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Original Research Article

The predictive value of nadir neutrophil count during treatment of cervical cancer: Interactions with tumor hypoxia and interstitial fluid pressure (IFP)

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ABSTRACT

Background and purpose: Hypoxia, high interstitial fluid pressure (IFP) and immune effects have individually been shown to modulate radiotherapy (RT) response in cervical cancer. The aim of this study was to investigate the interplay between hypoxia or IFP and circulating neutrophil levels, and their combined effect on survival following RT.
Material and methods: A total of 287 FIGO stage IB to IIIB cervical cancer patients treated with RT or RT and cisplatin (RTCT) were included. Tumor hypoxia and IFP were measured at baseline prior to treatment. Absolute neutrophil count (ANC) was measured at baseline and weekly during treatment. Median follow up was 7.1 years.
Results: High nadir ANC at the point of maximal myelosuppression was a stronger predictor of inferior survival than high baseline ANC after adjusting for clinical prognostic factors and treatment (RT vs. RTCT). The predictive effect of nadir ANC was most evident in patients with well-oxygenated tumors or tumors with high IFP at diagnosis.
Conclusions: This study provides new information about the combined influence of the tumor microenvironment and myeloid cells on the survival of cervical cancer patients treated with RT/RTCT to motivate the development of new treatments based on molecular targeting of immune–based radioresistance pathways.

Introduction

Cervical cancer is a global health problem [1]. At diagnosis, many patients have locally extensive disease with or without lymph node involvement, but nevertheless may be cured with radiotherapy (RT) and concurrent cisplatin chemotherapy (RTCT) [2]. Local control of the primary tumor has improved in recent years because of radiation dose escalation facilitated by better imaging and more targeted treatment delivery [3]. Nevertheless, the 5-year survival rate remains in the range of 60–70%, and only about 20% of patients are alive five years after a diagnosis of recurrence [3,4]. This highlights the importance of developing new strategies for targeting both radiation resistance pathways and occult metastatic disease.

The tumor microenvironment is recognized as an important determinant of tumor behavior and response to treatment in patients with cervical cancer [5–9]. Tumor hypoxia and high interstitial fluid pressure (IFP), both functional consequences of unregulated angiogenesis, abnormal stromal content and high tumor cell density, are independent predictors of local progression and the

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development of metastases in patients receiving potentially curative radiotherapy [8]. In addition, cervical tumors frequently contain immune cells, including lymphocytes, monocytes, macrophages, neutrophils and myeloid derived suppressor cells (MDSCs), which also influence disease behavior in complex and dynamic ways [10]. In particular, several recent studies have implicated pretreatment circulating and tumor-associated neutrophil (TANs) levels as being important predictors of cervical cancer recurrence following radiotherapy [11–15]. Furthermore, treatment-induced accumulation of TANs and MDSCs during radiotherapy has been shown in gliomas and other cancers to promote radiation resistance and early disease progression [16–18]. Preclinical studies have suggested biologically important interactions between hypoxia (or IFP) and the immune cell environment that promote cancer development, progression and treatment resistance [19]. However, there have been very few clinical studies exploring these relationships directly in patients, and none to our knowledge in cervical cancer.

The aim of this study was to investigate the interplay between circulating neutrophil levels before and during RT or RTCT and primary tumor hypoxia or IFP in a large cohort of cervical cancer patients with long follow-up.

Materials and methods

Study population

The study cohort was comprised of 287 patients with FIGO stage IB to IIIB (TNM cT1b-T3b, N0-1, M0) cervical squamous cell carcinoma or adenocarcinoma diagnosed and treated with radiotherapy between 1994 and 2010 at the Princess Margaret Cancer Centre. All had participated in a prospective, REB-approved clinical study of the effect of tumor hypoxia and IFP on outcome. Staging investigations included examination under anesthesia (EUA), chest X-ray or chest CT, abdominal/pelvic CT and pelvic MR. Tumor size was defined as the maximum linear dimension from EUA or MR. Pelvic and para-aortic lymph nodes were classified as positive for metastatic disease if the short axis nodal dimension was >1 cm on CT or MR [20].

None of the patients had hematological comorbidities apart from anemia due to bleeding. The characteristics of the patients are summarized in Table 1.

Patient treatment and follow-up

External beam RT was delivered to the pelvis (183 patients) or pelvis and para-aortic region (104 patients), depending on the results of staging, using a planned dose of 45–50.4 Gy in 1.8–2 Gy daily fractions with 18–25 MV photons. External beam RT was followed by two-dimensional intrauterine brachytherapy to a dose of 35–40 Gy using a pulsed dose rate technique. The mean International Commission on Radiation Units and Measures (ICRU) Point-A dose was 87 Gy from external beam and brachytherapy. All patients were treated prior to the current era of image-guided intracavitary and interstitial brachytherapy. Patients treated after February 1999 received cisplatin 40 mg/m^2 administered weekly during external beam RT. Suitability for chemotherapy was assessed weekly prior to each cycle. Cisplatin was withheld if the absolute neutrophil count (ANC) was $<1.5 \times 10^9$/L or the platelet count was $<100 \times 10^9$/L and reassessed the following week. All patients completed 3–5 weekly cycles of chemotherapy. Adjuvant or neoadjuvant chemotherapy was not used.

Patients were assessed every 3 months for the first 2 years following treatment, and every 4–6 months during years 3, 4 and 5. The median follow-up was 7.1 years (range 0.5–19.0 years).

Table 1
Patient characteristics.

Attribute	Group	All eligible patients (n = 287)
Age	Median and range (cm)	51.1 (19.5–78.7)
FIGO Stage	IB/IIA	93 (32%)
	IIB/IIIA	97 (34%)
	IIIB	97 (34%)
Histology	Squamous	224 (78%)
	Adenocarcinoma	40 (14%)
	Other	23 (8%)
Tumor Size (Missing n = 8)	<5 cm	95 (34%)
	≥5 cm	184 (66%)
Pelvic Lymph Node Status	Negative	190 (66%)
	Positive	97(34%)
Hemoglobin Concentration	≤120 g/l	93 (32%)
	>120 g/l	194 (68%)
Treatment Type	RT alone	90 (31%)
	RT + cisplatin (RTCT)	197 (69%)
HP_5 (Missing n = 23)	Median and range (%)	55 (0–99)
IFP (Missing n = 28)	Median and range (mmHg)	18 (−3 to 59)
Baseline ANC (Missing n = 10)	Median and range ($\times 10^9$/L)	5.2 (0.9–19.5)
Nadir ANC (Missing n = 10)	Median and range ($\times 10^9$/L)	2.5 (0.3–8.5)

FIGO, International Federation of Gynecologists and Obstetricians.
IFP, Interstitial fluid pressure.
RT, Radiotherapy.
RTCT, Radiotherapy + weekly cisplatin.

Measurement of tumor hypoxia and IFP

Pretreatment tumor hypoxia and IFP were measured transvaginally during EUA using polarographic and hydraulic needle-based approaches respectively, as described previously [8,21]. Multiple, spatially separated measurements were made in each tumor to account for heterogeneity. The hypoxic fraction (HP_5 – the percentage of the measurements in each patient <5 mmHg) ranged from 0% to 99% in individual tumors, and the grand median was 55%. The mean IFP in individual tumors was between −3 and 59 mmHg and the median across all tumors was 18.0 mmHg. There was no correlation between HP_5 and IFP.

Measurement of absolute neutrophil count (ANC)

Absolute neutrophil counts (ANCs) were measured at baseline and weekly during external beam RT as part of routine clinical practice. The baseline ANC was defined as the one closest to the start of RT and prior to the first dose of cisplatin, within a window from 30 days before to six days after the start of RT. A total of 277 patients had a baseline ANC that met this definition, and 71% were within six days of the first RT fraction. ANCs were also measured weekly during external beam RT, with 95% having at least three measurements. The nadir ANC was defined as the lowest value over the course of external beam RT.

Measurement of tumor-associated neutrophils (TANs)

In addition to blood counts, pretreatment TANs were assessed histologically in a subset of 93 patients with available paraffin-embedded tumor biopsies. Two gynecologic oncology pathologists (BC and KS) who were blinded to the hypoxia and IFP measurements reviewed the hematoxylin and eosin (H&E) stained sections. The presence of neutrophils based on the characteristic appearance of multi-lobed nuclei and pink cytoplasm were noted. Cases with any visible neutrophils were classified as positive for neutrophil infiltration.