 cells/μl), or grade 4 (min ALC <200 cells/μl).

We compared the differences of variables, such as age, pathology, FIGO stage, pelvic node status, Hb, SCC Ag, baseline ALC, histories of extended field RT (EFRT), partial BID, OT, central EBRT dose, HDR BT ratio and TD, as well as treatment outcomes between three groups of patients with grade 2, 3 and 4 CTCAE lymphopenia using Fisher's exact test or χ^2 test for categorical data. The categories of continuous variables like age, Hb, SCC Ag, baseline ALC and OT were determined by the value around the first or third quartiles according to relevance to DSS. The cut-off value of central EBRT dose, HDR BT ratio and TD was the median value of each group. The DSS and PFS between patients with grade 4 lymphopenia and those with grade 2 or 3 lymphopenia were compared using the Kaplan-Meier method. We conducted univariate and multivariate analyses using the Cox proportional hazards model. The variables with p-values ≤0.1 in univariate analyses, age and FIGO stage were included in multivariate analyses. All p-values were obtained using two-sided tests and those less than 0.05 were considered statistically significant. These statistical analyses were performed using R software version 3.2.3 (the R foundation for Statistical Computing, available at: http://www.r-project.org).

Results

The characteristics of the 124 patients are presented in Table I. The median patients' age was 57 years (first quartile=47, third quartile=68, range=28-82); 33% were elderly patients. Most patients (96%) had SCC. The number of patients with FIGO IB or IIA, IIB and III lesions were 26 (21%), 84 (68%) and 14(11%), respectively. In total, 79 patients (64%) had pelvic LN enlargement and 34 patients (27%) had Hb ≤11g/dl (median value (range)=11.7 (5.2-14.5), first quartile=10.7, third quartile=12.4). SCC Ag >10 ng/ml and baseline ALC ≤1,500 cells/μl were present in 29% (median value (range)=4.75 (0.3-105), first quartile=1.7, third quartile=11.8) and 17% (median value (range)=1,961 (432–3,696), first quartile=1,592, third quartile=2,258), respectively. EFRT was applied to 14 patients (11%). The median OT was 51 days (range=42–73, first quartile=49, third quartile=56) and 25 patients had OT greater than 8 weeks (56 days). The median total EQD2 and central EBRT EQD2 were 68.1Gy (range=64.6-82.3 Gy, first quartile=68.1, third quartile=71.9) and 39 Gy (range=35.4-59.4 Gy, first quartile=35.4, third quartile=44.3), respectively. The median HBR BT ratio was 0.445 (range=0.18-0.487, first quartile=0.383, third quartile=0.48). The number of patients with CTCAE grade 2, grade 3 and grade 4 lymphopenia were 14 (11%), 90 (73%) and 20 (16%), respectively. The grade 4 lymphopenia group included more patients having histories of EFRT, undergoing partial BID, and finishing treatment within 8 week than did the grade 2 and 3 groups (p=0.001, p<0.001 and p=0.048, respectively). The percentage of patients with stage III, Hb ≤11g/dl, SCC Ag >10 ng/ml, baseline ALC ≤1,500 cells/μl and central pelvic EBRT dose >39 EQD2 in each group tended to increase in parallel with the lymphopenia grade (p=0.153, p=0.168, p=0.107, p=0.071 and p=0.141, respectively). ALCs during pelvic CCRT declined abruptly and reached a nadir in the fourth or fifth week, as presented in Figure 1. The median min ALCs were 317 cells/μl (range=67–758, first quartile=238, third quartile=402).

In this study, 33 of the 124 patients experienced disease progression. There were 7 patients with locoregional disease progression (LRP), 5 patients with both local progression and distant metastasis (DM) and 21 patients with DM only. Among the 12 patients with LRP, 6 patients experienced relapse in the cervix (2 patients), lymph nodes (2 patients) and both (2 patients), while 6 patients displayed progression of the residual tumor in the cervix. In the 26 patients with DM, the disease had spread to the following sites: PALNs (12 patients), lungs (9 patients), peritoneum (5patients), bone

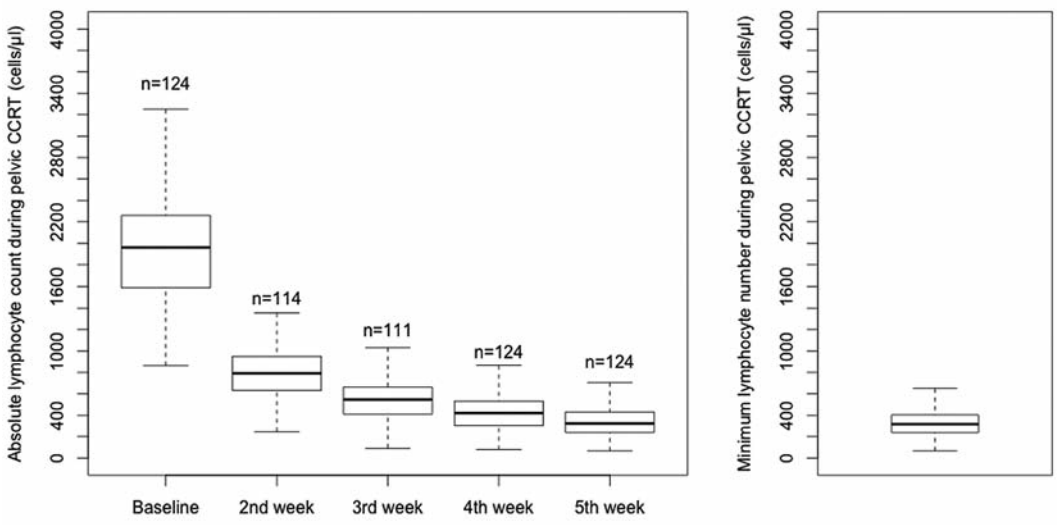

Figure 1. *The change in absolute lymphocyte count and minimum lymphocyte number during pelvic concurrent chemoradiotherapy (CCRT).*

(5 patients), liver (2 patients), muscle (2 patients), supraclavicular lymph node (1 patient), brain (1 patient) and adrenal gland (1 patient). In addition, 26 of 124 patients died due to cervical cancer in the follow-up period, 24 of them within 5 years after initial diagnosis. The 3-year DSS and 5-year DSS (5DSS) for all patients were 86.1% and 79.4%, respectively. The 5DSS rates for patients with FIGO stages IB or IIA, IIB and III lesions were 88.3, 79.4 and 64.3%, respectively. Patients with stage I or II disease had a 5DSS of 81.5%, whereas those with stage III disease had a 5DSS of 64.3% (p=0.126). The 3-year PFS (3PFS) and 5-year PFS for all patients were 75.6% and 73.4%, respectively. The 3PFS rates for patients with FIGO stages IB or IIA, IIB and III lesions were 83.5, 78.7 and 42.9%, respectively. Patients with stage I or II disease had a 3PFS of 79.9%, compared to 42.9% of those with stage III disease (p<0.001).

Table II presents the differences of treatment outcomes by lymphopenia grade. Patients with grade 4 lymphopenia had significantly more progressions and disease-specific deaths than patients with grade 2 or 3 lymphopenia (p=0.014 and p=0.01), mainly resulting from DMs (p=0.004). Of the 33 patients who experienced disease progression, 22 received more than three cycles of second-line chemotherapy, while the remaining patients received conservative treatment because they were either unable to tolerate or refused chemotherapy. The 5DSS and 3PFS were significantly lower among patients with grade 4 lymphopenia than among those with grade 2 or 3 lymphopenia, as illustrated in Figure 2 (50.4% *vs*. 84.8%, p<0.001, 50% *vs*. 80.7%, p=0.002, respectively). The results of the univariate and multivariate analyses of DSS and PFS are presented in Table III. Grade 4 lymphopenia was the only significant predictor of DSS and PFS on multivariate analysis (adjusted hazard ratio (AHR) (95% confidence interval (CI))=3.6 (1.37-9.44), p=0.009 and 3.28 (1.27-8.48), p=0.014, respectively).

Table IV shows that the ALCs at baseline and in the second, third, fourth and fifth week during pelvic CCRT in patients who did or did not receive EFRT were higher in patients with grade 2-3 lymphopenia than in those with grade 4. These differences increased and standard deviations of all groups decreased as pelvic CCRT proceeded. In particular, the patients with grade 4 lymphopenia in the EFRT group had a relatively lower baseline ALC and a more rapid decrease from baseline in the second week than did the grade 2-3 group, resulting in ALCs in the third week <300 cells/μl.

Discussion

Patients with a min ALC <200 cells/μl (grade 4 lymphopenia) had a significant lower 5DSS and 3PFS than those with a min ALC ≥200 cells/μl; thus, ALC was a significant predictor of DSS and PFS on multivariate analysis (Figure 2 and Table III). In addition, the grade 4 lymphopenia group included more factors that reflected a modification of RT dose and field, such as EFRT and partial BID, as shown in Table I (p=0.001 and p<0.001). There was no prolongation of OT in the grade 4 lymphopenia group because the patients with grade 4 lymphopenia included more patients treated with partial BID than those with grade 2–3 in a group of 99 patients with OT ≤8 weeks (grade 4: 13 of 20 patients (65%) *vs*. grade 2-3: 20 of 79 patients (25%), p=0.002). This association between OT and partial BID was consistent with a previous study about partial dose modification during CCRT (7). The influence on lymphopenia of EFRT and

Table I. *Comparison of all variables by CTCAE grade of minimum absolute lymphocyte count during pelvic concurrent chemoradiotherapy.*

Factors		Grade 2 (500 to <800 cells/μl)	Grade 3 (200 to <500 cells/μl)	Grade 4 (<200 cells/μl)	
	n=124	n=14 (11%)	n=90 (73%)	n=20 (16%)	*p*-Value
Age (years)					0.328
>65	41 (33%)	6 (57%)	31(66%)	4(80%)	
≤65	83 (67%)	8 (43%)	59 (34%)	16 (20%)	
Pathology					0.713
SCC	119 (96%)	14 (100%)	86 (96%)	19 (95%)	
Adeno subtype	5 (4%)	0 (0%)	4 (4%)	1 (5%)	
FIGO stage					0.153
IB or IIA	26 (21%)	4 (29%)	20 (22%)	2 (10%)	
IIB	84 (68%)	10 (71%)	61 (68%)	13 (65%)	
III	14 (11%)	0 (0%)	9 (10%)	5 (25%)	
Pelvic LN enlargement					0.484
No	45 (36%)	6 (43%)	34 (38%)	5 (25%)	
Yes	79 (64%)	8 (57%)	56 (62%)	15 (75%)	
Hemoglobin (g/dl)					0.168
>11	90 (73%)	13 (93%)	64 (71%)	13 (65%)	
≤11	34 (27%)	1 (7%)	26 (29%)	7 (35%)	
SCC Ag (ng/ml)					0.107
>10	36 (29%)	1 (7%)	27 (30%)	8 (40%)	
≤10	88 (71%)	13 (93%)	63 (70%)	12 (60%)	
Baseline ALC (cells/μl)					0.071
>1,500	103 (83%)	14 (100%)	75 (83%)	14 (70%)	
≤1,500	21 (17%)	0 (0%)	15 (17%)	6 (30%)	
EFRT					0.001
No	110 (89%)	14 (100%)	83 (92%)	13 (65%)	
Yes	14 (11%)	0 (0%)	7 (8%)	7 (35%)	
Partial BID					<0.001
No	86 (69%)	14 (100%)	65 (72%)	7 (35%)	
Yes	38 (31%)	0 (0%)	25 (28%)	13 (65%)	
OT (weeks)					0.048
>8	25 (20%)	3 (21%)	22 (24%)	0 (0%)	
≤8	99 (80%)	11 (79%)	68 (76%)	20 (100%)	
Central EBRT dose					0.141
>39 EQD2	62 (50%)	7 (50%)	41 (46%)	14 (70%)	
≤39 EQD2	62 (50%)	7 (50%)	49 (54%)	6 (30%)	
HDR BT ratio					0.666
>0.445	61 (49%)	7 (50%)	46 (51%)	8 (40%)	
≤0.445	63 (51%)	7 (50%)	44 (49%)	12 (60%)	
Total dose					0.225
>68.1 EQD2	53 (43%)	6 (43%)	35 (39%)	12 (60%)	
≤68.1 EQD2	71 (57%)	8 (57%)	55 (61%)	8 (40%)	

CTCAE, Common terminology criteria for adverse events; SCC, squamous cell carcinoma; FIGO, Federation of Gynecology and Obstetrics; LN, lymph node; SCC Ag, squamous cell carcinoma antigen; ALC, absolute lymphocyte count; RT, radiation therapy; EFRT, extended field RT; partial BID, fractionation twice a day in the third week during pelvic RT; OT, overall treatment time; EBRT, external beam RT; EQD2, an equivalent dose in 2 Gy using an α/β ratio of 10 Gy; central EBRT dose, EBRT EQD2 at central pelvis; HDR BT, high dose rate intracavitary brachytherapy; Total dose, the sum of central EBRT dose and HDR BT dose at A point; HDR BT ratio, the ratio of HDR BT dose to total dose.

Table II. *Comparison of treatment outcomes by CTCAE grade of minimum absolute lymphocyte count during pelvic concurrent chemoradiotherapy.*

Factors		Grade 2 (500 to <800 cells/μl)	Grade 3 (200 to <500 cells/μl)	Grade 4 (<200 cells/μl)	
	n=124	n=14 (11%)	n=90 (73%)	n=20 (16%)	*p*-Value
Progression					0.014
No	91 (73%)	13 (92.9%)	68 (75.6%)	10 (50%)	
Yes	33 (27%)	1 (7.1%)	22 (24.4%)	10 (50%)	
Locoregional progression (LRP)					0.963
No	116 (94%)	13 (92.9%)	85 (94.4%)	19 (95%)	
Yes	7 (6%)	1 (7.1%)	5 (5.6%)	1 (5%)	
Distant metastasis (DM)					0.004
No	104 (83%)	14 (100%)	77 (85.6%)	12 (60%)	
Yes	21 (17%)	0 (0%)	13 (14.4%)	8 (40%)	
LRP+ DM					0.713
No	119 (96%)	14 (100%)	86 (95.6%)	19 (95%)	
Yes	5 (4%)	0 (0%)	4 (4.4%)	1 (5%)	
Disease-specific death					0.01
No	98 (79%)	13 (92.9%)	74 (82.2%)	11 (55%)	
Yes	26 (11%)	1 (7.1%)	16 (17.8%)	9 (45%)	
Second treatment	N=33				0.726
No	11 (33%)	0 (0%)	8 (36.4%)	3 (30%)	
Yes	22 (67%)	1 (100%)	14 (63.6%)	7 (70%)	

CTCAE, Common terminology criteria for adverse events.

partial BID might come primarily from an increase in lymphocyte toxicity by the RT field, including the abdominal aorta and vena cava, and by shortening of lymphocyte recovery time. However, disease extent and RT response were likely to be contributors of grade 4 lymphopenia, since EFRT and partial BID were used for the patients considered to be high-risk based on tumor size, SCC Ag and lymph node status. In addition, stage III, Hb ≤11 g/dl, SCC Ag >10 ng/ml, baseline ALC ≤1,500 cells/μl and central EBRT dose >39 EQD2 correlated with grade 4 lymphopenia. High stage, anemia, pretreatment lymphopenia and high SCC Ag were associated with poor survival in past reports (10-13) and were significant predictors of DSS and/or PFS on univariate analyses, as displayed in Table III. Central EBRT dose implied RT response evaluated by physical examination and/or MRI during pelvic CCRT and was associated with poor survival in this study. Patients with grade 4 lymphopenia had more rapid progression and earlier disease-specific deaths than did those with grade 2-3 lymphopenia as they mainly developed DMs (Figure 2 and Table II). This revealed that drastic lymphopenia during pelvic CCRT might influence

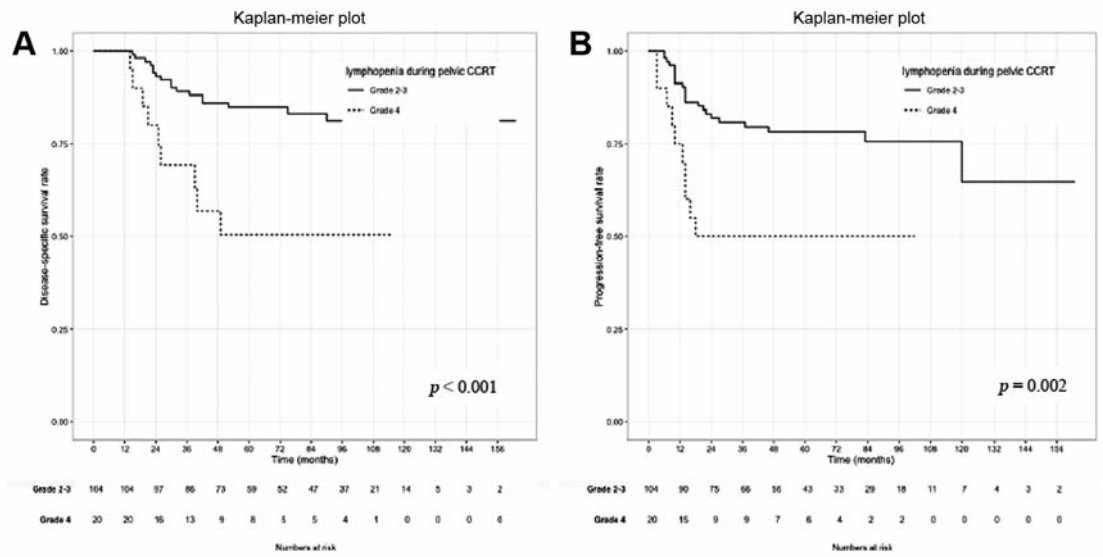

Figure 2. *Kaplan-Meier plots for (A) disease-specific survival and (B) progression-free survival compare a group of patients with grade 4 lymphopenia (min ALC <200 cells/μl) with those with grade 2-3 (min ALC ≥200 cells/μl). Min ALC, Minimum absolute lymphocyte during pelvic concurrent chemoradiotherapy (CCRT).*

systemic recurrence by undermining patients' immune function quantitatively. In this aspect, modification of RT dose or field in patients with high stage, adverse biomarkers and poor RT response might bring adverse effects through lymphopenia. However, partial BID was less relevant to the risk of poor survival than was EFRT (Table III), since the group of patients who underwent EFRT included more patients treated with partial BID than did not a group of EFRT, together with shortening of OT relevant to good treatment result (9 of 14 patients (64%) *vs*. 29 of 110 patients (26%), p=0.01) (14). HDR BT ratio was consistent regardless of lymphopenia grade; however, a low relative HDR BT dose tended to be associated with poor survival (Table III). Acceptable treatment results of Japanese trials based on both low total EQD2 at A point (52 Gy for stage I-II and 62 Gy for stage III-IVA) and a high HDR BT ratio (0.61 for stage I-II and 0.51 for stage III-IVA) let us appreciate the importance of HDR BT ratio (15, 16). Despite implications that high HDR BT ratio could improve treatment outcome, to date no study has proven this point. This approach could be suggested as a method to overcome the poor survival associated with severe lymphopenia if escalating relative HDR BT dose had a positive effect. Therefore, it needs to be investigated further.

In brief, severe lymphopenia of CTCAE grade 4, significantly associated with partial BID and EFRT, could predict poor survival arising from DM. BID treatment, which increased in a group of patients with EFRT history and OT ≤8 weeks, might make clinical impact of lymphopenia by dose modification unclear. Apart from lymphopenia, we estimated that the increase of relative HDR BT dose might be connected to an improvement of treatment outcome. Therefore, the use of MRI-guided HDR BT instead of partial BID might help to shorten OT and increase relative HDR BT dose with minimal effect on circulating lymphocytes. Although the benefit of prophylactic EFRT (P-EFRT) during CCRT for patients with locally advanced cervical cancer is controversial (8, 17, 18), our data supported that, withdrawing P-EFRT for patients with a low ALC at baseline, a rapid drop of ALC at the second week and an ALC below 300 cells/μl at the third week might be a more reasonable choice (Table IV). Adjuvant/consolidation chemotherapy (ACT) followed by pelvic CCRT and HDR BT could be carefully suggested for patients with grade 4 lymphopenia, although the clinical significance of ACT is not well known (19, 20).

This study had several limitations. First, central EBRT dose is not a general method to evaluate RT response, although its clinical relevance was described in this study. Second, we did not study whether HDT BT ratio was relevant to survival, although past studies presented a benefit of a high HDR BT ratio (15, 16). Third, this was a small-sized retrospective study containing data that had been collected over a prolonged period. Therefore, a well-designed, prospective study is warranted to confirm the clinical significance of grade 4 lymphopenia. Despite these limitations, an intimate association between poor survival and severe lymphopenia during CCRT provides clues to improve treatment results.

In conclusion, grade 4 lymphopenia during CCRT, that might reflect treatment toxicity, disease extent and RT

Table III. *Cox regression analyses for disease-specific survival and progression-free survival.*

Factors	Disease-specific survival				Progression-free survival			
	HR (95%CI)	*p*-Value	AHR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value	AHR (95%CI)	*p*-Value
Age (years) >65	1.45 (0.66-3.21)	0.356	1.76 (0.74-4.18)	0.2	1.14 (0.55-2.36)	0.734	1.59 (0.68-3.74)	0.285
Pathology Adeno subtype	2.35 (0.56-9.96)	0.245			1.54 (0.37-6.51)	0.554		
FIGO stage Stage III	2.11 (0.79-5.60)	0.134	0.99 (0.34-2.93)	0.99	3.94 (1.83-8.49)	<0.001	1.63 (0.65-4.05)	0.294
Lymph node Positive	1.45 (0.61-3.44)	0.404			1.51 (0.7-3.25)	0.293		
Hemoglobin (g/dl) ≤11	1.71 (0.78-3.77)	0.183			2.93 (1.03-4.2)	0.04	1.53 (0.65-3.6)	0.33
SCC Ag (ng/ml) >10	2.54 (1.18-5.48)	0.017	2.22 (0.91-5.45)	0.08	2.93 (1.48-5.83)	0.002	1.75 (0.74-4.12)	0.203
Baseline ALC ≤1,500 cells/μl	1.92 (0.8-4.55)	0.144			2.7 (1.3-5.88)	0.009	1.89 (0.75-4.76)	0.175
Lymphopenia Grade 4	3.63 (1.61-8.18)	0.002	3.6 (1.37-9.44)	0.009	3.06 (1.45-6.48)	0.003	3.28 (1.27-8.48)	0.014
EFRT Yes	2.34 (0.94-5.84)	0.067	1.11 (0.33-3.71)	0.868	2.84 (1.28-6.3)	0.01	1.56 (0.54-4.55)	0.415
Partial BID Yes	0.92 (0.39-2.19)	0.85			1.3 (0.63-2.7)	0.483		
OT (weeks) >8	1.62 (0.65-4.08)	0.301			2.02 (0.96-4.24)	0.065	2.53 (0.98-6.52)	0.054
Central EBRT dose >39 EQD2	3.28 (1.41-7.66)	0.006	2.11 (0.4-11.2)	0.38	4.32 (1.92-9.73)	<0.001	1.32 (0.25-7.05)	0.742
HDR BT ratio ≤0.445	3.01 (1.29-7.02)	0.011	1.8 (0.42-7.77)	0.433	4.06 (1.81-9.12)	0.001	2.35 (0.61-9.05)	0.213
Total dose >68.1 EQD2	1.92 (0.88-4.15)	0.1	0.7 (0.23-2.1)	0.522	2.87 (1.4-5.87)	0.004	0.85 (0.28-2.55)	0.776

HR, Hazard ratio; AHR, adjusted HR; CI, confidence interval; FIGO, Federation of Gynecology and Obstetrics; SCC Ag, squamous cell carcinoma antigen; ALC, absolute lymphocyte count; RT, radiation therapy; EFRT, extended field RT; partial BID, fractionation twice a day on third week during pelvic RT; OT, overall treatment time; EBRT, external beam RT; EQD2, an equivalent dose in 2 Gy using an α/β ratio of 10 Gy; central EBRT dose, EBRT EQD2 at central pelvis; HDR BT, high dose rate intracavitary brachytherapy; Total dose, the sum of central EBRT dose and HDR BT dose at A point, HDR BT ratio, the ratio of HDR BT dose to total dose.

Table IV. *Absolute lymphocyte count during pelvic concurrent chemoradiotherapy according to the history of extended field radiation therapy and CTCAE lymphopenia grade.*

ALC (cells/μl)	Extended field radiation therapy (n=14)			No Extended field radiation therapy (n=110)		
	Grade 2-3 n=7	Grade 4 n=7	*p*-Value	Grade 2-3 n=97	Grade 4 n=13	*p*-Value
Baseline	1942±575	1693±235	0.319	2069±597	1942±575	0.046
2nd week	801±404	403±196	0.036	861±283	656±208	<0.001
3rd week	484±254	206±49	0.044	609±221	349±148	<0.001
4th week	414±167	147±50	0.003	384±127	189±39	<0.001
5th week	300±99	130±38	0.004	370±121	171±28	<0.001

CTCAE, Common terminology criteria for adverse events; ALC, absolute lymphocyte count. Mean±standard deviation is shown; *t*-test was used for mean comparison.

response, was a significant predictor of DSS and PFS. From these observations, we can expect better survival than conventional treatment through a strategy to prevent severe lymphopenia or supplement ACT. Therefore, this finding should be further studied.

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References

1 Gooden MJ, de Bock GH, Leffers N, Daemen T and Nijman HW: The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer *105*: 93-103, 2011.

2 Ray-Coquard I, Cropet C, Van Glabbeke M, Sebban C, Le Cesne A, Judson I, Tredan O, Verweij J, Biron P, Labidi I, Guastalla JP, Bachelot T, Perol D, Chabaud S, Hogendoorn PC, Cassier P, Dufresne A, Blay JY, European Organization for R, Treatment of Cancer Soft T and Bone Sarcoma G: Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. Cancer Res *69*: 5383-5391, 2009.

3 Dunn GP, Bruce AT, Ikeda H, Old LJ and Schreiber RD: Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol *3*: 991-998, 2002.

4 Lissoni P, Meregalli S, Bonetto E, Mancuso M, Brivio F, Colciago M and Gardani G: Radiotherapy-induced lymphocytopenia: changes in total lymphocyte count and in lymphocyte subpopulations under pelvic irradiation in gynecologic neoplasms. J Biol Regul Homeost Agents *19*: 153-158, 2005.

5 Cho O, Oh YT, Chun M, Noh OK, Hoe JS and Kim H: Minimum absolute lymphocyte count during radiotherapy as a new prognostic factor for nasopharyngeal cancer. Head Neck *38(Suppl 1)*: E1061-1067, 2016.

6 Cho O, Oh YT, Chun M, Noh OK and Lee HW: Radiation-related lymphopenia as a new prognostic factor in limited-stage small cell lung cancer. Tumour Biol *37*: 971-978, 2016.

7 Chun M, Kang S, Ryu H, Chang K, Oh Y, Ju H and Lee E: Modified partial hyperfractionation in radiotherapy for bulky uterine cervical cancer: reduction of overall treatment time. Int J Radiat Oncol Biol Phys *47*: 973-977, 2000.

8 Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, Rotman M, Gershenson DM and Mutch DG: Pelvic Radiation with Concurrent Chemotherapy Compared with Pelvic and Para-Aortic Radiation for High-Risk Cervical Cancer. N Engl J Med *340*: 1137-1143, 1999.

9 Ogino I, Nakayama H, Okamoto N, Kitamura T and Inoue T: The role of pretreatment squamous cell carcinoma antigen level in locally advanced squamous cell carcinoma of the uterine cervix treated by radiotherapy. Int J Gynecol Cancer *16*: 1094-1100, 2006.

10 Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, Heintz AP, Ngan HY and Pecorelli S: Carcinoma of the cervix uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet *95(Suppl 1)*: S43-103, 2006.

11 Caro JJ, Salas M, Ward A and Goss G: Anemia as an independent prognostic factor for survival in patients with cancer. Cancer *91*: 2214-2221, 2001.

12 Duk JM, Groenier KH, De Bruijn H, Hollema H, ten Hoor KA, Van Der Zee A and Aalders JG: Pretreatment serum squamous cell carcinoma antigen: a newly identified prognostic factor in early-stage cervical carcinoma. J Clin Oncol *14*: 111-118, 1996.

13 Hoskin PJ, Rojas AM, Peiris SN, Mullassery V and Chong IY: Pre-treatment haemoglobin and peripheral blood lymphocyte count as independent predictors of outcome in carcinoma of cervix. Clin Oncol (R Coll Radiol) *26*: 179-184, 2014.

14 Song S, Rudra S, Hasselle MD, Dorn PL, Mell LK, Mundt AJ, Yamada SD, Lee NK and Hasan Y: The effect of treatment time in locally advanced cervical cancer in the era of concurrent chemoradiotherapy. Cancer *119*: 325-331, 2013.

15 Toita T, Kato S, Niibe Y, Ohno T, Kazumoto T, Kodaira T, Kataoka M, Shikama N, Kenjo M, Tokumaru S, Yamauchi C, Suzuki O, Sakurai H, Numasaki H, Teshima T, Oguchi M, Kagami Y, Nakano T, Hiraoka M and Mitsuhashi N: Prospective multi-institutional study of definitive radiotherapy with high-dose-rate intracavitary brachytherapy in patients with nonbulky (<4-cm) stage I and II uterine cervical cancer (JAROG0401/JROSG04-2). Int J Radiat Oncol Biol Phys *82*: e49-56, 2012.

16 Toita T, Kitagawa R, Hamano T, Umayahara K, Hirashima Y, Aoki Y, Oguchi M, Mikami M, Takizawa K and Cervical Cancer Committee of Japanese Gynecologic Oncology G: Phase II study of concurrent chemoradiotherapy with high-dose-rate intracavitary brachytherapy in patients with locally advanced uterine cervical cancer: efficacy and toxicity of a low cumulative radiation dose schedule. Gynecol Oncol *126*: 211-216, 2012.

17 Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, Rotman M, Gershenson D and Mutch DG: Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of Radiation Therapy Oncology Group Trial (RTOG) 90-01. J Clin Oncol *22*: 872-880, 2004.

18 Park SG, Kim JH, Oh YK, Byun SJ, Kim MY, Kwon SH and Kim OB: Is prophylactic irradiation to para-aortic lymph nodes in locally advanced cervical cancer necessary? Cancer Res Treat *46*: 374-382, 2014.

19 Kim YB, Cho JH, Keum KC, Lee CG, Seong J, Suh CO and Kim GE: Concurrent chemoradiotherapy followed by adjuvant chemotherapy in uterine cervical cancer patients with high-risk factors. Gynecol Oncol *104*: 58-63, 2007.

20 Miše BP, Jelavić TB, Strikic A, Hrepić D, Tomić K, Hamm W, Tomić S, Prskalo T and Vrdoljak E: Long follow-up of patients with locally advanced cervical cancer treated with concomitant chemobrachyradiotherapy with cisplatin and ifosfamide followed by consolidation chemotherapy. Int J Gynecol Cancer *25*: 315-319, 2015.

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