Interstitial fluid pressure

Peritumoral interstitial fluid flow velocity predicts survival in cervical carcinoma

Tord Hompland a, Kjersti V. Lund a,b, Christine Ellingsen a, Gunnar B. Kristensen c,d, Einar K. Rofstad a,*

a Department of Radiation Biology, Institute for Cancer Research, b Department of Radiology and Nuclear Medicine, c Department of Gynecological Cancer, d Department of Radiation Biology, Institute for Cancer Research, and e Institute for Medical Informatics, Oslo University Hospital, Oslo, Norway

Article info

Article history:
Received 7 June 2014
Received in revised form 1 September 2014
Accepted 6 September 2014
Available online 15 October 2014

Keywords:
Cervical carcinoma
Disease-free and overall survival
Interstitial fluid pressure
Peritumoral fluid flow

Abstract

Background and purpose: High tumor interstitial fluid pressure (IFP) is associated with poor outcome in locally advanced carcinoma of the uterine cervix. We have recently developed a noninvasive assay of the IFP of tumors, and in this assay, the outward interstitial fluid flow velocity at the tumor surface \( v_0 \) is measured by Gd-DTPA-based DCE-MRI and used as a parameter for IFP. Here, we investigated the independent prognostic significance of \( v_0 \) in cervical cancer patients given cisplatin-based concurrent chemo- and radiation therapy with curative intent.

Patients: The study involved 62 evaluable patients from a cohort of 74 consecutive patients (Stage IB through IIIb) with a median follow-up of 5.5 years.

Results: The actuarial disease-free survival (DFS) and overall survival (OS) at 5 years were 67% and 76%, respectively. Significant associations were found between \( v_0 \) dichotomized about the median value and DFS and OS, both in the total patient cohort and a subcohort of 40 Stage IB patients. Multivariate analysis involving stage, tumor volume, lymph node status, and \( v_0 \) revealed that only \( v_0 \) provided independent prognostic information about DFS and OS.

Conclusion: This investigation demonstrates a strong, independent prognostic impact of the pretreatment peritumoral fluid flow velocity in cervical cancer.

© 2014 Elsevier Ireland Ltd. All rights reserved. Radiotherapy and Oncology 113 (2014) 132–138

The interstitial fluid pressure (IFP) is in general higher in malignant solid tumors than in most normal tissues [1]. Tumor tissues typically show IFP values of 5–50 mmHg, whereas the IFP measured in normal tissues usually ranges from −3 to +3 mmHg. Experimental studies have revealed that the IFP may differ significantly among individual tumors of the same line, even when transplanted to the same site and being of the same size [2]. Clinical investigations have shown that the intertumor heterogeneity in IFP is substantial in all tumor types studied thus far, including lymphoma, cutaneous melanoma, breast carcinoma, head and neck carcinoma, and cervical carcinoma [3]. Preclinical studies have provided strong evidence that high IFP in tumors may be a significant therapeutic problem [1–4]. First, highly elevated IFP may cause low and heterogeneous uptake of conventional and macromolecular chemical therapeutic agents, leading to poor response to many forms of chemotherapy [5]. Second, tumor interstitial hypertension may lead to resistance to radiation therapy through hypoxia-dependent as well as hypoxia-independent mechanisms [6,7]. Third, high IFP in tumors may promote hematogenous and lymphogenous metastatic dissemination [8,9].

Clinical investigations have revealed that the IFP of the primary tumor may be an important prognostic parameter in locally advanced carcinoma of the uterine cervix [10–12]. In these investigations, high IFP was associated with poor disease-free survival independent of conventional prognostic factors, such as tumor volume, stage, and lymph node status. Patients with tumors with high IFP showed an increased probability of developing recurrences both locally within the irradiated pelvic region and at distant non-irradiated sites. Interestingly, Fyles et al. [12] observed that the independent prognostic effect of IFP for recurrence and survival was strong, whereas the independent prognostic effect of tumor hypoxia was of borderline significance and was limited to patients without nodal metastases.

Tumor IFP was measured invasively with the wick-in-needle method in these clinical investigations [10–12]. A noninvasive method for assessment of the IFP of tumors has recently been developed in our laboratory [13]. Because the IFP of tumors drops steeply to normal tissue values at the tumor surface, interstitial fluid oozes continuously out from tumors into the surrounding normal tissue [4,14]. Our IFP assay is based on the assumption that the velocity of this fluid flow is determined by the IFP drop at the tumor surface.

* Corresponding author at: Department of Radiation Biology, Institute for Cancer Research, Norwegian Radium Hospital, Box 4953 Nydalen, 0424 Oslo, Norway.

E-mail address: einar.k. rofstad@rr-research.no (E.K. Rofstad).

http://dx.doi.org/10.1016/j.radonc.2014.09.011
0167-8140/© 2014 Elsevier Ireland Ltd. All rights reserved.
surface. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) with gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) as contrast agent is used to detect the peritumoral interstitial fluid flow. By using mouse xenograft models of several types of human cancer, we have demonstrated that the velocity of the fluid flow at the tumor surface ($v_0$) correlates strongly with tumor IFP [13]. Furthermore, we have shown that the $v_0$ of the primary tumor can be measured accurately in cervical cancer patients and that $v_0$ is higher in patients with pelvic lymph node metastases than in patients without lymph node involvement [13].

In the present work, a different cohort of patients was studied to investigate whether $v_0$ may be an important prognostic factor for the long-term outcome of locally advanced cervical cancer after definitive cisplatin-based concurrent chemoradiotherapy. The study confirmed that the primary tumor $v_0$ is higher in patients with than in patients without pelvic lymph node involvement and showed that $v_0$ has a strong prognostic effect for disease-free and overall survival, independent of stage, tumor volume, and lymph node status.

**Materials and methods**

**Patients**

Seventy-four previously untreated patients recruited to the chemoradiotherapy protocol for locally advanced cervical cancer (FIGO Stage IB through IIIB) at the Norwegian Radium Hospital between October 2004 and June 2007 were included in the study. A total of 12 patients were excluded from the analysis, 8 because of severe motion artifacts in the MR images and 4 because the peak signal intensity of the Gd-DTPA-enhanced rim used to measure $v_0$ was poorly defined. The characteristics of the remaining 62 patients are summarized in Table 1.

Table 1. Patient characteristics ($N = 62$).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54</td>
</tr>
<tr>
<td>Median</td>
<td>27–81</td>
</tr>
<tr>
<td>Histogram, #patients</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>53</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>9</td>
</tr>
<tr>
<td>Volume, cm$^3$</td>
<td>42.7</td>
</tr>
<tr>
<td>Median</td>
<td>4.8–319</td>
</tr>
<tr>
<td>FIGO stage, #patients</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>5</td>
</tr>
<tr>
<td>IIA</td>
<td>3</td>
</tr>
<tr>
<td>IIB</td>
<td>40</td>
</tr>
<tr>
<td>IIBA</td>
<td>2</td>
</tr>
<tr>
<td>IBII</td>
<td>12</td>
</tr>
<tr>
<td>Pelvic lymph node status, #patients</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
</tr>
</tbody>
</table>

Standard diagnostics and staging involved $T_2$-weighted MRI, $T_1$-weighted MRI, and Gd-DTPA-based DCE-MRI of the pelvis in addition to gynecological examination and biopsy. Positron emission tomography and/or computed tomography were not carried out routinely.

All patients were treated with concurrent chemoradiotherapy with curative intent. External beam radiation therapy was given in 25 fractions during a period of 5 weeks to a total dose of 50 Gy to the primary tumor, parametria, and adjacent pelvic wall and 45 Gy to the rest of the pelvic region. In addition, 5–6 fractions of intracavitary brachytherapy with a dose of 4.2 Gy per fraction were given to Point A. Chemotherapy with cisplatin (40 mg/m$^2$) was given weekly with a maximum of 6 courses during the radiation therapy period.

The patients were followed up by clinical examinations every third month for the first 2 years and thereafter every sixth month. The primary endpoints were disease-free survival (DFS), defined as the time to relapse or death from any cause measured from the date of diagnosis, and overall survival (OS), defined as the interval from diagnosis to death from any cause. DFS and OS curves were generated by using the Kaplan–Meier method. Median follow-up was 5.5 years (range 1.7–7.3 years).

The investigations were approved by the regional committee of medical research ethics in southern Norway and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

**Magnetic resonance imaging**

A 1.5-T whole-body scanner (Signa; General Electric) and a 4-channel phased-array surface coil were used for MRI. The entire pelvic region was scanned with an axial $T_2$-weighted fast spin echo sequence (TR = 4960 ms, TE = 84 ms, field of view: 20 × 20 cm$^2$, image matrix: 512 × 512, number of excitations: 1.5, slice thickness: 5 mm, slice spacing: 6 mm). DCE-MRI was carried out at a temporal resolution of 29 s by using an axial $T_1$-weighted spoiled gradient recalled sequence (TR = 160 ms, TE = 3.5 ms, $\alpha_{T1} = 90^\circ$, field of view: 20 × 20 cm$^2$, image matrix: 256 × 256, number of excitations: 1, slice thickness: 5 mm, slice spacing: 6 mm). Three $T_1$-weighted images were acquired before a bolus of 0.1 mmol/kg Gd-DTPA was administered, and $T_1$-weighted images were recorded for 10 min after the Gd-DTPA administration. The MRI was carried out before treatment was initiated.

**Tumor volume and metastatic status**

Primary tumor volume and metastatic status were determined by examining MR images in the open source dicom viewer Osirix [15]. A region of interest (ROI) encompassing the tumor area was drawn in each $T_2$-weighted image, and tumor volume was reconstructed and calculated from these ROIs with a built-in function of Osirix. Metastatic status was assessed by examining the internal, external, and lower common iliac chains. A lymph node was scored as metastasis-positive when its shortest diameter in the $T_2$-weighted images was longer than 1.0 cm and the $T_1$-weighted images showed a contrast enhancement pattern similar to that of the primary tumor.

**Assessment of peritumoral interstitial fluid flow velocity**

The procedure used to measure peritumoral interstitial fluid flow velocity has been described in detail [13]. Briefly, the $T_1$-weighted image recorded immediately after the administration of Gd-DTPA showed a high-signal-intensity rim in the tumor periphery, and this rim moved outward with time. Movies showing the outward movement of the high-signal-intensity rim have been presented elsewhere [13]. The signal intensity across the rim was measured in line-shaped ROIs at different time points after the Gd-DTPA administration, as illustrated earlier [13]. The position of the peak signal intensity was identified by fitting a polynomial of the sixth degree to signal intensity data points calculated from four neighboring pixels by bilinear interpolation. The rim distance of Osirix. The signal intensity of the sixth degree to signal intensity data points calculated from four neighboring pixels by bilinear interpolation. The rim distance from the tumor surface ($S$), calculated from the position of the peak signal intensity, was measured as a function of time ($t$). The tumor surface ($S = 0$) was defined as the position of the peak signal intensity in the first $T_1$-weighted image recorded after the Gd-DTPA administration. Curves of $S(t) = S_0(1 - e^{-bt})$ were fitted to the data by regression analysis to determine $S_0$ and $b$, where $S_0$ is the
maximum distance the interstitial fluid can flow from the tumor surface and \( b \) is the attenuation coefficient (i.e., \( b \) describes the decrease in fluid flow velocity with time). Each curve fitting was based on 19–21 points, one for each time frame of the DCE-MRI series. By using the definition of velocity, fluid flow velocity was calculated as \( v(t) = \frac{dS}{dt} = S_0b(e^{-at} - e^{-bt}) \), where \( v_0 = v(t = 0) = S_0b \) is the fluid flow velocity at the tumor surface. Intratumor heterogeneity in \( v_0 \) was studied by measuring \( v_0 \) in 3–5 ROIs in the tumor periphery. The ROIs were positioned perpendicular to the tumor periphery in regions where a well-defined high-signal-intensity peak could be identified throughout the entire DCE-MRI series. Minor tumor movements during the DCE-MRI were corrected for by coordinate mapping.

### Statistical analysis

The Pearson product moment correlation test was used to search for correlations between parameters. Comparisons of data were carried out by using the Student \( t \) test (single comparisons) or by one-way ANOVA (multiple comparisons) when the data complied with the conditions of normality and equal variance. Under other conditions, comparisons were carried out by nonparametric analysis using the Mann–Whitney rank-sum test (single comparisons). The Kolmogorov–Smirnov method was used to test for normality. The random-effects ANOVA model was used to assess the proportions of the total variation in \( v_0 \) attributable to inter- and intratumor heterogeneity. Kaplan–Meier curves were compared by using the log-rank test. Univariate and multivariate Cox proportional hazard analyses were used to evaluate prognostic parameters with respect to DFS and OS. \( P \) values <0.05 were considered significant. Data are presented as mean ± standard deviation or median ± 95% confidence interval unless otherwise stated.

### Results

**The correlation coefficients of the curve fits were high**

The expression \( S(t) = S_0(1 - e^{-at}) = v_0(1 - e^{-bt})/b \) gave good fits to the measured values of \( S(t) \). Representative examples of curve fits are shown in Fig. 1a, which presents data from three tumors differing substantially in peritumoral interstitial fluid flow velocity. In general, the curve fits showed correlation coefficients of \( R^2 = 0.94–0.99 \).

**The intertumor heterogeneity in \( v_0 \) was larger than the intratumor heterogeneity**

Measurements of \( v_0 \) were carried out in 3–5 positions in each tumor. Mean \( v_0 \) of the individual tumors varied among the 62 patients from 0.007 to 0.043 mm/s with a median value of 0.020 ± 0.03 mm/s and a mean value of 0.021 ± 0.009 mm/s (Fig. 1b). The coefficient of variation ranged from 0.3% to 60% and increased with increasing mean \( v_0 \) (\( P = 0.00010, R^2 = 0.31 \)). A logarithmic transformation was applied to \( v_0 \) to fulfill the assumption of constant variance required by the random-effects ANOVA model, and by using the transformed data, the variation in \( v_0 \) between and within tumors was found to account for 85% and 15% of the total variation, respectively. The intertumor heterogeneity in \( v_0 \) was significantly larger than the intratumor heterogeneity (\( P < 0.00001 \)).

Mean \( v_0 \) was weakly associated with tumor stage and tumor volume

Mean \( v_0 \) varied only slightly with tumor stage (Fig. 1b). Significant differences in mean \( v_0 \) in an ANOVA test of Stage IB/IIA versus Stage IIB versus Stage IIIA/IIIB tumors (\( P > 0.05 \)). However, tumors in Stage IIIA/IIIB had slightly higher mean \( v_0 \) than tumors in Stage IB/IIA (\( P = 0.011, t \) test). Furthermore, there was a weak correlation between mean \( v_0 \) and tumor volume, primarily attributable to the 4 tumors with volume >150 cm\(^3\) (\( P = 0.00027, R^2 = 0.20, \) Fig. 1c). For the 58 tumors with volume <150 cm\(^3\), there was no correlation between mean \( v_0 \) and volume. Mean \( v_0 \) was not associated with tumor histology or patient age.

**High value of mean \( v_0 \) was associated with pelvic lymph node metastasis**

Positive lymph nodes were detected in 29 of the 62 patients, whereas the other 33 patients did not show evidence of metastatic...
growth. Forty of the 62 patients had tumors in Stage IIB, and 14 of the Stage IIB patients were metastasis-positive. Mean \( v_0 \) of the primary tumor was higher in the metastasis-positive than in the metastasis-negative patients, both in the total patient cohort (\( P < 0.00001 \), Fig. 2a) and in the subcohort of Stage IIB patients (\( P = 0.0044 \), Fig. 2b).

High value of mean \( v_0 \) was a strong prognostic factor for poor DFS and OS

The actuarial DFS and OS at 5 years were 67% and 76%, respectively. Tumor progression or recurrence was documented in 17 patients, 4 in the pelvis alone, 2 in the pelvis and para-aortic or distant lymph nodes, and 11 in para-aortic or distant lymph nodes alone. One patient was clinically disease-free after further treatment, whereas the other 16 patients died from their recurrences. There were 3 deaths from intercurrent diseases.

Univariate analysis revealed a significant association between mean \( v_0 \) dichotomized about the median value (0.020 ± 0.003 mm/s) of mean \( v_0 \) and outcome of treatment. The patients with low and those with high mean \( v_0 \) showed 5-year DFSs of 93% and 40%, respectively (\( P < 0.00001 \), Fig. 3a), and 5-year OSs of 97% and 55%, respectively (\( P = 0.00002 \), Fig. 3b). DFS and OS were also influenced by stage [IIB/III versus IIB versus II/III: \( P = 0.037 \) (DFS, Supplementary Fig. 1a), \( P = 0.048 \) (OS, Supplementary Fig. 1b)] and tumor volume dichotomized about the median value of 43 cm\(^3\) \( P = 0.060 \) (DFS, Supplementary Fig. 1c), \( P = 0.033 \) (OS, Supplementary Fig. 1d)], but were not influenced significantly by pelvic lymph node status \( P > 0.05 \) (DFS, Supplementary Fig. 1c), \( P > 0.05 \) (OS, Supplementary Fig. 1d)]. Multivariate analysis involving stage, tumor volume, lymph node status, and mean \( v_0 \) revealed that mean \( v_0 \) was the only parameter that provided independent prognostic information about DFS \( P < 0.00001 \) (mean \( v_0 \), \( P = 0.17 \) (stage)), \( P = 0.49 \) (tumor volume), \( P = 0.63 \) (lymph node status)] and OS \( P = 0.00003 \) (mean \( v_0 \), \( P = 0.47 \) (stage)), \( P = 0.89 \) (tumor volume), \( P = 0.36 \) (lymph node status)].

Forty patients had Stage IIB tumors, and this more homogenous patient subpopulation was analyzed separately. Mean \( v_0 \) was the only parameter that was associated with outcome of treatment in univariate analysis. The patients with low and those with high mean \( v_0 \) [mean \( v_0 \) below and above the median value (0.021 ± 0.003 mm/s) of mean \( v_0 \)] had 5-year DFSs of 100% and 44%, respectively (\( P = 0.00008 \), Fig. 3c), and 5-year OSs of 100% and 60%, respectively (\( P = 0.00026 \), Fig. 3d).

Discussion

Interstitial hypertension is a characteristic feature of malignant tissues that has been shown to be associated with poor outcome in locally advanced cervical cancer [10–12]. We have recently developed a novel assay for noninvasive assessment of the IFP of tumors [13]. In this assay, the outward interstitial fluid flow velocity at the tumor surface (\( v_0 \)) is measured by Gd-DTPA-based DCE-MRI and used as a parameter for tumor IFP.

The IFP of tumors drops steeply to normal tissue values at the tumor surface and fluid oozes out into the surrounding tissue, primarily because the draining lymphatics are located within the peritumoral normal tissue and not within the malignant tissue [3,5]. The velocity of this fluid flow is a complex function of several tissue parameters, including the location, density, and efficiency of the peritumoral lymphatics, the curvature of the tumor surface, and the hydraulic conductivity of the peritumoral normal tissue.

In our noninvasive assay of IFP, \( v_0 \) is calculated by using a simple model that gives excellent fits to experimental data (i.e., from the best fits of \( S(t) = S_0(1 – e^{-kt}) = v_0(1 – e^{-kt/b}) \) [13].

Correct assessment of \( v_0 \) requires that tumors do not move during DCE-MRI or that any movement can be corrected for and, moreover, that a high-signal-intensity rim with a well-defined intensity peak can be detected throughout a DCE-MRI series of ~10 min. The high-signal-intensity rims seen in DCE-MRI images of tumors are often diffuse, may have low intensity, may fade out with time, and may occasionally change in space and time, particularly when the DCE-MRI is carried out at high temporal resolution at the expense of high signal intensity. In the present study, this problem was diminished by using a \( T_1 \)-weighted sequence giving high signal intensity rather than high temporal resolution (cf. Supplementary Movie S2 in Ref. [13]). Furthermore, \( v_0 \) was measured in ROIs that were positioned manually in regions showing a well-defined high-signal-intensity peak throughout the DCE-MRI series, thus avoiding regions where the peak signal intensity was poorly defined. Routine assessment of \( v_0 \) may require a fully automated procedure. An ideal automated procedure should correct for any tumor movement during the DCE-MRI and should consider the entire three-dimensional tumor surface in the \( v_0 \) calculations.

Fig. 2. Measurements of \( v_0 \) were carried out in 3–5 positions in the primary tumors of 62 patients with locally advanced carcinoma of the uterine cervix. Mean \( v_0 \) versus metastatic status for the 62 patients (a) and the subcohort of 40 patients with Stage IIB disease (b). Symbols represent individual patients. Horizontal lines show mean values. The \( P \) values were determined by the Mann–Whitney rank-sum test. Neg, negative; Pos, positive.
Attempts to develop a procedure for automated assessment of $v_0$ have been initiated in our laboratory, and if we succeed, widespread investigations of the prognostic power of $v_0$ in malignant diseases will be possible.

Although the procedure used in the present study is not suitable for routine assessment of $v_0$, our investigation is an important proof-of-principle study showing that $v_0$ has a strong prognostic effect for DFS and OS in cervical cancer patients treated with concurrent chemoradiotherapy. The prognostic power of $v_0$ was independent of established clinical prognostic factors including stage, tumor volume, and lymph node status, implying that $v_0$ is a clinically relevant parameter of the tumor microenvironment that provides unique information about the outcome of cervical carcinoma.

It should be noticed that only 62 evaluable patients were included in our study, and therefore, it needs validation by other centers. However, 40 of our patients had Stage IIB tumors, which enabled separate analysis of the prognostic power of $v_0$ in a relatively homogenous patient subpopulation. The high fraction of Stage IIB tumors may be the reason why standard criteria like tumor volume, lymph node status, and stage were not found to provide independent prognostic information about DFS and OS when the total population of 62 patients were subjected to multivariate analysis.

Lymph node status was assessed by MRI using established size, morphological, and contrast-enhancement criteria. Several investigations have shown that these MRI criteria can detect lymph node metastases in locally advanced cervical carcinoma with high specificity whereas the sensitivity, on the other hand, is relatively low [16]. Consequently, it cannot be excluded that the limitations of MRI in assessing lymph node metastatic status accurately may represent a source of error in this study. However, it should be pointed out that MRI is an accepted and commonly used technique for defining lymph node status in gynecological cancer [16].

Our study has other limitations also. First, measurements attempting to find correlations between $v_0$ and IFP were not carried out. Such measurements are warranted although strong correlations between $v_0$ and IFP have been found in preclinical studies of melanoma and cervical carcinoma xenografts [13]. Second, $v_0$ was not measured during or after completed radiation therapy. The possibility that the $v_0$ of irradiated tumors and changes in $v_0$ during radiation therapy may provide information on radiation-induced changes in IFP and have prognostic effect for DFS and OS should be investigated.

In the seminal investigations showing that cervical cancer patients with primary tumors with high IFP have increased probability of developing local and distant recurrences, the patients were treated with radiation therapy alone [10–12]. In a recent study of cervical cancer patients receiving concurrent chemoradiotherapy, Milosevic et al. [17] found that only patients with high tumor IFP benefitted from adding chemotherapy to the radiation therapy.

Fig. 3. Kaplan–Meier curves for disease-free (left) and overall (right) survival of patients with locally advanced carcinoma of the uterine cervix stratified by mean $v_0$. The $P$ values were determined by univariate Cox regression analysis. (a, b) Sixty-two patients with FIGO Stage IB, IIA, IIB, IIIA, or IIIB disease. Low $v_0$, mean $v_0 < \text{median of mean of 0.020 mm/s (} N = 31)$; high $v_0$, mean $v_0 > \text{median of mean of 0.020 mm/s (} N = 31)$. (c, d) The subcohort of 40 patients with FIGO Stage IIIb disease. Low $v_0$, mean $v_0 < \text{median of mean of 0.021 mm/s (} N = 20)$; high $v_0$, mean $v_0 > \text{median of mean of 0.021 mm/s (} N = 20)$.
protocol and, hence, that the DFS of patients with high tumor IFP did not differ significantly from that of patients with low tumor IFP. A similar chemoradiotherapy protocol was used in our study, and in contrast to Milosevic et al. [17], we found that the patients with high \( v_0 \) showed both poorer DFS and OS than those with low \( v_0 \). Interestingly, peritumoral interstitial convection is not determined solely by the tumor IFP, but is also influenced by the hydraulic conductivity of the adjacent normal tissue and the density and functionality of the peritumoral lymphatics [18].

IFP has been shown to differ between tumor regions in human cervical carcinomas, and the intratumor heterogeneity in IFP has been suggested to be a consequence of regional variations in microvascular hydrostatic pressure and restricted interstitial flow of fluid from one perfused region to another [19]. Milosevic et al. [19] measured IFP by the wick-in-needle method in 1–5 positions in 77 cervical carcinomas and found that the variation in IFP between and within tumors accounted for 41% and 59% of the total sample variance, respectively. In comparison, \( v_0 \) was assessed in 3–5 positions in 62 tumors in the present study, and the inter- and intratumor heterogeneities were found to account for 85% and 15% of the total variation, respectively. To be clinically useful, an assay of a microenvironmental tumor parameter must reliably discriminate between patients, implying that the intratumor heterogeneity of the parameter has to be smaller than the actual intratumor heterogeneity. This requirement is clearly fulfilled by our DCE-MRI assay of \( v_0 \). Furthermore, from the data reported here and those reported by Milosevic et al. [19], it seems that assessment of \( v_0 \) by DCE-MRI has a greater potential to discriminate between patients than measurement of IFP by the wick-in-needle method.

There are several possible biological explanations of a link between IFP/\( v_0 \) and clinical outcome in cervical carcinoma. In a small study involving 3 patients, Roh et al. [20] observed an inverse relation between IFP and tumor oxygenation and suggested that high IFP is associated with tumor hypoxia and, hence, may provide an indication of tumor radiation resistance caused by hypoxia. However, this suggestion has not been verified in more recent studies. In a comprehensive study involving 104 patients, Fyles et al. [12] could not find a significant relationship between IFP and tumor hypoxia, and moreover, IFP was found to have a strong independent prognostic impact for DFS whereas the prognostic impact of hypoxia was of borderline significance. Similarly, preclinical studies of the IFP and oxygenation of tumors have failed to show a correlation between these microenvironmental parameters [9,21,22], and studies of human melanoma xenografts have revealed that high tumor IFP can cause resistance to radiation therapy and promote pulmonary and lymph node metastasis by hypoxia-independent mechanisms [7,8].

It has also been suggested that high IFP may be a consequence of high angiogenic activity and, hence, that the prognostic effect of IFP in cervical cancer may reflect the general aggressive behavior of highly angiogenic tumors [3]. Moreover, the proangiogenic factor vascular endothelial growth factor-A (VEGF-A) is up-regulated in tumors with high angiogenic activity, and it has been suggested that VEGF-A may cause resistance to radiation therapy by inhibiting radiation-induced endothelial cell inactivation [23,24]. These suggestions are consistent with preclinical studies, which have revealed that high IFP is associated with high concentrations of VEGF-A, decreased radiocurability, and increased metastatic propensity in human melanoma xenografts [7,8]. The expression of VEGF-A was not measured in the cervical carcinomas included in this study. However, studies of possible associations between \( v_0 \) and VEGF-A expression are highly warranted to shed light on the mechanisms causing interstitial hypertension in tumors and those linking high IFP/\( v_0 \) to poor outcome in cervical carcinoma.

In the present investigation, the majority of the recurrences occurred outside the radiation field, suggesting that the observed associations between high \( v_0 \) and poor outcome were primarily a result of high \( v_0 \) being associated with increased metastatic propensity rather than decreased radiocurability. Several mechanisms may link \( v_0 \) to lymph node metastasis. First, fluid flow from tumors into surrounding tissues may direct tumor cells toward peritumoral lymphatics by autologous chemotaxis [25]. Second, proteolytic enzymes and chemokines transported by the interstitial fluid may facilitate tumor cell migration by remodeling the extracellular matrix [25]. Third, interstitial fluid flow may drive fibroblast differentiation and migration, leading to enhanced tumor cell invasion [26]. Fourth, the interstitial fluid may carry lymphangiogenic factors that promote metastasis by dilating peritumoral lymphatics and inducing lymphangiogenesis [27]. Fifth, increased interstitial and lymphatic fluid flow may decrease the sentinel lymph node immunity to metastatic tumor cells [28,29].

Our study suggests that \( v_0 \) may be an important biomarker of poor outcome in cervical cancer and, consequently, that the peritumoral interstitial fluid flow/IFP may be important targets for improved therapy. The IFP of experimental tumors has been shown to decrease after irradiation [22], and a study of human cervical cancer revealed that tumor IFP decreased during the course of radiation therapy and that the decrease was significant only in patients with complete responses [11]. These observations suggest that irradiation prior to standard treatment may be a useful strategy for decreasing the IFP of tumors that are generally given chemotherapy. Antiangiogenic treatment may be another useful strategy for decreasing the IFP of tumors. Preclinical studies have suggested that antiangiogenic treatment may cause a time-dependent normalization of the vasculature of tumors and, hence, decreased IFP [24,30]. Furthermore, antiangiogenic treatment with an anti-VEGF-A antibody has been shown to decrease the IFP and improve the outcome of concurrent chemoradiotherapy in human rectal carcinoma [31]. However, an optimal therapeutic strategy for targeting peritumoral interstitial fluid flow/IFP in cervical cancer can probably not be developed without detailed understanding of the mechanisms linking IFP/\( v_0 \) to outcome of treatment. Regardless of therapeutic strategy, assessment of \( v_0 \) by DCE-MRI may be essential for selecting patients with high-IFP tumors for targeted treatment as well as for monitoring the effect of the treatment.

In summary, our study provides significant evidence that assessment of \( v_0 \) by Gd-DTPA-based DCE-MRI yields important prognostic information about patients with locally advanced carcinoma of the uterine cervix treated with concurrent cisplatin-based chemoradiotherapy. The prognostic power of \( v_0 \) for DFS and OS was independent of established clinical prognostic factors such as tumor volume and lymph node status, both in the total cohort of patients with Stage IB, IIA, IIB, IIIA, or IIIB disease and in the subcohort with Stage IIB tumors, implying that \( v_0 \) may be an important biomarker of interstitial hypertension-induced aggressiveness and treatment resistance in cervical cancer. The possibility that \( v_0 \) may be a strong prognostic factor also in malignant diseases other than cervical carcinoma merits comprehensive prospective clinical studies.

**Conflict of interest**

None declared.

**Acknowledgments**

Financial support was received from the Norwegian Cancer Society and the South-Eastern Norway Regional Health Authority.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2014.09.011.

References