

# Different prognostic effects of nadirs of neutrophils and lymphocytes during radical (chemo)radiotherapy for cervical cancer

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DOI: [10.31083/j.ejgo4206184](https://doi.org/10.31083/j.ejgo4206184)

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Submitted: 17 August 2021 Revised: 15 September 2021 Accepted: 29 September 2021 Published: 15 December 2021

**Objective:** To study prognostic effects of nadirs of neutrophils and lymphocytes during radical (chemo)radiotherapy for cervical cancer. **Methods:** Patients with International Federation of Gynecology and Obstetrics (FIGO) IB1-IVA cervical cancer from January 2015 through December 2019 were retrospectively analyzed. All patients were treated with radical (chemo)radiotherapy and had available baseline and weekly complete blood counts with differentials before and during treatment. **Results:** A total of 107 patients were eligible. Receiver operating characteristic (ROC) curve determined the cutoff values predicting overall survival (OS) of nadirs of absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) during treatment were  $2.62 \times 10^9/L$  and  $0.2 \times 10^9/L$ , respectively. Compared with ANC nadir  $\leq 2.62 \times 10^9/L$  (N = 94), patients with ANC nadir  $> 2.62 \times 10^9/L$  (N = 13) had lower 2-year OS rate (51.9% vs 88.7%,  $p = 0.01$ ) and lower 2-year progression-free survival (PFS) rate (52.7% vs 80.8%,  $p = 0.003$ ); compared with ALC nadir  $> 0.2 \times 10^9/L$  (N = 65), patients with ALC nadir  $\leq 0.2 \times 10^9/L$  (N = 42) had lower 2-year OS rate (74.7% vs 90.0%,  $p = 0.004$ ) and lower 2-year PFS rate (67.8% vs 83.3%,  $p = 0.038$ ). Multivariate COX analysis showed that ANC nadir ( $> 2.62$  vs  $\leq 2.62 \times 10^9/L$ ) (hazard ratio (HR) 4.729, 95% confidence interval (CI) 1.355–16.505,  $p = 0.015$ ), ALC nadir ( $\leq 0.2$  vs  $> 0.2 \times 10^9/L$ ) (HR 4.463, 95% CI 1.616–12.321,  $p = 0.004$ ) were associated with OS. **Conclusions:** Peripheral ANC nadir and ALC nadir during radical (chemo)radiotherapy have different prognostic effects in predicting treatment outcome. ANC and ALC nadir during radical (chemo)radiotherapy may serve as a guide to treatment of cervical cancer.

## Keywords

Absolute lymphocyte count nadir; Absolute neutrophil count nadir; Cervical cancer; Prognosis; Radiotherapy

## 1. Introduction

Cervical cancer is common in women [1]. Definitive concurrent chemoradiotherapy (CCRT) is the preferred treatment for locally advanced cervical cancer [2]. However, the reported 5-year survival is less than 50% with CCRT [3]. It is possible to improve the prognosis of patients by optimizing the treatment modalities if predictive or prognostic factors

were revealed before or during treatment. Therefore, it is of great clinical significance to look for biomarkers related to efficacy or prognosis in routine clinical practice.

Immune system plays an important role in the development of malignant tumors [4] and is also essential for tumor killing and eradication. The body's immune response is necessary to prevent tumor recurrence after radical treatment for malignant tumors [5]. Neutrophil and lymphocyte are two major important components of immune system. Meta-analyses showed that neutrophil/lymphocyte ratio (NLR) before treatment was prognostic for cervical cancer [6]. Higher NLR correlated with poor survival [6] and worse response to neoadjuvant chemotherapy of cervical cancer [7]. Neutropenia and lymphopenia are common in cervical cancer patients during CCRT [8, 9]. High absolute neutrophil count (ANC) during CCRT was associated with poor local control and survival in cervical cancer patients [10]. Lymphopenia at baseline, during treatment and post treatment was associated with decreased survival in patients with cervical cancer [11, 12]. However, data on prognostic effects of neutrophil nadir and lymphocyte nadir remain scanty. Therefore, the authors aimed to study the prognostic significance of neutrophil nadir and lymphocyte nadir in patients with International Federation of Gynecology and Obstetrics (FIGO) IB1-IVA cervical cancer receiving radical radiotherapy with or without chemotherapy and to provide reference data for the future study on the optimal treatment modality according to the clinical parameters during treatment.

## 2. Methods

### 2.1 Patients

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics committee of the University

of Hong Kong-Shenzhen Hospital (NO: [2019]049) and individual consent for this retrospective analysis was waived. The authors retrospectively identified patients with newly diagnosed cervical cancer treated at the University of Hong Kong-Shenzhen Hospital from January 2015 through December 2019 for initial review. All patients provided informed consent form for anti-cancer treatments and for the use of personal medical data for academic research. According to departmental practice guideline since 2015, all patients had vaginal and rectal examination by experienced gynecologists, contrast magnetic resonance imaging (MRI) of pelvis (if no contraindication of MRI) and contrast computed tomography (CT) of thorax, abdomen and pelvis or positron emission tomography (PET)-CT for baseline staging. Serial complete blood counts (CBC) were obtained before RT and weekly during treatment. Patients were included in this study if they met the following criteria: (1)  $\leq 18$  years old, (2) performance status  $\leq 2$ , (3) pathology-confirmed cervical squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma, (4) FIGO (2018) stage IB1-IVA (FIGO (2009) before 2018 and FIGO (2018) since 2018 were used in clinical practice. Re-staging of the included patients before 2018 was performed based on FIGO (2018) by experienced oncologist before final analyses in this study), (5) initial treatment with external beam radiotherapy (RT) followed by brachytherapy with or without concurrent cisplatin or carboplatin, (6) had available CBC including differentials before RT and weekly during treatment. The ANC and absolute lymphocyte counts (ALC) measured as cells  $\times 10^9/L$ . Patients were not selected if they had adjuvant RT after surgery or had concomitant secondary primary malignant tumor.

## 2.2 Treatment

### 2.2.1 Radiation therapy

All patients received external beam RT followed by brachytherapy with or without concurrent platinum. Two external beam RT techniques, three-dimensional conformal radiotherapy (3DCRT) or RapidArc, were used. GTV (Gross Tumor Volume)-T was the primary tumor. GTV-N was the locoregional pathological lymph nodes. CTV (Clinical Target Volume)-T included cervix, uterus, bilateral parametrium and part of vagina. CTV-N was GTV-N + 3 mm margin. CTV-E encompassed regional lymphatics (common, internal iliac and external iliac, presacral  $\pm$  inguinal  $\pm$  para-aortic). ITV (Internal Target Volume)-T was CTV-T plus margins (anterior-posterior 10 mm, superior-inferior 10 mm and lateral 5 mm). ITV45 = ITV-T + CTV-N + CTV-E. PTV45 = ITV45 + 5 mm margin. PTV-N = CTV-N + 5 mm margin. Dose prescription: PTV45: 45 Gy/25 Fr in 1.8 Gy/Fr daily; using simultaneous integrated boost (SIB) to PTV-N: 55 Gy/25 Fr in 2.2 Gy/Fr daily if lymph nodes were within the pelvis, or 57.5 Gy/25 Fr in 2.3 Gy/Fr daily if lymph nodes were outside the pelvis. There were two sequential phases in 3DCRT technique: phase I, PTV45: 45 Gy/25 Fr to whole pelvis; phase II, boosting to pelvic wall, FIGO IIIB 16 Gy/8 Fr, FIGO IB1-IIIA 10 Gy/5 Fr. All external beam

RT was 5 Fr/week. Brachytherapy started 4–5 weeks after the start of external beam RT, under CT or MRI guidance,  $^{192}\text{Ir}$  (iridium) high dose rate, 6 Gy to point A, once a week for a total of 4 times. Cumulative equivalent of  $\sim 80$ –85 Gy was set for point A.

### 2.2.2 Chemotherapy

Chemotherapy was delivered concurrently with external beam RT, however not recommended to patients aged over 70 or FIGO stage IB1. Patients received cisplatin (40 mg/m<sup>2</sup>) weekly, up to 5–6 weeks. Carboplatin (Area Under the Curve (AUC) = 2 mg/mL/min) weekly was used as an alternative if creatinine clearance  $\leq 50$  mL/min. CBC was obtained weekly prior to administration of chemotherapy. If ANC  $< 1.5 \times 10^9/L$  or platelet  $< 100 \times 10^9/L$ , chemotherapy was withheld until the recovery of bone marrow function. Granulocyte colony-stimulating factor (G-CSF) was administered when ANC  $< 1.5 \times 10^9/L$  at the discretion of the treating physicians. In cases involving long RT waiting time, induction chemotherapy with paclitaxel plus carboplatin was given.

### 2.3 Statistical analysis

Statistical analyses were conducted by R version 3.6.1 (R Core Team, Vienna, Austria) and SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA) statistical software. Descriptive statistics were used to demonstrate patient demographics, tumor clinicopathologic factors and treatment related factors. Overall survival (OS) was defined as the time between the start of RT and the date of death from any cause or the last date with confirmed survival. The Progression-free survival (PFS) was defined as the time between the start of RT and the date of the first occurrence of progressive disease or death from any cause. The authors performed OS and PFS analyses in different ANC and ALC nadir subgroups using Kaplan-Meier curves with the log-rank test. Univariate and multivariate Cox regression was used to investigate the potential prognostic factors of OS and PFS. Univariate and multivariate linear regression were used to analyze variables associating with ANC and ALC nadir. Receiver operating characteristic (ROC) curves were constructed to find out the cut-off values of ANC nadir and ALC nadir for OS prediction and pre-RT ANC and ALC for predicting ANC nadir  $> 2.62 \times 10^9/L$  and ALC nadir  $> 0.2 \times 10^9/L$  during RT, respectively.  $p$  values less than 0.05 was considered clinically significant.

## 3. Results

### 3.1 Patient characteristics

From January 2015 to December 2019, one hundred and seven patients were included in this study. Thirty-two patients (29.9%) were older than 60 years old. One hundred and three (96.3%) had squamous cell carcinoma. Seventy-four patients (69.2%) had FIGO stage III-IVA. Eighteen patients (16.8%) received induction chemotherapy before RT. Fifteen patients (14.0%) had RT alone and seventy-three (68.2%) patients received at least 5 cycles of weekly concurrent platinum. Table 1 summarizes patients' clinical characteristics.

**Table 1. Patients' clinical characteristics.**

Characteristic	No. (%)
All pts (Median (IQR))	52 (46–63)
Age	
≤60	75 (70.1)
>60	32 (29.9)
ECOG	
0–1	94 (87.9)
2	13 (12.1)
FIGO stage	
IB1–IIB	33 (30.8)
IIIA–IVA	74 (69.2)
Histology	
Squamous	103 (96.3)
Non-squamous	4 (3.7)
Concurrent platinum cycles	
0–4	34 (31.8)
5–6	73 (68.2)
Induction chemotherapy	
Yes	18 (16.8)
No	89 (83.2)
EBRT techniques	
3DCRT	27 (25.2)
RapidArc	80 (74.8)
Pre-RT CBC (Median (IQR))	
WBC ( $\times 10^9/L$ )	6.8 (5.5–8.1)
ANC ( $\times 10^9/L$ )	4.4 (3.3–5.7)
ALC ( $\times 10^9/L$ )	1.7 (1.3–2.0)
PLT ( $\times 10^9/L$ )	268 (218–330)
HGB (g/L)	119 (103–131)
During-RT CBC nadir (Median (IQR))	
WBC ( $\times 10^9/L$ )	2.1 (1.6–3.0)
ANC ( $\times 10^9/L$ )	1.3 (1.0–2.0)
ALC ( $\times 10^9/L$ )	0.22 (0.17–0.30)
PLT ( $\times 10^9/L$ )	98 (72–150)
HGB (g/L)	92 (79–101)

ECOG, Eastern Cooperative Oncology Group; FIGO, Federation of Gynecology and Obstetrics; EBRT, external beam radiotherapy; 3DCRT, 3-dimensional conformal radiation therapy; CBC, complete blood count; WBC, white blood cell; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; PLT, platelet; HGB, hemoglobin; IQR, interquartile range.

### 3.2 ANC nadir and ALC nadir were associated with survivals

Two-year OS and PFS for the whole population were 84.0% and 77.4% respectively after a median follow-up of 29.7 months (range, 3.0–66.3). According to the time-dependent ROC, the cutoff values of ANC nadir and ALC nadir for OS prediction was  $2.62 \times 10^9/L$  and  $0.2 \times 10^9/L$ , respectively. Compared with ANC nadir  $\leq 2.62 \times 10^9/L$  ( $N = 94$ ), patients with ANC nadir  $> 2.62 \times 10^9/L$  ( $N = 13$ ) had lower 2-year OS rate (51.9% vs 88.7%,  $p = 0.01$ ) and lower 2-year PFS rate (52.7% vs 80.8%,  $p = 0.003$ ) (Fig. 1A,B). Patients with ALC nadir  $\leq 0.2 \times 10^9/L$  ( $N = 42$ ) had lower 2-year OS rate

(74.7% vs 90.0%,  $p = 0.004$ ) and lower 2-year PFS rate (67.8% vs 83.3%,  $p = 0.038$ ) than patients with ALC nadir  $> 0.2 \times 10^9/L$  ( $N = 65$ ) (Fig. 1C,D).

### 3.3 Univariate and multivariate Cox regression on OS and PFS

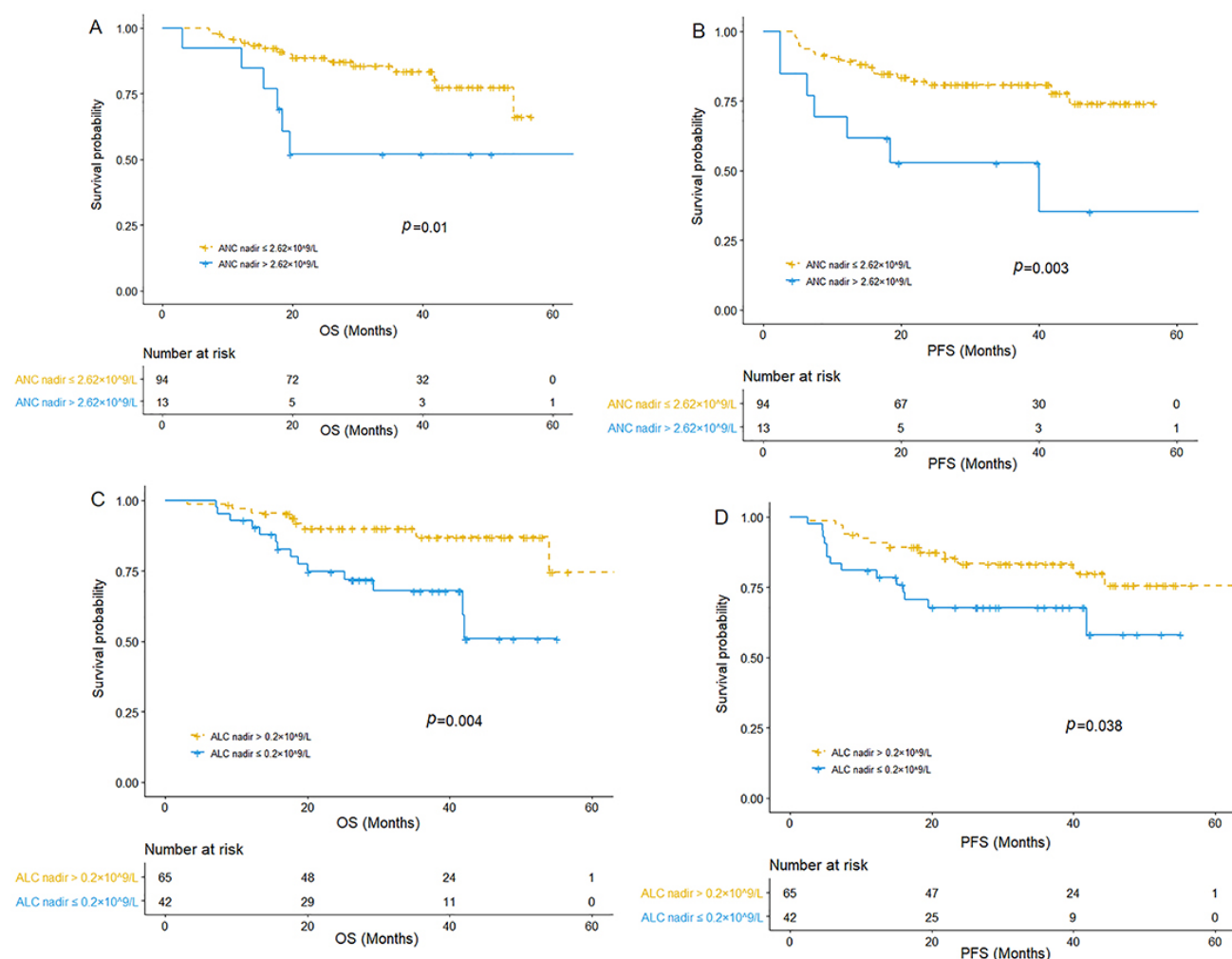
The impact of prognostic factors on treatment results by univariate and multivariate analysis was shown in Table 2.

Both univariate and multivariate analyses demonstrated that ANC nadir and ALC nadir were significant prognostic factors for predicting OS and PFS. ANC nadir  $> 2.62 \times 10^9/L$  and ALC nadir  $\leq 0.2 \times 10^9/L$  were associated with poorer OS (hazard ratio (HR) 4.729, 95% confidence interval (CI) 1.355–16.505,  $p = 0.015$ ; and HR 4.463, 95% CI 1.616–12.321,  $p = 0.004$ ; respectively) and shorter PFS (HR 4.055, 95% CI 1.375–11.962,  $p = 0.011$ ; and HR 3.926, 95% CI 1.570–9.816,  $p = 0.003$ ; respectively) after adjustment in the multivariate Cox regression models. In addition, pre-RT ANC was also found to be of prognostic value (HR 1.109, 95% CI 1.008–1.220,  $p = 0.033$ ) in multivariate model. Other studied prognosticators, including age, ECOG, FIGO stage, histology, induction chemotherapy, concurrent platinum cycles, RT techniques, pre-RT hemoglobin and pre-RT ALC, did not have statistically significant effects on survival in univariate or multivariate Cox regression models.

### 3.4 Characteristics of ANC nadir and ALC nadir during RT

The median (interquartile range (IQR)) pre-RT CBC and CBC nadir during RT were shown in Table 1. The median (IQR) pre-RT ANC, pre-RT ALC, ANC nadir and ALC nadir during RT for the whole population were  $4.4 (3.3–5.7) \times 10^9/L$ ,  $1.7 (1.3–2.0) \times 10^9/L$ ,  $1.3 (1.0–2.0) \times 10^9/L$  and  $0.22 (0.17–0.30) \times 10^9/L$ , respectively. Paired  $t$  test showed that ANC ( $t = -11.503$ ,  $p = 0.000$ ) and ALC ( $t = -27.295$ ,  $p = 0.000$ ) decreased significantly during RT compared with those before RT. The median time from the start of RT to ANC nadir and ALC nadir were 38 days (IQR, 32–48) and 34 days (IQR, 28–41). Univariate and multivariate linear regressions showed that  $\geq 5$  cycles of concurrent platinum significantly associated with lower ANC nadir (odds ratio (OR)  $-0.587$ , 95% CI  $-0.939$ – $-0.235$ ,  $p = 0.001$ ) and ALC nadir (OR  $-0.074$ , 95% CI  $-0.118$ – $-0.029$ ,  $p = 0.001$ ) (Table 3). In addition, pre-RT ANC was associated with ANC nadir (OR 0.088, 95% CI 0.040–0.135,  $p = 0.000$ ) and pre-RT ALC was associated with ALC nadir (OR 0.076, 95% CI 0.044–0.109,  $p = 0.000$ ). No other demographic or clinical factors correlated significantly with ANC nadir or ALC nadir in multivariate linear regression models. RT technique did not affect ANC or ALC nadirs.

According to the cutoff value predicting OS, ANC nadir was divided into two groups:  $> 2.62 \times 10^9/L$  and  $\leq 2.62 \times 10^9/L$  group; ALC nadir was divided into two groups:  $> 0.2 \times 10^9/L$  and  $\leq 0.2 \times 10^9/L$  group. ROC analysis of pre-RT ANC and pre-RT ALC showed that the cutoff value of pre-RT ANC was  $3.64 \times 10^9/L$  with AUC = 0.654 ( $p = 0.073$ ), sensitivity = 92.3% and specificity = 36.2%, which indicated that patients with pre-RT ANC  $> 3.64 \times 10^9/L$  were more



**Fig. 1. Kaplan–Meier curves of overall survival (OS) and progression-free survival (PFS).** (A) OS curve of patients with ANC nadir  $>2.62 \times 10^9/L$  ( $N = 13$ ) or  $\leq 2.62 \times 10^9/L$  ( $N = 94$ ) during treatment (2-yr 51.9% vs 88.7%,  $p = 0.01$ ). (B) PFS curve of patients with ANC nadir  $>2.62 \times 10^9/L$  ( $N = 13$ ) or  $\leq 2.62 \times 10^9/L$  ( $N = 94$ ) during RT (2-yr 52.7% vs 80.8%,  $p = 0.003$ ). (C) OS curve of patients with ALC nadir  $\leq 0.2 \times 10^9/L$  ( $N = 42$ ) or  $>0.2 \times 10^9/L$  ( $N = 65$ ) during RT (2-yr 74.7% vs 90.0%,  $p = 0.004$ ). (D) PFS curve of patients with ALC nadir  $\leq 0.2 \times 10^9/L$  ( $N = 42$ ) or  $>0.2 \times 10^9/L$  ( $N = 65$ ) during RT (2-yr 67.8% vs 83.3%,  $p = 0.038$ ).

likely to have ANC nadir  $>2.62 \times 10^9/L$  during RT (Fig. 2A). The cutoff value of pre-RT ALC was  $2.05 \times 10^9/L$  with AUC = 0.743 ( $p = 0.000$ ), sensitivity = 38.5% and specificity = 97.6%, which implied that patients with pre-RT ALC  $>2.05 \times 10^9/L$  were more likely to have ALC nadir  $>0.2 \times 10^9/L$  during RT (Fig. 2B).

#### 4. Discussion

Immune system plays an important role in the fight against tumor. In 2013, Chen and Mellman demonstrated cancer-immune cycle and revealed the mechanism of killing tumor cells by immune system [13]. This study found that patients with ANC nadir  $>2.62 \times 10^9/L$  or ALC nadir  $\leq 0.2 \times 10^9/L$  during RT had worse OS and PFS. Previous study showed that patients with higher neutrophil levels one week after the start of CCRT had worse local control and survival [10]. Lymphopenia at baseline, during treatment and post

treatment was associated with decreased survival in patients with cervical cancer [11, 12]. The findings of this study were consistent with those of previous studies.

Neutrophils are the major components of blood leukocytes and are part of the earliest cellular components in the body to fight against tissue injury and infection [14]. Neutrophils exist in the tumor micro-environment [14] and tumor-associated neutrophil signatures were predictors of survival for diverse solid tumors [15]. Wisdom *et al.* [10] reported that neutrophils promoted tumor resistance to radiation therapy. In 1233 patients undergoing radiotherapy, baseline ANC  $>7 \times 10^9/L$  was associated with worse OS [16]. Compared to RT alone, CCRT increased locoregional tumor control and survival in a variety of cancer types, at the same time, caused a decline in neutrophil count [17]. Likewise, this study showed that ANC decreased significantly during RT, and patients who received 5 or more cy-



**Table 2. Univariate and multivariate analysis of potential factors associated with the overall survival and progression-free survival for all 107 patients.**

Variable	Overall survival				Progression-free survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
Age (>60/≤60 years old)	0.438 (0.148–1.298)	0.136	NI		0.611 (0.245–1.522)	0.290	NI	
ECOG (2/0–1)	1.307 (0.386–4.430)	0.667	NI		1.052 (0.315–3.508)	0.935	NI	
FIGO stage (IIIA–IVA/IB1–IIB)	2.994 (0.885–10.130)	0.078	NI		1.573 (0.631–3.921)	0.331	NI	
Histology (Squamous/Non-squamous)	0.680 (0.091–5.081)	0.707	NI		0.953 (0.129–7.055)	0.962	NI	
Induction chemotherapy (Yes/No)	0.781 (0.180–3.393)	0.742	NI		0.505 (0.119–2.152)	0.356	NI	
Concurrent platinum cycles (5–6/0–4)	0.406 (0.174–0.947)	0.037	0.449 (0.153–1.319)	0.145	0.419 (0.193–0.911)	0.028	0.487 (0.198–1.199)	0.118
EBRT techniques (3DCRT/RapidArc)	1.263 (0.514–3.103)	0.610	NI		1.220 (0.531–2.804)	0.639	NI	
Pre-RT HGB (Continuous)	0.972 (0.949–0.995)	0.017	0.974 (0.948–1.001)	0.056	0.984 (0.963–1.004)	0.123	NI	
Pre-RT ANC (Continuous)	1.098 (1.007–1.198)	0.035	1.109 (1.008–1.220)	0.033	1.072 (0.985–1.168)	0.109	NI	
Pre-RT ALC (Continuous)	0.583 (0.270–1.260)	0.170	NI		0.704 (0.354–1.401)	0.317	NI	
ANC nadir (>2.62/≤2.62 × 10 <sup>9</sup> /L)	3.228 (1.260–8.274)	0.015	4.729 (1.355–16.505)	0.015	3.432 (1.440–8.177)	0.005	4.055 (1.375–11.962)	0.011
ALC nadir (≤0.2/>0.2 × 10 <sup>9</sup> /L)	3.344 (1.385–8.071)	0.007	4.463 (1.616–12.321)	0.004	2.224 (1.023–4.833)	0.044	3.926 (1.570–9.816)	0.003

ECOG, Eastern Cooperative Oncology Group; FIGO, Federation of Gynecology and Obstetrics; EBRT, external beam radiotherapy; 3DCRT, 3-dimensional conformal radiation therapy; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; HGB, hemoglobin; HR, hazard ratio; CI, confidence interval; NI, not included.

**Table 3. Univariate and multivariate linear regression associating variables with ANC nadir and ALC nadir during radiotherapy.**

Variables	ANC nadir (×10 <sup>9</sup> /L)				ALC nadir (×10 <sup>9</sup> /L)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Age (>60/≤60 years old)	0.399 (0.040–0.759)	0.030	0.178 (–0.190–0.546)	0.340	0.089 (0.043–0.135)	0.000	0.030 (–0.016–0.076)	0.202
ECOG (2/0–1)	0.162 (–0.352–0.676)	0.533	NI		0.033 (–0.035–0.101)	0.341	NI	
FIGO stage (IIIA–IVA/IB1–IIB)	–0.086 (–0.450–0.278)	0.641	NI		–0.035 (–0.084–0.013)	0.147	NI	
Histology (Squamous/Non-squamous)	–0.135 (–1.021–0.751)	0.763	NI		0.036 (–0.082–0.154)	0.545	NI	
Concurrent platinum cycles (5–6/0–4)	–0.519 (–0.866– –0.172)	0.004	–0.587 (–0.939– –0.235)	0.001	–0.075 (–0.121– –0.029)	0.002	–0.074 (–0.118– –0.029)	0.001
EBRT techniques (3DCRT/RapidArc)	–0.118 (–0.504–0.269)	0.548	NI		–0.009 (–0.061–0.043)	0.731	NI	
Pre-RT HGB (Continuous)	0.003 (–0.006–0.012)	0.476	NI		0.002 (0.000–0.003)	0.010	0.001 (0.000–0.002)	0.129
Pre-RT ANC (Continuous)	0.080 (0.032–0.128)	0.001	0.088 (0.040–0.135)	0.000	0.000 (–0.007–0.006)	0.907	NI	
Pre-RT ALC (Continuous)	0.355 (0.092–0.618)	0.009	0.201 (–0.062–0.464)	0.133	0.088 (0.056–0.120)	0.000	0.076 (0.044–0.109)	0.000

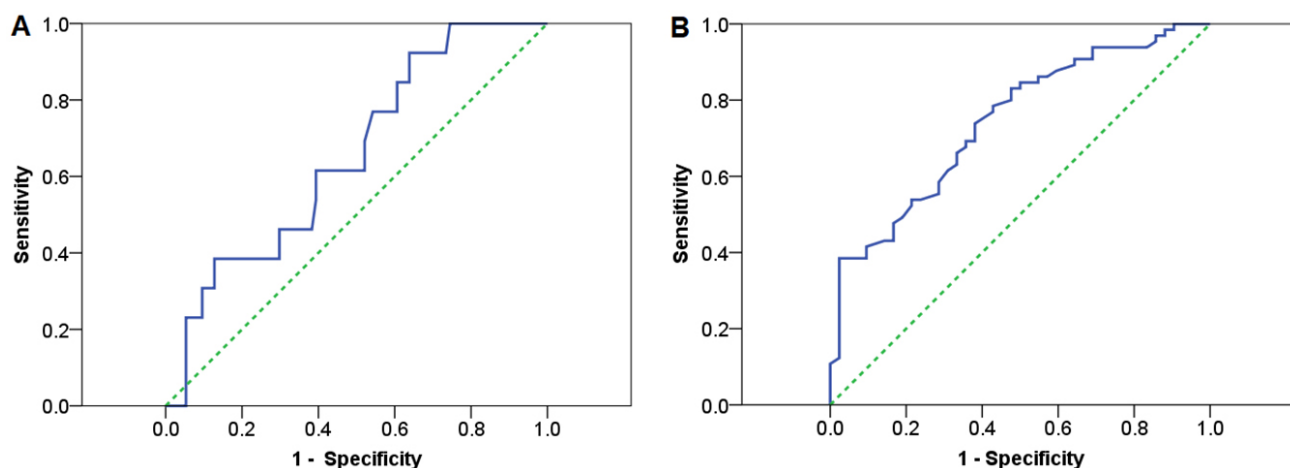
ECOG, Eastern Cooperative Oncology Group; FIGO, Federation of Gynecology and Obstetrics; EBRT, external beam radiotherapy; 3DCRT, 3-dimensional conformal radiation therapy; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; HGB, hemoglobin; OR, odds ratio; CI, confidence interval; NI, not included.

cles of platinum chemotherapy had lower ANC nadir during RT. The hypothesis is that decreased neutrophil count may contribute to the positive effects of adding concurrent chemotherapy to RT on treatment outcome, as high levels of neutrophil in peripheral blood during RT may increase radiation resistance and promote tumor recurrence.

In this study increased number of cycles of weekly platinum showed a tendency to correlate with better OS and PFS. In clinical practice, when ANC <1.5 × 10<sup>9</sup>/L during CCRT, chemotherapy is usually withheld until ANC ≥1.5 × 10<sup>9</sup>/L. In order to give sufficient chemotherapy during CCRT period, clinicians usually administer G-CSF to achieve rapid neutrophil recovery rather than watchful waiting. However, some research showed that G-CSF may affect the prognosis

of cancer patients. In a prospective trial for head and neck cancer, patients who received prophylactic G-CSF during treatment had significantly poorer locoregional tumor control [18]. Studies in cervical cancer showed that increased production of G-CSF from tumor cells was associated with resistance to CCRT or RT alone [19, 20]. Therefore, there was a paradox between G-CSF injection to increase the level of ANC to meet the requirements for chemotherapy and the radiation resistance induced by G-CSF and ANC, which should be taken into consideration by physicians when making decision on G-CSF prescription.

Lymphocytes are the main effector cells for killing tumor cells and lymphopenia is consistently reported as an adverse prognostic factor for survival in a variety of solid tumors



**Fig. 2. ROC curves of pre-RT ANC and ALC.** (A) ROC curve of pre-RT ANC predicting ANC nadir  $>2.62 \times 10^9/\text{L}$  during RT, cutoff value =  $3.64 \times 10^9/\text{L}$ , AUC = 0.654 ( $p = 0.073$ ), sensitivity = 92.3%, specificity = 36.2%. (B) ROC curve of pre-RT ALC predicting ALC nadir  $>0.2 \times 10^9/\text{L}$  during RT, cutoff value =  $2.05 \times 10^9/\text{L}$ , AUC = 0.743 ( $p = 0.000$ ), sensitivity = 38.5%, specificity = 97.6%.

[21, 22]. The present study also demonstrated that ALC nadir  $\leq 0.2 \times 10^9/\text{L}$  during RT predicted poor survival for cervical cancer patients. Lymphocytes are extremely sensitive to radiation. The LD<sub>50</sub> of lymphocytes (lethal dose required to reduce the survival rate of lymphocytes by 50%) is 2 Gy and LD<sub>90</sub> is only 3 Gy [23]. Some research showed that the role chemotherapy played in lymphopenia during chemoradiation was not significant. It was reported that concurrent cisplatin could not change the degree and characteristics of radiation induced immunosuppression in patients with cervical cancer [24]. There were similar results in non-small cell lung cancer (NSCLC) [25]. In NSCLC patients treated with different regimens as neoadjuvant and concurrent chemotherapy, the ALC did not decrease significantly after two cycles of neoadjuvant chemotherapy and the changes of ALC during chemoradiation were similar with different concurrent chemotherapy regimens [25]. However, in this study patients with 5–6 cycles of weekly platinum were more likely to have lower ALC nadir than those with fewer than 5 cycles of weekly platinum. A study assessed in vitro effects of platinum compounds on lymphocyte proliferation and demonstrated that certain platinum salts affected lymphocyte proliferation [26]. It is worthwhile to study the effect of chemotherapy on immunosuppression to assist in the choice of chemotherapy regimens. It was reported that radiation dose and radiation techniques may affect the risk and severity of radiation-induced lymphopenia [27]. Therefore, the risk and severity of lymphopenia might be decreased by optimizing radiation parameters.

There are some limitations in this retrospective study. First, the time points of blood tests were not completely consistent. Although in clinical practice CBC should be tested before RT and weekly during RT, the blood test time schedules will be disrupted once chemotherapy was withheld due to bone marrow suppression. As the treatment of bone mar-

row suppression and decision of chemotherapy delay was decided completely by the clinician in charge, there were some variations in the time points of blood tests. Second, once the ANC  $<1.5 \times 10^9/\text{L}$ , the clinician in charge usually prescribed G-CSF with the aim to make the ANC  $\geq 1.5 \times 10^9/\text{L}$ , which affected the trends of ANC changes during treatment. Third, some research showed that G-CSF may affect the prognosis of cancer patients as mentioned previously in this discussion. As there was no standard use of G-CSF in this study, it is difficult to evaluate the impact of use of G-CSF on the results of this study. Fourth, some confounding factors of bone marrow suppression and survival, such as age, induction chemotherapy, and the cycles of concurrent platinum, might impact the interpretation of the results in this study. However, we took into account all these 3 factors in the COX regression analysis. Our main results that ANC nadir and ALC nadir during radical (chemo)radiotherapy were prognostic factors for survival were consistent with previous studies as we described in the introduction section [6, 10–12]. Finally, with regard to the power of this study, we calculated the power using the following method: A Cox regression of the log hazard ratio on a covariate with a standard deviation of 0.5000 based on a sample of 107 observations achieves 92% power at a 0.05000 significance level to detect a regression coefficient equal to 1.4960. The sample size was adjusted since a multiple regression of the variable of interest on the other covariates in the Cox regression was expected to have an R-Squared of 0.1100. The sample size was adjusted for an anticipated event rate of 0.2100. To validate the prognostic significance of these commonly used clinical parameters, prospective clinical trials with larger sample size are needed.

## 5. Conclusions

Peripheral blood ANC and ALC, which are widely available inexpensive tests and convenient for dynamic mon-

itoring, are potential prognostic biomarkers during radical (chemo)radiotherapy for cervical cancer patients. ANC nadir and ALC nadir in peripheral blood during radical (chemo)radiotherapy have different prognostic effects. In order to improve the prognosis of cervical cancer patients treated with radical (chemo)radiotherapy, clinicians can try to optimize treatment plan with consideration of the change of peripheral blood ANC and ALC.

## Author contributions

ZYX, LY designed the research study. ZYX, LY, and QL performed the research. LY and ZYX analyzed the data. LY, QL wrote the manuscript. ZYX revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All subjects gave their informed consent form for the use of personal medical data for academic research purpose before treatment and individual consent for inclusion before they participated in the study was waived. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University of Hong Kong-Shenzhen Hospital (approval number: [2019]049).

## Acknowledgment

The authors thank those doctors, nurses, therapists and physicists of the department for their help in finishing this work.

## Funding

This project was supported by the grants from Health Commission of Guangdong Province, China (Grant No.: B2020100); HKUSZH Seed Funding, High Level-Hospital Program, Health Commission of Guangdong Province, China (Grant No.: HKUSZH201902031); and Shenzhen Key Medical Discipline Construction Fund (Grant No.: SZXK014).

## Conflict of interest

The authors declare no conflict of interest.

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