

Interstitial Fluid Pressure Predicts Survival in Patients with Cervix Cancer Independent of Clinical Prognostic Factors and Tumor Oxygen Measurements¹

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ABSTRACT

The purpose of this study was to determine the independent prognostic significance of interstitial fluid pressure (IFP) measurements in cervix cancer. A total of 102 patients with newly diagnosed cervix cancer were accrued to this prospective study. There were 31 International Federation of Gynecology and Obstetrics stage IB or IIA tumors, 40 IIB tumors, and 31 IIIB tumors. The median size was 5 cm (range, 2–10 cm). Pelvic lymphadenopathy was identified radiographically in 20 patients. IFP was measured at examination under anesthesia using a wick-in-needle technique. Multiple measurements were made in each tumor. The mean IFP in individual tumors ranged from –3 to 48 mm Hg, and the median for the entire cohort was 19 mm Hg. Treatment consisted of external beam and intracavitary radiation without chemotherapy. Median follow-up was 2.5 years. The 3-year disease-free survival of all of the patients was 53%. Disease-free survival was 34% in patients with IFP >19 mm Hg, and 68% in those with lower IFP ($P = 0.002$). To evaluate rigorously the independent prognostic significance of IFP measurements relative to established clinical factors, a multivariate model was first developed using stepwise selection of clinical covariates. Tumor size ($P = 0.0003$) and pelvic lymph node status ($P = 0.0016$) comprised the clinical model. IFP, when added to this model, provided additional independent prognostic information ($P = 0.0013$). IFP was also significant ($P = 0.0027$) when the clinical factors and hypoxic proportion as determined with the Eppendorf electrode were analyzed together. Patients with high IFP were more likely to recur both locally and at distant sites. This study is the first to document a strong, independent prognostic importance of pretreatment IFP measurements in cervix cancer. Patients with high IFP are significantly more likely than those with low IFP to recur after radiotherapy and die of progressive disease, independent of clinical prognostic factors and the results of tumor oxygen measurements.

INTRODUCTION

IFP³ is elevated in most solid malignant tumors because of high capillary permeability and impaired lymphatic drainage (1–3). High IFP may impair the delivery of macromolecules to the central regions of tumors (1) and may alter tumor blood flow (4–6). A prospective clinical study has been ongoing at PMH since 1994 with the aim of determining the independent prognostic significance of IFP and oxygen measurements in patients with cervix cancer (7–9). An earlier analysis of the first 77 patients accrued to the study revealed average IFPs in individual tumors ranging from 3 to 48 mm Hg, significant variation in IFP from region to region in some tumors, a weak inverse

correlation with tumor oxygenation, and an association between high pretreatment IFP and residual pelvic tumor after the completion of radiotherapy (7). Accrual to the study was completed in January 1999. This report, based on the entire cohort, describes the relationship between tumor IFP and patient survival. To our knowledge, it is the first to demonstrate a strong independent prognostic effect of IFP. The relationship between oxygen measurements and treatment outcome for these patients is discussed in a separate publication.⁴

MATERIALS AND METHODS

Patient Population. A total of 116 patients were accrued to this study between April 1994 and January 1999. Fourteen patients were excluded from the analysis, seven because distant metastases were present at diagnosis and only palliative treatment was administered, one because the histological diagnosis was later changed to sarcoma, one because treatment consisted of radical hysterectomy rather than radiotherapy, one because chemotherapy was administered concurrently with radiation, and four because IFP measurements were not performed because of equipment malfunction. The characteristics of the remaining 102 eligible patients are summarized in Table 1. The maximum tumor size was determined clinically at examination under anesthesia and ranged from 2–10 cm with a median of 5 cm. Pelvic and para-aortic lymph node status was evaluated by magnetic resonance imaging of the pelvis, computed tomographic scan of the abdomen and pelvis, and/or bipedal lymphangiography and was classified as negative, positive, or suspicious according to published criteria (10, 11). Pelvic lymph nodes were negative in 61 patients, positive in 20 patients, and suspicious in 21 patients. Para-aortic nodes were negative in 91 patients, positive in two patients, suspicious in seven patients, and not assessable in two patients. All of the patients with positive or suspicious para-aortic lymph nodes also had positive pelvic lymph nodes. None of the patients had metastases to other lymph node groups or distant sites.

This study was approved by the Clinical Trials Committee of the PMH and the Human Subjects Review Committee of the Office of Research Services at the University of Toronto. Written informed consent was obtained from each patient before the measurements.

Measurement of IFP and Oxygen Tension. IFP was measured using a wick-in-needle apparatus, and oxygen tension was measured using a polarographic needle electrode system (Eppendorf-Netheler-Hinz, Hamburg, Germany), as described in detail elsewhere (7–9). All of the measurements were made during examination under anesthesia with the patients in the lithotomy position. Anesthesia was administered using i.v. propofol and inhaled nitrous oxide, and the inspired oxygen concentration was maintained at 40%. Clinical examination and magnetic resonance imaging of the pelvis were used to assure that the IFP and oxygen measurements were made in tumor and not inadvertently in adjacent normal tissue.

IFP was measured at one to five spatially separated locations around the circumference of the visible cervical tumor, at a depth of approximately 2 cm from the surface. Four or more measurements were performed in 87 of the 102 tumors. Multiple measurements of IFP are necessary in each tumor to account for regional heterogeneity, as described previously (7). The mean of the individual IFP measurements was calculated for each tumor and ranged from –2.8 to 48 mm Hg. The mean IFP was <0 mm Hg in only one patient. It is

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³ The abbreviations used are: IFP, interstitial fluid pressure; PMH, Princess Margaret Hospital; DFS, disease-free survival; HP₅, hypoxic proportion (percentage of oxygen measurements <5 mm Hg).

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Table 1 Patient characteristics

Parameter ^a	Category	Value
Age	Range	23–78 years
	Median	52 years
Histology	Squamous	83
	Adenocarcinoma	13
	Adenosquamous	6
Grade	1	14
	2	54
	3	25
	Unknown	9
Size	Range	2–10 cm
	Median	5 cm
FIGO Stage	IB/IIA	31
	IIB	40
	IIIB	31
Pelvic LN	Negative	61
	Positive	20
	Suspicious	21
PA-LN	Negative	91
	Positive	2
	Suspicious	7
	Unknown	2
Pre-treatment Hgb	Range	82–152 g/liter
	Median	126 g/liter
HP ₅	Range	0–93%
	Median	47%
IFP	Range	–2.8 to 48 mm Hg
	Median	19 mm Hg

^a FIGO, International Federation of Gynecology and Obstetrics; Hgb, hemoglobin concentration; LN, lymph nodes; PA-LN, para-aortic lymph nodes.

possible that the measurements in this patient were inadvertently made in the normal tissue surrounding the tumor, because negative IFP values are uncommon in tumors (7, 12–18). However, all of the IFP measurements from all of the eligible patients were included in the analysis to avoid potential bias. Tumor IFP was approximately normally distributed among the patients, with mean and median values for the entire cohort of 20 and 19 mm Hg, respectively.

Oxygen tension was measured immediately before IFP at approximately the same circumferential positions in the tumor. Measurements were made along one to seven linear tracks, with 20 to 30 measurements/track. At least four measurement tracks were used in 88% of the tumors. Oxygenation was expressed as the proportion of the individual oxygen measurements along all of the tracks that were <5 mm Hg (hypoxic proportion or HP₅). The HP₅ in the individual tumors ranged from 0% to 93%, and the mean and median values for the entire cohort were 46% and 47%, respectively.

The IFP and pO₂ measurements were well tolerated in all of the patients, and there were no side effects.

Treatment of Cervix Cancer. Radiation treatment was prescribed according to the policies of the PMH Gynecological Oncology Group. External beam pelvic radiation was administered using anterior-posterior fields or a four-field box technique and 18–25 MV photons. The irradiated pelvic volume included the primary tumor as well as the internal, external, and lower common iliac lymph nodes. Typically, a dose of 45 to 50 Gy in 1.8- to 2-Gy daily fractions was prescribed at the isocenter, and a two half-value-layer posterior attenuator was used to reduce the rectal dose by 10 to 20%. An external beam pelvic dose of at least 45 Gy was delivered to 97 of the 102 patients. In addition, external beam para-aortic radiation was administered concurrently to 20 patients with involved pelvic or para-aortic lymph nodes at doses ranging from 12.6 to 50 Gy. Chemotherapy was not used.

After external beam radiation, 97 patients were treated with a single intracavitary low-dose-rate or pulsed-dose-rate brachytherapy application using an intrauterine line source alone without colpostats. A dose of 40 Gy at a dose rate of 0.5–0.8 Gy/h was administered 2 cm lateral to the midpoint of the active sources. Brachytherapy was not feasible in five patients because of unfavorable tumor anatomy. These patients received an external beam boost to the primary tumor using a four-field conformal technique to deliver an additional dose of between 10 and 20 Gy.

Patients were followed at three monthly intervals for the first 2 years after completing radiotherapy and at six monthly intervals thereafter. At each visit, the clinical history was updated, and a physical examination, including pelvic examination, was performed. Laboratory and radiographic tests were obtained

as required based on clinical findings. The follow-up of surviving patients ranged from 6 months to 5.5 years, and the median follow-up was 2.5 years.

Analysis. The primary end point of the study was DFS measured from the date of diagnosis of cervix cancer. Patients who were documented to have progressive or recurrent disease after treatment or who died of any cause were counted as events. Patients who failed to respond to treatment were classified as having a disease-free interval of zero. Other patients were counted as failures at the time of first recurrence. Those who remained alive and free of disease were censored at the time of last follow-up. DFS was estimated using the Kaplan-Meier method, and survival curves were compared using the log-rank test.

The primary aim of the study was to determine whether IFP measurements added additional prognostic information to that readily obtained from routine clinical and radiographic staging investigations. Therefore, a clinical multivariate prognostic factor model was first developed using the Cox proportional hazards model with step-wise selection of clinical covariates and DFS as the end point. IFP was then added to this model to determine its independent prognostic effect. The relative importance of IFP and HP₅ was examined by repeating the analysis and adding both IFP and HP₅ to the clinical model. The α level for rejecting IFP as an important prognostic factor was set at 0.025 to minimize the risk of a false-positive result with multiple challenges to the data. Age, size, pretreatment hemoglobin concentration, IFP, and HP₅ were tested in the Cox model as continuous variables. Stage, grade, histology, and pelvic lymph node status were tested as categorical variables. The interaction terms between IFP and the significant clinical factors were also tested.

Overall survival, defined as the interval from diagnosis to death from any cause, local recurrence, defined as a first recurrence in the irradiated pelvic volume, and distant recurrence, defined as a first recurrence outside of the irradiated pelvic volume, were secondary end points of the study. Local and distant recurrences defined in this way are competing events. Local recurrence cannot by definition occur after distant recurrence and *vice versa*, although both can occur simultaneously. Kaplan-Meier estimates of the local or distant recurrence-free rate assume true survival data without competing events and, therefore, overestimate the true recurrence rate. To deal with this problem, the cumulative marginal probabilities of local and distant recurrence were calculated using Gray's method (19), which accounts for competing risks. Differences between cumulative recurrence curves were tested for statistical significance using the Wald test with robust standard error. Factors predictive of local and distant recurrence were identified using the Cox proportional hazards model adjusted for competing risks as described by Lunn and McNeil (20). Patients who recurred initially at distant sites were treated palliatively and not subjected to routine pelvic examination or pelvic imaging. Therefore, the predictive value of IFP for secondary pelvic recurrence (after initial distant recurrence) was not evaluated.

RESULTS

There was no apparent relationship between the mean tumor IFP and patient age, tumor size, stage, lymph node status at diagnosis, pretreatment hemoglobin concentration, or HP₅. The relationship between IFP and pelvic lymph node status is shown in Fig. 1a, and the relationship between IFP and HP₅ is shown in Fig. 1b.

Tumor progression or recurrence was documented in 42 patients. Four were clinically free of disease after further treatment, 12 were alive with progressive disease, and 26 had died. There were two deaths from intercurrent disease, and 64 patients were alive and continuously free of disease from the completion of treatment. The actuarial DFS was 53% at 3 years, and the overall survival was 68%.

Univariate analysis identified a significant association between IFP dichotomized about the median value of 19 mm Hg and the long-term outcome of patients after radiotherapy. Patients with low and high IFPs had 3-year DFSs of 68% and 34%, respectively ($P = 0.002$), as shown in Fig. 2. DFS was also influenced by clinical factors, including age (≤ 53 versus > 53 years; $P = 0.0096$), maximum tumor size (≤ 5 versus > 5 cm; $P = 0.0008$), stage (IB/IIA versus IIB versus IIIB; $P = 0.0003$), pelvic lymph node status (negative versus suspicious versus positive; $P < 0.0001$), pretreatment hemoglobin concentration

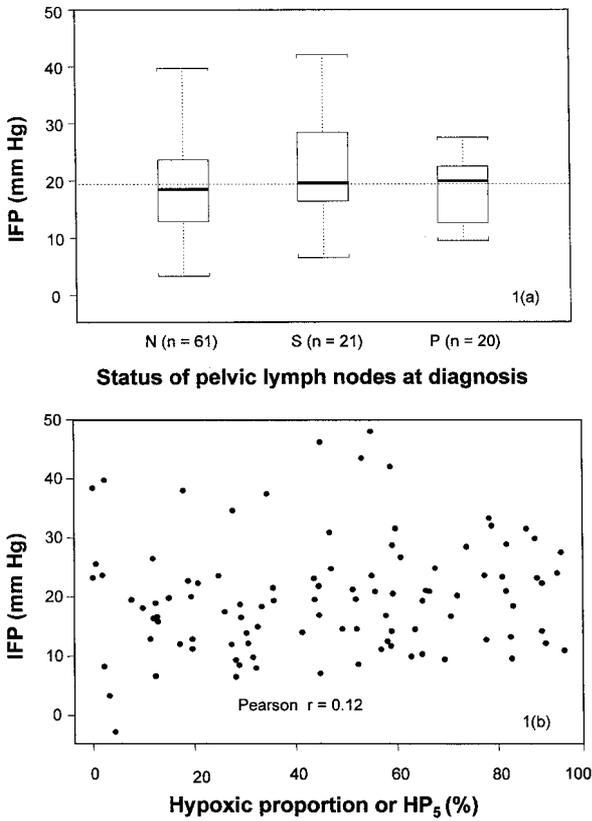


Fig. 1. Bar-plot (a) of IFP stratified by the status of pelvic lymph nodes at diagnosis [N, negative (no radiographic evidence of lymph node enlargement); P, positive (enlarged pelvic lymph nodes indicative of metastatic disease); S, suspicious (pelvic lymph nodes suspicious for metastatic disease)]. Scattergram (b) showing IFP and HP₅ in 102 patients with cervix cancer.

(≤ 126 versus > 126 g/liter, $P = 0.059$), and HP₅ (≤ 50 versus $> 50\%$; $P = 0.0065$). The DFS of patients with suspicious pelvic lymph nodes was not statistically different from that of patients with negative nodes; therefore, the two groups were combined (negative/suspicious versus positive) in the multivariate analyses.

The main goal of the study was to determine the additional prognostic value of IFP measurements in relation to established clinical and radiographic staging procedures. A clinical multivariate prognostic factor model was initially developed. Age, maximum tumor size, stage, pelvic lymph node status, pretreatment hemoglobin concentration, grade, and histology were examined using the Cox proportional hazards model. Only size ($P = 0.0003$) and pelvic lymph node status ($P = 0.0016$) were independently predictive of DFS and formed the clinical model. IFP, when added to this model, provided additional independent prognostic information ($P = 0.0013$). The relative risk of recurrence or death increased by a factor of 1.06 for each mm Hg increase in IFP, independent of tumor size and lymph node status. The combined influence of IFP and tumor size on DFS is shown in Fig. 3, and the combined influence of IFP and lymph node status is shown in Fig. 4.

The prognostic effect of IFP was also evaluated in relation to clinical factors and tumor oxygenation. A third multivariate model was constructed in which both IFP and HP₅ were added to the clinical model. IFP remained significant (risk ratio = 1.05; $P = 0.0027$) and was a stronger independent predictor of DFS than HP₅ (risk ratio = 1.004; $P = 0.52$). As described elsewhere,⁴ a detailed analysis of the relationship between HP₅ alone and the significant clinical factors of tumor size and pelvic lymph node status revealed HP₅ to be an important determinant of outcome only in patients with negative

nodes. Therefore, the importance of IFP was also tested in this subgroup. IFP and HP₅ both contributed independently to outcome and provided additional prognostic information compared with clinical evaluation of tumor size alone. Fig. 5 shows DFS stratified by both IFP and HP₅ in node-negative patients. The multivariate models for DFS are summarized in Table 2.

With respect to overall survival, tumor size was the only clinical variable predictive of outcome. There was no detectable influence of pelvic lymph node status. There was a suggestion that IFP, when added to size in the Cox model, provided additional prognostic information, but this failed to achieve statistical significance (risk ratio = 1.04; $P = 0.07$; Table 3).

The site of first recurrence was pelvis alone in 18 patients, pelvis plus distant (outside of the irradiated pelvic volume) in 13 patients, and distant alone in 14 patients. The cumulative incidence curves for local and distant recurrence are shown in Fig. 6, a and b, respectively. There was a trend toward a higher likelihood of local recurrence in patients with IFPs greater than the median value of 19 mm Hg (38% versus 24% at 3 years; $P = 0.068$) and a significantly higher risk of distant recurrence (47% versus 13%; $P = 0.0014$). Multivariate analysis showed IFP to be significantly associated with both local recurrence ($P = 0.011$) and distant recurrence ($P = 0.011$), independent of tumor size and lymph node status. The multivariate analyses for local and distant recurrence are summarized in Table 3. The fact that IFP appears to be only marginally predictive of local control by univariate analysis but strongly predictive by multivariate analysis mainly re-

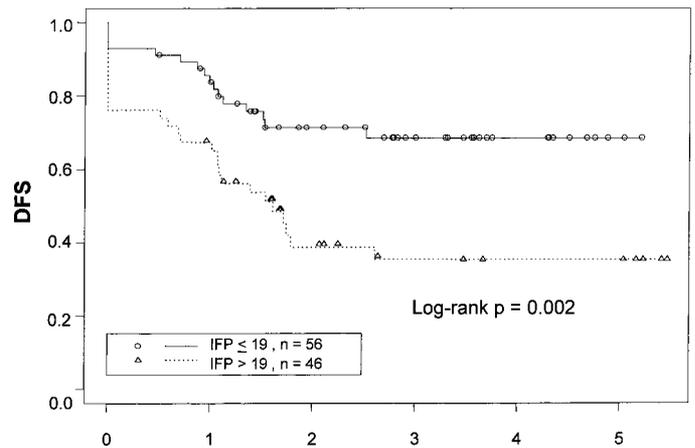


Fig. 2. DFS stratified by IFP.

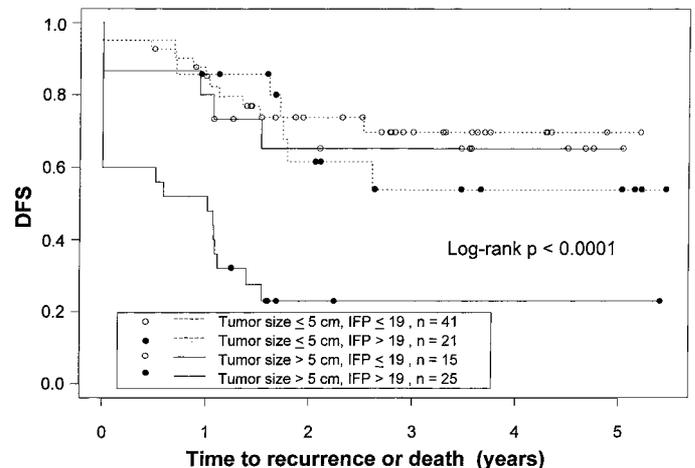


Fig. 3. DFS stratified by IFP and maximum tumor size.

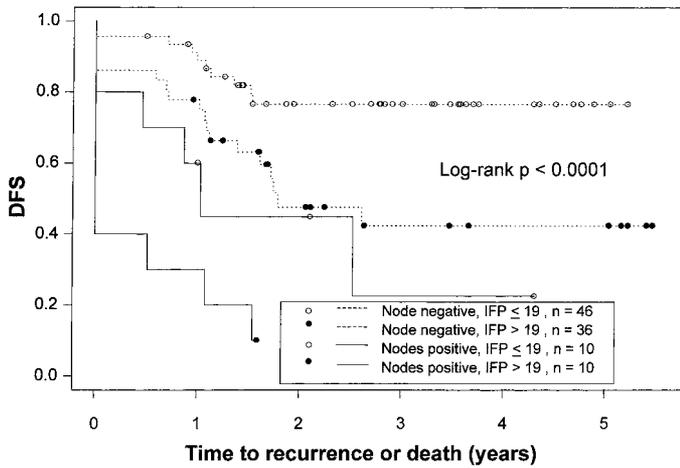


Fig. 4. DFS stratified by IFP and pelvic lymph node status.

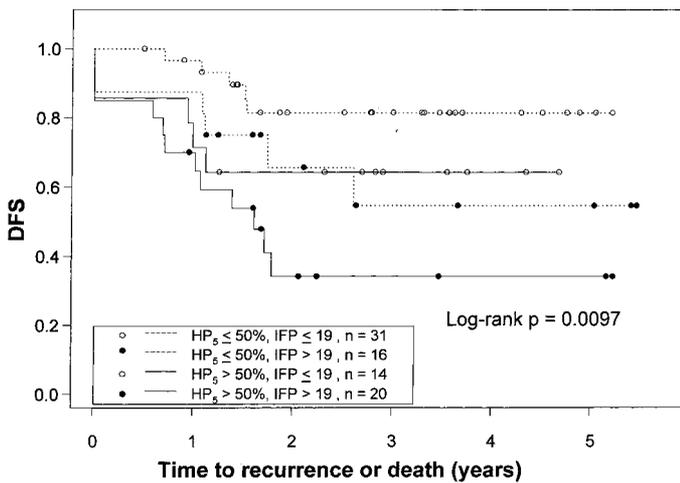


Fig. 5. DFS stratified by IFP and HP₅ in patients with negative or suspicious pelvic lymph nodes at diagnosis.

Table 2 Multivariate prognostic factor models for DFS

Parameter	Clinical ^a	Clinical + IFP ^a	Clinical + HP ₅ + IFP ^a	Clinical + HP ₅ + IFP ^a (node neg)
Tumor size ^b	0.0003	0.0027	0.0028	0.0014
Pelvic LN status ^c	0.0016	0.0002	0.0015	
HP ₅ ^b			0.5183	0.027
IFP ^b		0.0013	0.0027	0.01

^a P, Cox proportional hazards model.
^b Continuous variable.
^c Categorical variable; LN, lymph node.

flects inherent differences in the two techniques of analysis. Patients were divided into two groups above and below the median IFP value for the univariate analysis as reflected in Fig. 6a, with loss of some information about the relationship between IFP and local control. However, IFP was treated as a continuous variable in the multivariate analysis with preservation of all of the possible information in the data set.

DISCUSSION

Elevated IFP has long been recognized as a characteristic of solid malignant tumors (21). Measurements in a variety of human tumors have yielded values in the range of 0 to 100 mm Hg (7, 12–18, 21).

However, the importance of IFP as a predictor of patient outcome after treatment has not been described previously. This report is the first to demonstrate a strong association between elevated pretreatment IFP in cervix cancer and patient survival after radiotherapy that is independent of both clinical prognostic factors and tumor oxygenation. The results imply that IFP is a clinically relevant physiological parameter of solid tumors that provides unique information about response to radiotherapy and overall patient prognosis. In addition, the results suggest that IFP may also be useful as a means of selecting patients for novel treatment strategies targeted at the tumor vasculature.

The IFP in any tissue is determined by the interaction of a variety of intrinsic physiological parameters that ultimately determine the capillary pressure, the resistance to fluid leakage across the capillary walls, and the resistance to fluid percolation through the interstitium to the periphery of the tumor (1, 5, 22, 23). IFP is near zero in most normal tissues, regardless of the capillary pressure, because capillary permeability is low. Also, there is an osmotic gradient that favors the flow of interstitial fluid back into the vasculature, and there is a rich lymphatic network that permeates the tissue and drains any residual interstitial fluid into the central venous circulation (24). In contrast, tumors usually have abnormal, highly permeable capillaries and lymphatic channels that, although present, have impaired function to the point of being unable to adequately drain excess fluid from the

Table 3 Multivariate prognostic factor models for overall survival, local relapse-free rate, and distant relapse-free rate

Parameter	Overall survival ^a	Local relapse-free ^a	Distant relapse-free ^a
Tumor size ^b	0.0081	0.009	0.0078
Pelvic LN status ^c		0.0069	0.033
IFP ^b	0.07	0.011	0.011

^a P, Cox proportional hazards model.
^b Continuous variable.
^c Categorical variable; LN, lymph node.

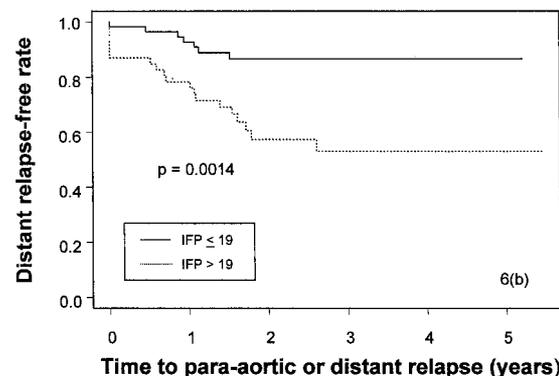
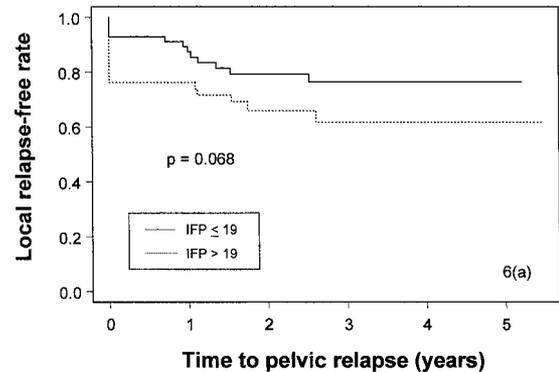


Fig. 6. Cumulative risk of local (a) and distant (b) recurrence stratified by IFP.

interstitium (2, 3, 25). Therefore, fluid that leaks from the vasculature accumulates in the interstitium and distends the elastic interstitial matrix causing the pressure to rise. In many tumors, the interstitial resistance greatly exceeds the capillary wall resistance, leading to a situation where the IFP nearly equals the capillary pressure (26).

Capillary pressure generally reflects capillary resistance to blood flow, which is higher in tumors than in the corresponding normal tissues for many reasons, including abnormal vessel structure and organization and high capillary blood viscosity (27–30). It has been hypothesized that the accumulation of excess fluid in the interstitium of tumors, which is the underlying cause of elevated IFP, may exert forces that lead directly to compression of tumor capillaries and increased flow resistance (4, 5, 31). Another theory suggests that tumor growth in a confined space leads to increased solid tissue pressure, which in turn causes capillary compression, elevated IFP, and reduced tumor blood flow (31–33). It is likely that all of these mechanisms contribute to a greater or lesser degree to the high flow resistance in tumors. Differences in IFP from tumor to tumor, by virtue of the fact that IFP closely tracks the average capillary pressure, provide a relative indication of flow resistance and tumor perfusion.

An earlier analysis of the first 77 patients accrued to this study showed a weak inverse correlation between IFP and tumor oxygenation (7), in keeping with an inverse relationship between IFP and blood flow. However, with further accrual, the strength of this association has diminished to the point where it is no longer significant, as indicated in Fig. 1. Studies of IFP and oxygenation in animal tumors have similarly failed to show a correlation between these parameters (34–36). This suggests that the strong prognostic effect of IFP seen in this study is not primarily mediated through differences in tumor oxygenation. However, it does not exclude the possibility that the high capillary resistance associated with elevated IFP is one of many factors contributing to the development of hypoxia in tumors. High IFP is probably indicative of a chronic limitation in tumor blood flow, superimposed on a background of multiple other factors that influence oxygenation independent of perfusion, including hemoglobin oxygen saturation, longitudinal oxygen gradients within the tumor microvasculature, long oxygen diffusion distances, and oxygen consumption by tumor cells (37–39). Random tumor-to-tumor variability in these other parameters would tend to mask any underlying association between IFP and oxygenation, making it difficult to detect in clinical or laboratory experiments.

Although it is possible that IFP and Eppendorf measurement provide independent information about different aspects of tumor oxygenation to explain the results observed in this study, it is also possible that IFP provides information about tumor physiology that is largely unrelated to perfusion and pretreatment oxygen status. Many of the characteristics of tumors that lead to elevated IFP, including abnormal vessel structure and organization and high vascular permeability, arise because of unregulated angiogenesis. This suggests that IFP measurements might provide an integrated measure of the physiological consequences of angiogenesis from one tumor to the next and unique functional information that is not provided by other indicators of angiogenesis such as histological vascular density. The prognostic effect of IFP seen in this study may reflect the more aggressive overall behavior of tumors with high angiogenic activity that is mediated through the interaction of a variety of fundamental physiological and molecular mechanisms including impaired tumor perfusion, the development of hypoxia and acidosis, greater genetic instability, and a greater capacity to form distant metastases. In addition, it has been demonstrated recently that vascular endothelial growth factor, a potent angiogenic protein that is important in the development of both the abnormal tumor vasculature and elevated IFP (40), is induced by ionizing radiation in some tumors and may protect the vasculature

from radiation-induced endothelial cell killing under both oxic and hypoxic conditions (40, 41). A clearer understanding of the relationship between IFP and the tumor vasculature would help to explain the strong prognostic effect of IFP that was observed in this study.

In summary, our results provide the first evidence that IFP measurements yield important, clinically relevant biological information about cervix cancer that is independent of both standard prognostic factors and tumor oxygenation. Patients in this study were treated with radiotherapy alone, and those with high-IFP tumors were more likely to recur both within the pelvis and at previously untreated distant sites. The results may or may not be applicable to cervix cancer patients treated with radiation and concurrent cisplatin chemotherapy, which is now standard practice in many North American centers, and to other tumors that may differ pathophysiologically from cervix cancer. Additional laboratory and clinical studies are needed to better understand the mechanisms by which IFP predicts survival of patients with cervix cancer who undergo radiotherapy and to evaluate the prognostic effect of IFP measurements in patients treated surgically. These studies are a prerequisite to using IFP measurements as a means of selecting patients for specific treatments aimed at overcoming the adverse prognostic effect of high IFP and improving survival.

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