

Oxygenation of Carcinomas of the Uterine Cervix: Evaluation by Computerized O₂ Tension Measurements

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ABSTRACT

Direct oxygen partial pressure (pO₂) readings in cancers of the cervix and in the normal cervix of nulliparous or parous women were obtained using a computerized pO₂ histography system. The oxygenation status of the tumors was evaluated as a function of clinical staging and histological grading. pO₂ measurements were performed with a customized electrode system in conscious pre- and postmenopausal, untreated patients with well-defined arterial blood gas status.

With this technique, pO₂ measurements in the normal cervix of nulliparous women resulted in oxygenation patterns which were characteristic for normal, adequately supplied tissues (median pO₂, 48 mm Hg) with approximately 1% of the pO₂ values grouped between zero and 2.5 mm Hg, *i.e.*, in a range with less than half-maximum radiosensitivity. As a rule, the mean (and median) pO₂ values were distinctly lower in the normal cervix of parous women (most probably due to scar formation following vaginal delivery) and in malignancies. In the normal cervix of parous women the median pO₂ value was 13 mm Hg (with approximately 14% of the pO₂ readings in the lowest class), 14 mm Hg in International Federation of Gynecologists and Obstetricians I/II tumors (2% of the readings in the lowest pO₂ class), and 11 mm Hg in International Federation of Gynecologists and Obstetricians III/IV cancers (1% of the pO₂ data in the lowest class). To date, 5 of 18 cervical cancers exhibited pO₂ values between zero and 2.5 mm Hg. The oxygenation pattern in cervical cancers and the occurrence of hypoxia and/or anoxia did not correlate with either the clinical stages and histological grades or with a series of clinically relevant parameters (*e.g.*, tumor size). No significant differences were found between pre- and postmenopausal tumors, between squamous cell carcinomas and adenocarcinomas, and between endophytic or exophytic tumors.

From these studies there is clear indication that the oxygenation status of individual tumors cannot be predicted on the basis of staging and/or grading, predominantly because of the pronounced tumor-to-tumor variabilities. Evaluation of the tissue oxygenation of individual tumors is thus mandatory to prove that tumor oxygenation can predict the overall prognosis and/or treatment outcome.

INTRODUCTION

It has recently been shown that the oxygenation pattern in human breast cancers and the occurrence of hypoxia and/or anoxia does not correlate with either the pathological stages or the histological grades of the tumors investigated (1). In earlier studies Kolstad (2, 3) demonstrated a correlation between the mean and lowest observed tissue oxygen tensions and the stage of disease in superficial regions of cervical cancers. Furthermore, Kolstad's data suggested that an association existed between large intercapillary distances, local recurrence and clinical staging. The latter finding has been confirmed by Awwad *et al.* (4) and by other investigations emphasizing the predictive value of the vascular density (which may reflect the tumor oxygenation) for survival after radiotherapy (5-8).

Thus far, pO₂ values determined in cervical cancers cannot be considered correct on an absolute scale (3, 9-12). Artifacts

due to large electrodes and thus tissue compression (11), problems with the calibration of the sensor (10), and the use of bare electrodes (9, 10, 12) are obvious in the earlier studies. Bare sensors monitor O₂ concentrations instead of O₂ partial pressures (O₂ tensions). For the evaluation of oxygen tension (pO₂) distribution in tissues, membrane-covered O₂ sensors are required (13). A direct conversion of O₂ concentrations (cO₂) into pO₂ values according to the Henry-Dalton law (cO₂ = $\alpha \cdot pO_2$) is not possible, since the O₂ solubility coefficient (α) is anisotropic in tissues (14). In addition, the validity of the O₂ data reported earlier is limited due to measurements in the tumor periphery only (3, 9-12) and due to a selection of patients with stage II malignancies (9-12). Reports on the oxygenation pattern of advanced malignancies are lacking. Furthermore, monitoring of the arterial blood gas status, which is mandatory for the evaluation of tissue oxygenation patterns, has not been performed in the earlier studies.

Reliable pO₂ readings in normal and malignant tissues are now possible using a novel, commercially available technique (computerized pO₂ histography) which allows for the systematic evaluation of the tissue oxygenation status. The measuring procedure uses a computerized O₂ sensor movement, minimizes compression artifacts, and has been shown to represent a valid method for analysis of tissue oxygenation patterns (for a recent description of this technique see Ref. 1).

Using this novel technique, the objective of this study was to investigate the oxygenation pattern in primary cancers of the uterine cervix in conscious patients, placing emphasis on a well defined arterial blood gas status and on the evaluation of the pO₂ distribution as a function of clinical staging and histological grading. Cervical carcinomas were selected as a tumor entity because radiation is a major treatment modality for this disease and tumor hypoxia is regarded an important factor for radioresistance in these tumors. In addition, accessibility of these malignancies allows systematic studies under clinical conditions.

The key questions were as follows: Do the pO₂ data obtained with this method match with earlier results suggesting low mean O₂ tensions or concentrations in cervical cancers? What are the absolute pO₂ values in cervical cancers as a function of clinical staging and histological grading? Are there differences in the oxygenation between squamous cell carcinomas and adenocarcinomas? Other key issues of this investigation were the comparison of the oxygenation pattern in normal cervical tissue and in malignancies, of the pO₂ distribution in the normal cervix of *para versus* nullipara, and the evaluation of O₂ patterns in central *versus* superficial tumor regions.

MATERIALS AND METHODS

Patients. As part of a prospective study on untreated patients with cancer of the uterine cervix which aims to assess the relevance of pO₂ measurements in individual tumors for local control after therapy, we have evaluated intratumor pO₂ measurements in 7 pre- and 11 postmenopausal conscious patients (age, 27-80 years) entering the study. Fifteen women presented with histologically confirmed squamous cell

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movement was immediately followed by a backward step of 30% of the forward motion in order to minimize compression effects caused by the forward motion of the O₂-sensitive needle electrode. This motion pattern led to an effective forward step length of maximally 0.7 mm. Depending on the tumor size, a sufficient number, *n*, of pO₂ readings (*n* > 50) were obtained in order to present the data by means of pO₂ frequency distributions (pO₂ histograms) which can reflect the oxygenation pattern of individual tumors.

The total length of the measuring canal corresponded to the length of the cervix or to the length of the cervical tumor if the latter did not exceed 40 mm. Otherwise a measuring length equal to one-half of the sagittal cervix/tumor diameter was used. At the end of the measurement the pO₂ probe was automatically removed from the tissue. In cancer patients the pO₂ measurements were carried out immediately before initiation of therapy. All measurements were conducted on conscious women not undergoing any cancer treatment.

Monitoring of Relevant Systemic Parameters. In order to guarantee that tissue pO₂ measurements were exclusively performed during arterial normoxemia, basic cardiovascular parameters (e.g., heart rate, arterial blood pressure) and factors determining the arterial O₂ concentration (e.g., hemoglobin concentration, hematocrit, arterial oxyhemoglobin saturation) were monitored during the measuring procedures (1).

Statistical Analysis. Using an on-line computing system, pO₂ histograms (i.e., pO₂ frequency distributions) were obtained with a class width of 2.5 mm Hg. The distribution of the measured pO₂ values was characterized by the mean pO₂, the median pO₂, and the 10 and 90 percentile values. For statistical evaluation of possible differences between groups the Mann-Whitney-Wilcoxon *U* test was used, if not stated otherwise.

RESULTS

Using the computerized pO₂ histography system, pO₂ values were recorded in the normal cervix of nullipara and para and in cancers of the uterine cervix of different clinical stages and histological grades. As a rule, the mean (and median) pO₂ values were distinctly lower in the malignancies and in the normal cervix of parous women than in the normal cervix of nulliparous women (see Figs. 1 and 2). The histograms for parous and nulliparous, healthy subjects were significantly different (*P* < 0.02). No differences in the oxygenation pattern of the normal uterine cervix were found between smokers and nonsmokers or users and nonusers of oral contraceptives.

In cancer patients, no differences in the pO₂ distribution were obvious between adenocarcinomas and squamous cell carcinomas; among endophytic (*n* = 4), exophytic (*n* = 8), and ulcerated tumors (*n* = 6); between para (*n* = 15) and nullipara (*n* = 3); or between pre- and postmenopausal patients. Pooled pO₂ histograms in cancer patients were similar to those obtained in the normal cervix of parous women. Thus far, 5 of 18 cervical cancers exhibited pO₂ values between zero and 2.5 mm Hg, i.e., tissue areas with less than half-maximum radiosensitivity. One of 7 normal cervixes in nulliparas and 3 of 7 cervixes in paras exhibited pO₂ values between zero and 2.5 mm Hg.

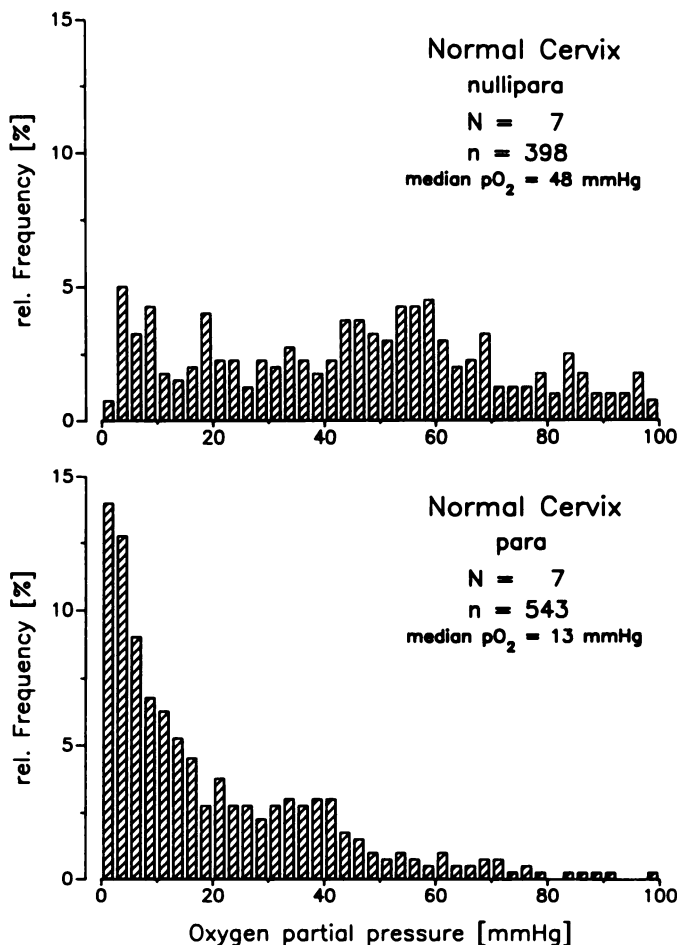


Fig. 1. Frequency distributions of measured oxygen partial pressures (pO₂ histograms) for normal cervix of nulliparous (top) and parous (bottom) women. *N*, number of patients; *n*, number of pO₂ measurements.

Pooled data for all cervical cancers of clinical stages FIGO I and II are presented in Fig. 2, top, whereas the respective pO₂ histogram for malignancies of stages FIGO III and IV are shown in Fig. 2, bottom. This compilation provides clear evidence that there are no statistically significant differences between the two groups (median pO₂ in FIGO I/II tumors, 14 mm Hg; median pO₂ in FIGO III/IV tumors, 11 mm Hg). This implies that the clinical stages of the cervical cancers investigated thus far cannot be the paramount factor determining tumor tissue oxygenation. Surprisingly, the number of pO₂ readings in the "hypoxic" class (0–2.5 mm Hg) is lower in FIGO III/IV than in FIGO I/II tumors.

Based on the oxygenation pattern of the malignancies investigated there is substantial experimental evidence that the occurrence of radiobiological hypoxia does not correlate with the

Table 2 Characteristics of healthy patients undergoing computerized pO₂ histography of the uterine cervix

	AK	BW	PF	JB	RM	FS	EG	UH	CH	NS	UM	NM	MM	KS
Age (yr)	29	27	35	50	42	83	44	23	23	22	29	23	26	41
Menopausal stage	Pre	Pre	Pre	Post	Pre	Post	Post	Pre	Pre	Pre	Pre	Pre	Pre	Pre
Parous status	p ^a	p	p	p	p	p	p	np	np	np	np	np	np	np
Mean pO ₂ (mm Hg)	13	24	11	8	25	41	22	59	11	48	81	40	26	59
Median pO ₂ (mm Hg)	4	18	4	7	18	39	14	53	7	46	84	34	19	57
10 percentile (mm Hg)	0	6	0	3	5	13	11	35	3	18	53	19	8	47
90 percentile (mm Hg)	38	43	32	13	64	64	47	89	24	86	108	69	55	74
pO ₂ readings between 0 and 2.5 mm Hg (%)	43	48	30	0	0	0	0	0	5	0	0	0	0	0

^a p, para; np, nullipara.

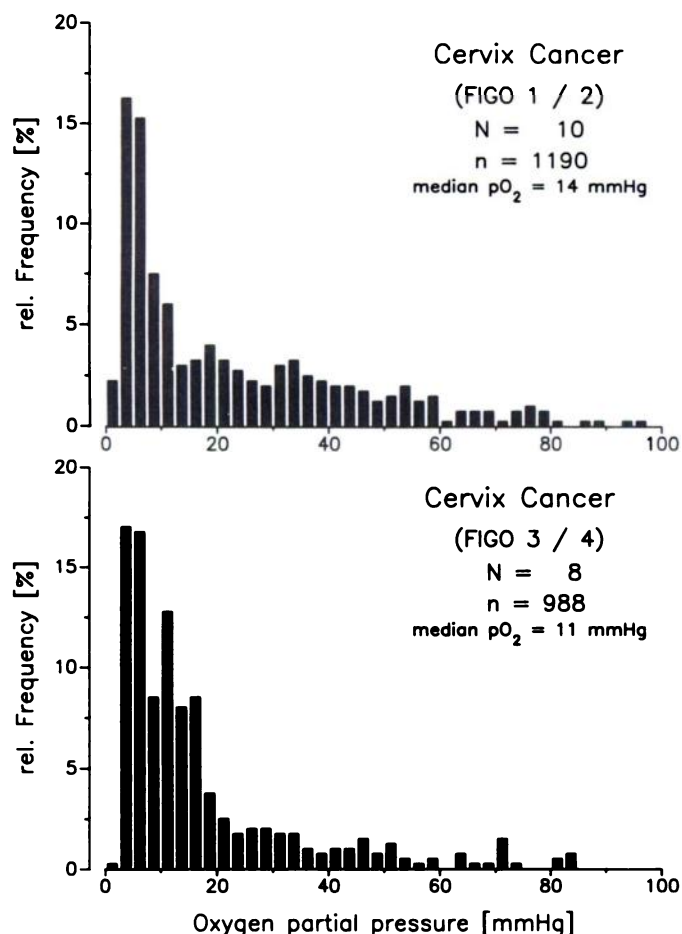


Fig. 2. pO_2 histograms derived from cervical cancers of different clinical stages (*top*, FIGO I/II; *bottom*, FIGO III/IV). N , number of patients; n , number of measured pO_2 values.

clinical stage and the histological grade and other clinically relevant parameters. Correlations between staging or grading and the number of pO_2 readings on zero level, the mean or median pO_2 , and the 10% or 90% percentiles could not be detected.

There is marked tumor-to-tumor variability, even if tumors of the same clinical stage (FIGO II), grade (G2), and histology (squamous cell carcinomas) are compared (see Fig. 3). In the case presented in Fig. 3, *top*, all measured pO_2 values are higher than 18 mm Hg and thus indicative of a relative radiosensitivity >2.4 (17). In contrast, oxygenation of the cervical cancer shown in Fig. 3, *bottom*, contains a significant volume of tissue (at least 20%) with less than half-maximum radiosensitivity.

As a result of intratumor heterogeneities, a certain number of pO_2 measurements in different electrode tracks through a tumor is required in order to obtain sufficient information on the oxygenation status of an individual tumor. In 5 patients we compared the pO_2 histograms obtained from 2 "standard" electrode tracks (at 6 and 12 o'clock positions) with those evaluated from multiple tracks (up to 10 additional electrode passages). No significant differences were found when >40 pO_2 values were recorded per track. If the lowest pO_2 class (0–2.5 mm Hg) is considered, there were no differences when multiple *versus* "standard" electrode tracks were compared. Additionally, similar pO_2 histograms were usually obtained when the same tumor region was measured repeatedly during a 1-day observation period using different O_2 -sensitive electrodes.

In the cervical cancers studied, high and low pO_2 values were randomly distributed in both tumor periphery and center.

DISCUSSION

Oxygen tension measurements were performed in the normal cervix and in cervical cancers of conscious women using a novel polarographic device capable of reliable tissue pO_2 determinations. In the normal cervix of nullipara, during controlled arterial blood gas status, a pO_2 distribution was found similar to that of most normal tissues (1, 17). In contrast, a left-shifted pO_2 histogram with a reduction of median oxygen levels and an asymmetry toward lower pO_2 values was observed in the cervix of para. The existence of tissue areas with less than half-maximum radiosensitivity (approximately 13% of the pO_2 readings) is a finding which has not been addressed previously and, in addition, had not been expected by earlier investigators. This pronounced left shift of the pO_2 histogram most probably is due to the scar formation in the cervix after vaginal delivery(ies). The biological and clinical significance of the lower oxygenation status of parous women is not clear at present and deserves further investigation.

When comparing the tissue oxygenation of cervical cancers (FIGO I-IV) with that found in the normal cervix of parous women, a clear-cut difference was not observed. In both instances, left-shifted pO_2 distributions were found with a higher percentage of pO_2 readings in lowest pO_2 class (0–2.5 mm Hg) in the normal cervix of parous women.

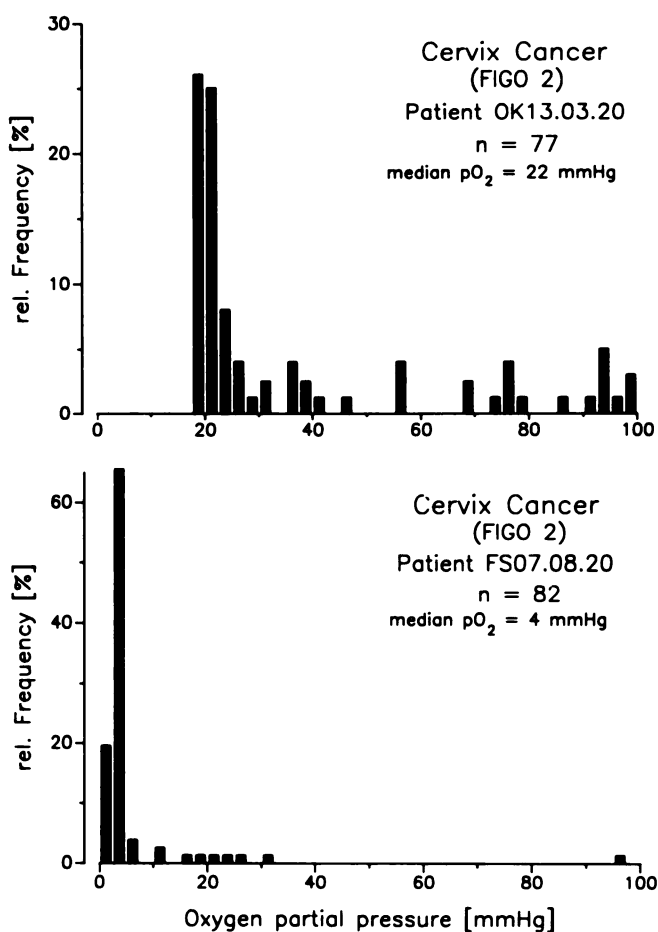


Fig. 3. pO_2 frequency distributions of two squamous cell carcinomas of the cervix (stage FIGO II, grade G2) substantiating marked tumor-to-tumor variability in the oxygenation status. n , number of pO_2 recordings.

Table 3 Tissue oxygen tension in the normal cervix and in cervical cancer of patients (n = number of patients)

Condition	pO ₂ (mm Hg)	n	Refs.
Normal cervix	41 (4-79) ^a	48	2, 3
Nullipara	48 ^b	7	This study
Para	13 ^b	7	This study
Cervix cancer			
Stage 0	26 ^a	33	2, 3
Stage I	17 ^a	16	2, 3
Stage II	19 ^a	19	2, 3
	14 ^b	9	This study
Stage III	12 ^b	7	This study

^a Estimated mean (range); measurement with bare electrodes in superficial tissue regions.

^b Median pO₂ value.

When stage II and III tumors are considered, almost identical median pO₂ values compared with the data evaluated for normal cervix of para were found (see Table 3). Only when the tumor data are related to the median pO₂ value of the normal cervix of nullipara is a ratio of approximately 3.4 for stage II tumors obtained. This latter ratio is distinctly higher than that reported earlier for stage II cervical cancers [2.2 (2) and 2.5 (10)]. This difference may partly be due to the fact that in the latter studies no distinction was made between nulliparous and parous patients.

As was the case with breast cancers (1), the oxygenation pattern and the occurrence of low pO₂ readings did not correlate with either the clinical stages and histological grades or with the tumor histology (squamous cell carcinomas *versus* adenocarcinomas), the location of the pO₂ measurements (center *versus* periphery), and a series of other clinically relevant factors. Again, from these clinical studies there is clear indication that the oxygenation status of individual tumors before therapy cannot be predicted solely on the basis of staging and/or grading. This is mostly due to pronounced tumor-to-tumor (and intratumor) pO₂ variabilities. Evaluation of the tissue oxygenation of individual tumors is thus mandatory to prove that tumor oxygenation can predict the overall prognosis and/or treatment outcome.

When comparing the oxygenation pattern of breast cancers (pathological stages T1-4) with that of cervical cancers (clinical stages FIGO I-IV), one striking difference has to be mentioned. Whereas the number of pO₂ readings in the lowest class is almost identical in the cervix and breast cancers investigated (2 *versus* 3%), the two pO₂ histograms are significantly different ($P < 0.005$), with a better oxygenation in the breast cancers (White test) (18, 19). This may be at least partially due to the larger size of the cervical cancers investigated (mean maximum diameter in cervical cancers, 6.1 cm *versus* 3.1 cm in breast cancers; $P < 0.0001$). However, when the cervix and breast

cancers are considered as separate groups there is thus far no indication that a correlation exists between median pO₂ values and tumor sizes.

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