Radiotherapy and Oncology 80 (2006) 132—137
www.thegreenjournal.com

Hypoxic markers

Long-term performance of interstial fluid pressure and hypoxia as prognostic factors in cervix cancer☆

Anthony Fyles*, Michael Milosevic, Melania Pintilie, Ami Syed, Wilf Levin, Lee Manchul, Richard P. Hill

Departments of Radiation Oncology, Clinical Study Coordination and Biostatistics, and Advanced Molecular Oncology, Princess Margaret Hospital/Ontario Cancer Institute, Canada, Departments of Radiation Oncology and Medical Biophysics, University of Toronto, Toronto, Canada

Abstract

***Objectives*: Hypoxia and high interstitial fluid pressure (IFP) have been shown to independently predict for nodal and distant metastases, as well as survival, in patients with cervix cancer. Using data from our prospective trial, we updated a cohort of patients treated with definitive radiation alone without chemotherapy, to assess the long-term prognostic impact of these microenvironmental features.**

***Methods*: Between April 1994 and January 1999, 107 eligible patients with cervix cancer were entered into a prospective study of tumor oxygenation and IFP prior to primary radiation therapy. Oxygenation data are presented as the hypoxic proportion, defined as the percentage of pO_2 readings <5 mm Hg (abbreviated as HP_5). Patients with HP_5 values >50% were considered to have hypoxic tumors. IFP is presented in mm Hg, divided into high and low IFP groups by the median value.**

Patients ranged in age from 23 to 78 years with a mean of 53 years. The maximum tumor size ranged from 2 to 10 cm, with a median diameter of 5 cm. FIGO stage was IB in 28 patients, IIA in 4, IIB in 42 and IIIB in 33 patients. Twenty-two patients (21%) had evidence of pelvic lymph node involvement on staging CT abdomen/pelvis or MR pelvis. HP_5 ranged from 0% to 99% with a median of 48%. IFP ranged from −3 to 48 mm Hg (median 19 mm Hg). Median follow-up was 6.7 years (range 0.9—10.6).

***Results*: Disease-free survival (DFS) at 5 years was 50%. Five year DFS was 42% for patients with hypoxic tumors (HP_5 > 50%), and 58% in patients with oxygenated tumors (HR 1.01 per %, $p = 0.05$). DFS at 5 years was 42% for patients with interstitial hypertension (IFP >19 mm Hg), and 63% in patients with IFP $\leqslant$19 mm Hg (HR 1.05 per mm Hg, $p = 0.001$). In a multivariate analysis only tumor size (HR 1.2, $p = 0.009$) pelvic nodal metastases (HR 3.3, $p = 0.0004$) and IFP (HR 1.06, $p = 0.0005$) were predictive of DFS. Because an interaction between nodal status and oxygenation was observed ($p = 0.03$), further analysis indicated a borderline significant impact of HP_5 in addition to tumor size in node negative patients (HR 1.01, $p = 0.06$). These results were similar when local or distant relapse was used as an endpoint.**

***Conclusions*: These results confirm our initial finding of the strong independent prognostic impact of IFP for relapse and survival in patients with cervix cancer. In contrast, the independent prognostic impact of HP_5 is of borderline significance and is limited to patients without imaging evidence of nodal metastases. However, these findings do not diminish the biologic significance of hypoxia, or the role of hypoxia and IFP as biomarkers of treatment response and as therapeutic targets.**

Keywords: Hypoxia; Interstitial fluid pressure; Angiogenesis; Cervix cancer

The tumor microenvironment has profound effects on malignant behaviour and treatment outcome following cancer therapy in multiple tumor types. Numerous studies have reported the impact of hypoxia [1—5], but less is known about the prognostic significance of elevated interstitial fluid pressure (IFP) [6]. In women with cervix cancer, hypoxia has generally been shown to have prognostic impact for survival following either surgery or radiation, consistent with its relationship to angiogenesis and metastasis [7,8]. In contrast, the relationship between hypoxia and pelvic control

☆ This work was supported by a Program Project grant from the National Cancer Institute of Canada with funds from the Terry Fox Run.

0167-8140/$ - see front matter doi:10.1016/j.radonc.2006.07.014

following radiation has been less clear, as it is strongly influenced by tumor volume.

IFP is elevated in tumors as a result of both the abnormal microvasculature that arises from unregulated angiogenesis, and a lack of functional lymphatics. The capillaries in tumors are structurally and functionally abnormal: they are often incompletely formed, arranged in chaotic patterns and hyper-permeable to fluid and serum proteins [9]. These anatomic and structural abnormalities contribute to high geometric and viscous vascular resistance [10,11], and impaired oxygen delivery [12]. Although lymphatic vessels may be present, they are usually compressed or otherwise functionally incapacitated [13]. High levels of VEGF-C, which is an important stimulus for lymphatic development, have been documented in animal and human tumors and have been related to poor treatment outcome in a number of malignancies, including cervix and head and neck cancers [14]. Overall, these abnormalities lead to a situation where excess fluid leaks from the vasculature, accumulates in the interstitium and distends the elastic interstitial matrix causing IFP to rise.

At Princess Margaret Hospital (PMH), a prospective trial evaluating these microenvironmental parameters in women with cervix cancer has been underway since 1994. Initial results with a median follow-up of 2.5 years showed a profound independent impact of interstitial hypertension on both relapse and survival for all patients, whereas the independent effect of hypoxia was limited to patients with negative nodes on imaging studies [1]. The results of this study were recently updated to reflect a median patient follow-up of almost 7 years. This report describes the importance of these microenvironmental parameters on tumor control with radiotherapy and long-term patient survival.

Methods

This was a single institution prospective study that included newly diagnosed women with grossly evident, biopsy proven carcinoma of the cervix, in whom definitive radiation therapy alone was the planned treatment. Patients were ineligible if the tumor was clinically occult, a prior malignancy had been diagnosed, or they had received chemotherapy as part of initial management. Written informed consent was obtained from each participant prior to study entry and the Research Ethics Board at the Princess Margaret Hospital approved the trial.

Investigation, staging and treatment of patients were performed according to the policies of the PMH Gynecologic Cancer Group as previously described [1]. An examination under anesthesia (EUA) was performed to determine the stage according to the guidelines established by the International Federation of Gynecologists and Obstetricians (FIGO). Imaging included chest X-ray, CT of abdomen and pelvis, pelvic MRI, and lymphangiography (performed prior to 1998). Pelvic and para-aortic lymph nodes were classified as positive for metastatic disease if the short axis dimension was >1 cm, in keeping with established imaging criteria.

External beam radiotherapy was delivered to the pelvis or pelvis and para-aortic region (in 21 of 22 patients with involved pelvic nodes), depending upon the results of staging, using a planned dose of 45–50 Gy in 1.8–2 Gy daily fractions with 18–25 MV photons. A four-field box technique was used to treat the pelvis, whereas anterior–posterior parallel-opposed fields were used for the pelvis and para-aortic nodes. External radiation was followed by a single intracavitary brachytherapy application using an intrauterine line source. A planned dose of 35–40 Gy was prescribed 2 cm lateral to the midpoint of the sources (equivalent to Point A) using low dose rate or pulsed dose rate equipment. Adjuvant or neo-adjuvant chemotherapy was not used, nor was concurrent chemo-radiation.

Oxygen and IFP measurements

Oxygen measurements were performed during examination under anesthesia (EUA) with the Eppendorf pO_2 histograph (Eppendorf-Netheler-Hinz, GmbH) as previously described [15]. Where feasible, 20–30 measurements per needle track were obtained at five positions symmetrically spaced around the circumference of the tumor in order to assure a reproducible estimate of tumor oxygenation in the setting of significant intra-tumor heterogeneity. Measurements of tumor interstitial fluid pressure were performed using a wick-in-needle apparatus as previously described [6]. Oxygenation and IFP were measured at one to five (typically 4 or 5) spatially separated locations around the circumference of the visible cervical tumor, at a depth of approximately 2 cm from the surface.

Follow-up clinical visits after completing radiotherapy were performed by the responsible physician every 3 months for the first 2 years and every 4–6 months during the third to fifth years. A general physical examination and pelvic examination were performed at each visit, as well as a pelvic MRI at 3–6 months following treatment. MRI and other diagnostic tests were performed thereafter as indicated clinically.

Data analysis

Oxygenation data are presented as the hypoxic proportion, defined as the percentage of pO_2 readings <5 mm Hg (abbreviated as HP_5). IFP was expressed as the mean of the individual values in each tumor. Linear correlation was assessed using a Spearman correlation coefficient. The main end-point was disease-free survival (DFS) defined as the time between diagnosis and the first event: relapse of any type or death. Patients whose disease was never controlled (i.e. had only a partial response to radiotherapy) were considered to have relapsed at the time of diagnosis. DFS was estimated using the Kaplan–Meier method and survival curves were compared using the log-rank test. The probability of relapse was calculated using a cumulative incidence approach [16]. A Cox proportional hazards model was employed to test the significance of HP_5 and IFP on DFS and relapse. An initial model was constructed based on