Oxygenation of Spontaneous Canine Tumors During Fractionated Radiation Therapy*

Roger E. Achermann[1], Stefanie M. Ohlerth[1], Carla Rohrer Bley[1], Max Gassmann[2], Natalie Inteeworn[1], Malgorzata Roos[3], Myriam Schärz[1], Melanie C. Wergin[1], Barbara Kaser-Hotz[1]

Background and Purpose: Tumor oxygenation predicts treatment outcome, and reoxygenation is considered important in the efficacy of fractionated radiation therapy. Therefore, the purpose of this study was to document the changes of the oxygenation status in spontaneous canine tumors during fractionated radiation therapy using polarographic needle electrodes.
Material and Methods: Tumor oxygen partial pressure (pO_2) measurements were performed with the Eppendorf-pO_2-Histograph. The measurements were done under general anesthesia, and probe tracks were guided with ultrasound. pO_2 was measured before radiation therapy in all dogs. In patients treated with curative intent, measurements were done sequentially up to eight times (total dose: 45–59.5 Gy). Oxygenation status of the palliative patient group was examined before each fraction of radiation therapy up to five times (total dose: 24–30 Gy).
Results: 15/26 tumors had a pretreatment median $pO_2 \leq 10$ mmHg. The pO_2 values appeared to be quite variable in individual tumors during fractionated radiation therapy. The pO_2 of initially hypoxic tumors (pretreatment median $pO_2 \leq 10$ mmHg) remained unchanged during fractionated radiotherapy, whereas in initially normoxic tumors the pO_2 decreased.
Conclusion: Hypoxia is common in spontaneous canine tumors, as 57.7% of the recorded values were ≤ 10 mmHg. The data of this study showed that initially hypoxic tumors remained hypoxic, whereas normoxic tumors became more hypoxic.

Key Words: Hypoxia · Polarographic needle electrode · Fractionated radiation therapy · Dog

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Oxygenierung spontaner Tumoren beim Hund unter fraktionierter Radiotherapie

Hintergrund und Ziel: Die Sauerstoffversorgung von Tumoren ist ein wichtiger prognostischer Faktor. Die Reoxygenierung von Tumoren nach Bestrahlung wird als wichtiger Mechanismus bei der fraktionierten Radiotherapie angesehen. Ziel der Studie war es, die Veränderungen in der Oxygenierung von spontan gewachsenen Tumoren des Hundes unter fraktionierter Radiotherapie zu beschreiben.
Material und Methodik: Der Sauerstoffpartialdruck (pO_2) wurde polarographisch mit dem Eppendorf-pO_2-Histographen gemessen. Die Messungen erfolgten unter Vollnarkose und Ultraschallkontrolle. Die pO_2-Werte wurden vor der Strahlentherapie gemessen. Bei Hunden mit kurativem Behandlungsschema wurden bis zu acht sequentielle Messungen während der Strahlentherapie durchgeführt (Gesamtdosis: 45–59.5 Gy). Der Oxygenierungsstatus der Tumoren mit palliativem Protokoll wurde vor jeder Fraktion bis zu fünfmal bestimmt (Gesamtdosis: 24–30 Gy).
Ergebnisse: 15/26 Tumoren hatten vor Beginn der Strahlentherapie einen medianen $pO_2 \leq 10$ mmHg. Der Verlauf der pO_2-Werte während fraktionierter Radiotherapie erschien in individuellen Tumoren ziemlich variabel. Wurden die Tumoren in initial hypoxisch (prätherapeutischer medianer $pO_2 \leq 10$ mmHg) und normoxisch unterteilt, so zeigte sich, dass die pO_2-Werte der hypoxischen Tumoren unverändert blieben, während normoxische Tumoren eine Abnahme des pO_2 zeigten.
Schlussfolgerung: Hypoxie ist normalerweise in spontanen Tumoren des Hundes messbar, lagen doch fast 60% der gemessenen Werte ≤ 10 mmHg. Die Daten dieser Studie zeigten, dass initial hypoxische Tumoren unter fraktionierter Radiotherapie hypoxisch blieben, während initial normoxische Tumoren vermehrt hypoxisch wurden.

Schlüsselwörter: Hypoxie · Polarographische Feinnadelsonden · Fraktionierte Bestrahlung · Hund

*This study was conducted in the context of a doctoral thesis (R.E. Achermann).

[1] Section of Diagnostic Imaging and Radiation Oncology, Veterinary School, University of Zurich, Switzerland,
[2] Institute for Veterinary Physiology, University of Zurich, Switzerland,
[3] Institute for Social and Preventive Medicine, University of Zurich, Switzerland.

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Introduction

Tumor oxygenation status plays a major role in cancer therapy. The success of radiation therapy is, in part, dependent on the oxygen supply to a tumor [19, 44]. The assessment of pretreatment oxygenation status has been shown to be of prognostic relevance for tumor recurrence, metastases, and survival [9, 15, 21, 36]. According to the oxygen fixation hypothesis, the radiation-induced damage to the DNA can be fixed in the presence of oxygen but repaired under hypoxic conditions [20]. Hypoxic tumors, therefore, need a two- to threefold higher radiation dose than normoxic tumors to kill the same amount of tumor cells [19]. Reoxygenation, the process by which the hypoxic cell fraction becomes oxygenated after ionizing radiation, is considered to be a major factor in the efficacy of fractionated radiotherapy. Proposed mechanisms thought to be responsible for the process of reoxygenation are changes in tumor blood flow and vascular architecture, reduced interstitial pressure, and a decrease in cellular oxygen consumption [5, 26, 28, 33, 44]. However, the complete mechanism of reoxygenation has not been fully understood yet. Contradictory results have been reported in a limited number of studies, in which changes in tumor oxygenation have been measured during fractionated radiotherapy. An increase of mean oxygen tension after irradiation has been reported in early clinical studies in the 1960s describing oxygen partial pressure (pO_2) measurements during fractionated radiation therapy [2, 10]. The value of these pioneer investigations is limited because of the technical difficulties in measuring pO_2 in the tumor tissue. The electrodes used caused compression artifacts, and the pO_2 measurements could not be performed at multiple sites within the tumor tissue. For example, Badib & Webster [2], only recorded one pO_2 value per tumor oxygen measurement. This study did not reflect the heterogeneity of pO_2 values in tumors shown by several other studies [35, 43, 45]. Various experimental studies measuring changes of pO_2 in rodent tumors documented a change of the oxygenation status during fractionated radiation therapy [3, 42, 48]. However, only a few clinical studies have been performed. Because of limited patient compliance, measurements often could only be performed pre-, mid- and posttreatment; therefore, interpretation of these results is difficult [11, 13, 17, 31].

While Lartigau et al. [29] reported an increase of tissue pO_2 after 32 Gy of accelerated radiation therapy of head and neck carcinoma, Stadler et al. [41] found a significant reduction of pO_2 levels after a radiation dose of 30 Gy in the same tumor group. Brizel et al. [7] performed a second set of pO_2 measurements in 27 human patients suffering from head and neck squamous cell carcinoma and reported no changes in oxygenation after exposure to 10–15 Gy. Vujaskovic et al. [47] measured tumor oxygenation before and after hyperthermia treatment in spontaneous canine soft tissue sarcomas. This study showed an increase of the median pO_2 after a low (< 44 °C) median tumor temperature mainly through improved perfusion, whereas a decrease of the median pO_2 was observed after a high (> 44 °C) median tumor temperature. The pO_2 values appeared to be quite variable in individual tumors during fractionated radiation therapy. Becker et al. [4] reported a significant increase of tumor oxygenation after hyperbaric oxygenation in seven human patients.

Pretreatment measurements of the oxygenation status in canine tumors have been previously performed by us using polarographic oxygen needle electrodes [1]. The study showed that four out of nine spontaneous canine soft tissue sarcomas were severely hypoxic ($pO_2 \leq 2.5$ mmHg). We concluded that the dog could serve as a reliable model for repeated measurements in order to get more information on changes of pO_2 during fractionated radiation therapy. Since there is a wide inter- and intratumoral heterogeneity, repeated measurements during radiotherapy are necessary to increase knowledge about tumor oxygenation [35, 43, 45]. Furthermore, information on the oxygenation status may play an important role when adjuvant anti-angiogenetic agents or hypoxic cell toxins are considered to be used in combination with radiation therapy, because tumor oxygenation status represents the most important stimulus for angiogenesis [14].

Therefore, the aim of our study was to document the changes of the oxygenation status in spontaneous canine tumors during fractionated radiation therapy using polarographic needle electrodes.

Material and Methods

Patients

26 dogs with malignant tumors of spontaneous origin were included in the study (Table 1). All dogs were scheduled for fractionated radiation therapy. Patient age ranged from 3 to 13 years with a mean of 8.7 years. The dogs' mean weight was 32.4 kg (range: 11.1–66 kg).

All dogs underwent physical examination, thoracic radiography, and complete blood analysis. Further diagnostic workup was performed as indicated. The mean value of erythrocyte concentration (Ec) was 6.6×10^6/µl (range: 4.3–8.9 $\times 10^6$/ml; normal: 5.5–8.5 $\times 10^6$/µl), the mean packed cell volume (PCV) 43.1% (range: 29–55%; normal: 37–55%), and the mean hemoglobin level (Hb) 15.1 g/dl (range: 10.0–20.1 g/dl; normal: 12–18 g/dl). The mean value of mean corpuscular volume (MCV) was 65.9 fl (range: 54–70.7 fl; normal: 60–77 fl), the mean level of corpuscular hemoglobin (MCH) 23.1 pg (range: 19–25 pg; normal: 19.5–24.5 pg), and mean corpuscular hemoglobin concentration (MCHC) 35.1 g/dl (range: 33–36.6 g/dl; normal: 32–36 g/dl). The length, width, and depth of the tumors were measured before radiation therapy. The volume was calculated using the rotation ellipsoid formula ($\pi abc/6$). The initial tumor response at the end of fractionated radiation therapy was defined as no response (unchanged tumor volume), partial response (decreased tumor volume), or complete response (no macroscopic tumor disease). Individual characteristics of the measured tumors are presented in Table 2.

In 20 of the 26 dogs, repeated oxygenation measurements could be performed during fractionated radiation therapy. In the remaining six patients, repeated measurements could not be done due to various reasons. Patient #18 was excluded from the longitudinal analyses because of unreliable repeated pO_2 measurements due to extensive bleeding from the tumor tissue. 13 of the 20 dogs with repeated measurements were treated with a palliative irradiation protocol, and seven dogs underwent curative radiotherapy.

First, for each tumor an individual pO_2 course was recorded. Then for all tumors combined, the changes of the oxygenation status during fractionated radiotherapy were analyzed. Further, initially hypoxic tumors were compared to normoxic tumors. Although we were aware of the patient and tumor heterogeneity, we attempted to analyze the influence of the dose per fraction.

Anesthesia Protocol

Before pO_2 measurements, all dogs had an intravenous catheter placed for induction of anesthesia. Since anesthesia affects tumor perfusion and, thus, tumor oxygenation, a standardized anesthetic protocol was used. Intravenously, midazolam (Dormicum®, Roche Pharma AG, Reinach, Switzerland) was injected at a dosage of 0.125 mg/kg immediately followed by propofol (Propofol®, Fresenius Kabi AG, Stans, Switzerland), slowly administered to effect. Anesthesia was maintained with isoflurane and oxygen (Forene®, Abott AG, Baar, Switzerland). Enflurane, which has an isomeric structure to isoflurane, did not alter polarographic measurements of oxygenation such as has been reported for halothane anesthesia [34]. During pO_2 measurements, dogs were monitored with pulse oxymetry and the sO_2 level was kept ≥ 95%. All patients received intravenous lactated Ringer's solution at a rate of 10–15 ml/kg/h.

pO_2 Measurements

For pO_2 measurements, dogs were positioned carefully, so that blood supply to the tumor was not impaired. Skin over the tumor area was clipped and surgically prepared. A small stab incision was made into the skin whenever needed. Measure-

Table 1. Characteristics of 26 dogs with spontaneous canine tumors. Ec: erythrocyte concentration; F: female intact; FS: female spayed; Hb: hemoglobin; M: male intact; MC: male castrated; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PCV: packed cell volume.

Tabelle 1. Patientendaten der 26 Hunde mit spontanen caninen Tumoren. Ec: Erythrozytenkonzentration; F: weiblich intakt; FS: weiblich kastriert; Hb: Hämoglobin; M: männlich intakt; MC: männlich kastriert; MCH: mittlerer korpuskulärer Hämoglobingehalt; MCHC: mittlere korpuskuläre Hämoglobinkonzentration; PCV: Hämatokrit.

Patient	Breed	Sex	Age (years)	Weight (kg)	Ec ($\times 10^6$/µl)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
1	Labrador retriever	M	10	32.4	6.37	43	15.2	68	24	35
2	German wirehair pointer	FS	6	26.5						
3	Maremmano	FS	10	66	8.06	49	16.9	61	21	34
4	Tervueren	MC	3	32	5.86	40	14.1	68	24	35
5	Flat coated retriever	M	10	34.9	4.7	30	10	64	22	35
6	Mixed-breed	MC	11	34.4		43				
7	Golden retriever	M	13	34	4.74	30.9	11.2	65	23.6	36.2
8	French bulldog	FS	10	11.1	6.69	44	15.8	66	24	36
9	Mixed-breed	MC	7	50.5	5.8	41	14.5	70	25	36
10	Mixed-breed	F	12	18.1	4.33	29	10.5	67	24	36
11	German shepherd	M	9	30	8.86	55	20.1	62	22.7	36.6
12	Flat coated retriever	M	11	30.8	7.01	46	16.1	66	23	35
13	Mixed-breed	M	8	43.5	7.77	52	18.2	67	23	35
14	Mixed-breed	F	12	34.2	7.13	39	13.7	54	19	36
15	Golden retriever	F	7	35.5	6.8	47	16.7	69	25	36
16	Malinois	FS	6	32	5.56	39	13.8	70	25	35
17	Mixed-breed	FS	10	22.9	8.32	48	16.5	58	20	35
18	Labrador retriever	FS	12	29.3	5.71	38	13.7	66	24	36
19	Golden retriever	M	6	40	5.58	39.5	13.5	70.7	24.2	34.3
20	Bernese mountain dog	MC	5	42	5.37	36	13	67	24	36
21	Dachshund	MC	11	13.7	6.55	43	14.9	65	23	35
22	Dalmatian	F	6	28	6.86	48	15.9	70	23.2	33.1
23	Golden retriever	F	7	34.5	7.54	52	18.3	70	24	35
24	Beauceron	MC	3	35.2	7.68	51	17.2	66	22	34
25	Standard poodle	M	8	12.4	7.34	48	15.9	65	22	33
26	Mixed-breed	FS	12	38.1	7.16	47	15.7	66	22	33

ments were initiated when focal bleeding was under control. Tumor oxygen partial pressure measurements were performed with the Eppendorf-pO2-Histograph (Helzel Medical System, Kaltenkirchen, Germany, previous Eppendorf Netheler Hinz GmbH, Hamburg, Germany). The technique and procedure have been described originally by Höckel et al. [22], Kallinowski et al. [25], and Vaupel et al. [45]. The measurement procedure in dogs has been previously described in detail [1]. The needle electrode was placed within the tumor tissue under ultrasound guidance (ATL 5000, Philips Medical Systems, Zurich, Switzerland). After approximately 1 min of adaptation to the tissue, stabilization of pO_2 values was observed. Subsequently, pO_2 measurements were collected, while the correct needle position was monitored ultrasonographically. Three different electrode tracks and a total of at least 50 recorded values were acquired for reliable statistical analysis [22]. The measurement inaccuracy of the device was ± 1.5 mmHg. Therefore, slightly negative values between –1.5 mmHg to 0 mmHg were defined as $pO_2 = 0$ mmHg [41].

Radiation therapy was given with a linear accelerator (BBC Dinaray 20) using 6-MV photons or 5- to 16-MeV electrons. If indicated, computer treatment planning was performed with CadPlan 6.0. The pO_2 measurements of the patients treated palliatively were done before each fraction of radiation therapy. 15 dogs were treated with a 5 × 6 Gy irradiation protocol on a Monday/Wednesday/Friday schedule. One dog was radiated with 8-Gy fractions on day 0, 7, and 21. The dogs scheduled for curative radiation therapy had oxygen measurements before fraction 1, 3, 6, 8, 10, 12, 14, and 16. The irradiation protocol for the curatively treated dogs was 14–17 × 3.5 Gy (n = 6) given on a Monday/Tuesday/Thursday/Friday schedule or daily 3-Gy fractions 15–17 times (n = 4).

Data Analyses

Data analysis was performed with StatView® (SAS Institute Inc., Version 6.0.1).

The oxygenation status was defined on the basis of the statistical, biological and quality parameters published by

Table 2. Characteristics of the 26 measured tumors. Tumor response at the end of fractionated radiation therapy was defined as no response (NR): unchanged tumor volume; partial response (PR): decreased tumor volume; complete response (CR): no macroscopic tumor disease.

Tabelle 2. Tumorcharakteristika der 26 gemessenen Tumoren. Therapieerfolg am Ende der Radiotherapie wurde definiert als kein Ansprechen (NR): unverändertes Tumorvolumen; partielles Ansprechen (PR): vermindertes Tumorvolumen; komplettes Ansprechen (CR): Tumor makroskopisch nicht mehr nachweisbar.

Patient	Histology	Location	Grade	Stage[a]	Volume[b] (cm^3)	Hypoxic subvolume[c] (cm^3)	Initial tumor response
1	Myxosarcoma	Extremities	I	3	356.26	54.15	PR
2	Spindle cell sarcoma	Oral cavity	III	3	15.66	6.59	PR
3	Myxosarcoma	Head	III	3	113.1	113.1	Surgery after first measurement
4	Fibrous histiocytoma	Head	Not applicable	4	65.44	42.37	PR
5	Histiocytic sarcoma	Extremities	Not applicable	3	331.83	309.26	PR
6	Melanoma	Oral cavity	Not applicable	3	27.49	22.91	PR
7	Fibrosarcoma	Head	I	3	40.32	40.32	NR
8	Hemangiopericytoma	Extremities	II	3	100.53	23.34	PR
9	Fibrosarcoma	Oral cavity	I	1	35.81	12.39	PR
10	Osteosarcoma	Oral cavity	III	3	64.8	18.74	PR
11	Fibrosarcoma	Oral cavity	I	2	31.42	26.7	PR
12	Histiocytic sarcoma	Extremities	Not applicable	3	53.6	40.98	Stopped XRT after first fraction
13	Squamous cell carcinoma	Oral cavity	Not applicable	1	6.29	0.66	CR
14	Osteosarcoma	Oral cavity	II	3	54.61	32.76	PR
15	Squamous cell carcinoma	Oral cavity	Not applicable	3	28.72	1.48	CR
16	Chondrosarcoma	Sacrum	I	3	250.14	129.1	PR
17	Fibrosarcoma	Extremities	II	3	111.91	100.19	PR
18	Myxosarcoma	Extremities	I	3	83.56	21.1	PR
19	Osteosarcoma	Oral cavity	II	2	17.65	2.1	NR
20	Fibrosarcoma	Head	I	3	50.3	24.7	PR
21	Fibrosarcoma	Oral cavity	I	1	12	12	PR
22	Acanthomatous epulis	Oral cavity	Not applicable	2	1.57	0.31	CR
23	Fibrosarcoma	Oral cavity	II	3	6.3	5.62	PR
24	Squamous cell carcinoma	Oral cavity	Not applicable	2	2.62	0.09	CR
25	Melanoma	Oral cavity	Not applicable	2	2.1	1.17	PR
26	Osteosarcoma	Oral cavity	Not done	3	21.99	21.99	PR

[a] WHO TNM classification; [b] $\pi abc/6$; [c] volume × % ≤ 5 mmHg

Thews & Vaupel [43]: statistical parameters were minimum of pO_2, median pO_2, mean pO_2, standard deviation of pO_2, the inter-quantile range (IQR) of pO_2, and the maximum of pO_2. The biological relevance was given by the relative frequency of values ≤ 2.5 mmHg, ≤ 5 mmHg, and ≤ 10 mmHg. Quality parameters were the minimum pO_2, maximum pO_2, count and percentage of measurements < 0 mmHg and > 100 mmHg, and the total count of recorded values (data not completely shown). In addition, the hypoxic subvolume (HSV), as proposed by Stadler et al. [40] and Stüben et al. [42], was determined before treatment by multiplying the initial total tumor volume with the relative frequency of values ≤ 5 mmHg. In this study, hypoxia was defined as a pretreatment median pO_2 ≤ 10 mmHg, as proposed by Thews & Vaupel [43]. For the longitudinal studies, differences to the pretreatment measurements were calculated. Furthermore, dose intervals were defined (6–9 Gy, 12–15 Gy, 16–21 Gy, 24–27 Gy, 30–33 Gy, 38.5–39 Gy, and 45–45.5 Gy) to compare the different curative and palliative irradiation protocols. All analyses with a p-value ≤ 0.05 were considered to be significant.

Results

15 of the 26 tumors were initially hypoxic (pretreatment median pO_2 ≤ 10 mmHg). Table 3 describes the pretreatment oxygenation status of the measured tumors. The mean of the pretreatment median pO_2 level of all measured tumors was 12 mmHg (range: 0–48 mmHg). In half of the tumors, > 47% of all measured pO_2 values were ≤ 2.5 mmHg. The mean total tumor volume before therapy was 72.5 cm^3 (range: 1.6–356.3 cm^3). The pretreatment HSV ranged between 0.1 and 309.3 cm^3 with a mean of 40.9 cm^3. Two tumors showed no response at the end of fractionated radiation therapy, in 18 tumors a partial response was seen, and four dogs had a complete response after radiaton therapy. Initial tumor response could not being evaluated in dogs #3 (surgery after first XRT) and #12 (stopped XRT after first fraction).

Pearson correlations between the mean pO_2, median pO_2, minimum and maximum pO_2, standard deviation and IQR of pO_2 were highly significant pretherapeutically ($p < 0.001$; $r = 0.74$ to 0.99 and -0.72 to -0.89). Pearson correlations between patient/tumor characteristics and pO_2 parameters are presented in Table 4. The mean corpuscular volume (MCV) showed a negative correlation to the hypoxic fraction ≤ 2.5, 5, and 10 mmHg. The packed cell volume (PCV) negatively correlated with the tumor volume as well as with the HSV. The other blood parameters reported did not correlate with pO_2 parameters. Interestingly, the dogs' age correlated with the HSV and the MCV. As expected, tumor stage correlated with the tumor volume as well as with the HSV. Neither stage nor tumor volume correlated with the pO_2 parameters.

Time course of pO_2 in each dog is presented in Table 5. The study demonstrated both a decrease or an increase of tumor oxygenation during radiation therapy. In individual tumors, a combination of increase and decrease of pO_2 was observed as well as constantly hypoxic pO_2 courses (Figure 1). When all measured tumors were combined, a decrease of the median pO_2 during the initial phase of radiation therapy was followed by an increase and again a decrease toward the end of therapy. However, since confidence intervals overlapped, the changes were not statistically significant (Figure 2).When the study population was grouped into initially hypoxic and initially normoxic tumors, a significant difference was seen (Figure 3). At a dose of 24–27 Gy (end of palliative treatment and middle of curative treatment), there was a significant difference in pO_2 between initially hypoxic tumors and normoxic tumors ($p = 0.002$). The difference in pO_2 for 45–45.5 Gy, which is toward the end of curative therapy, did not reach sta-

Table 3. Descriptive statistical analysis of the pretreatment oxygen partial pressure (pO_2) in 26 spontaneous canine tumors. % ≤ 2.5, 5, 10 mmHg: relative frequency of values ≤ 2.5, 5, 10 mmHg; IQR: interquantile range; SD: standard deviation; SE: standard error.

Tabelle 3. Deskriptive statistische Analyse des prätherapeutischen Sauerstoffpartialdruckes (pO_2) in 26 spontanen caninen Tumoren. % ≤ 2,5, 5, 10 mmHg: relative Frequenz der Messwerte ≤ 2,5, 5, 10 mmHg; IQR: Bereich Quantile 25–75%; SD: Standardabweichung; SE: Standardfehler.

	Mean	Median	SD	SE	Range
Minimum (mmHg)	0.2	0.0	0.5	0.1	0.0–1.9
Median (mmHg)	12.2	4.9	14.9	3.0	0.0–48.3
Mean (mmHg)	17.7	16.4	14.7	2.9	0.2–53.6
SD (mmHg)	17.5	19.5	10.5	2.1	0.3–31.7
IQR (mmHg)	22.5	20.2	19.9	4.0	0.3–60.2
Maximum (mmHg)	66.1	82.8	34.4	6.9	0.9–100.0
% ≤ 2.5 mmHg	47.4	47.3	33.0	6.6	1.8–100.0
% ≤ 5 mmHg	52.8	51.6	33.6	6.7	3.5–100.0
% ≤ 10 mmHg	57.8	60.6	32.1	6.4	3.9–100.0

Table 4. Pretreatment correlation analysis of patients and pO_2 parameters. % ≤ 2.5, 5, 10 mmHg: relative frequency of values ≤ 2.5, 5, 10 mmHg; HSV: hypoxic subvolume; MCV: mean corpuscular volume; PCV: packed cell volume.

Tabelle 4. Korrelationen der prätherapeutischen Patientendaten und Sauerstoffmessungen. % ≤ 2,5, 5, 10 mmHg: relative Frequenz der Messwerte ≤ 2,5, 5, 10 mmHg; HSV: hypoxisches Subvolumen; MCV: mittleres korpuskuläres Volumen; PCV: Hämatokrit.

	r	p
MCV, % ≤ 2.5 mmHg	−0.46	0.025
MCV, % ≤ 5 mmHg	−0.47	0.022
MCV, % ≤ 10 mmHg	−0.44	0.035
PCV, Tumor volume	−0.51	0.012
PCV, HSV	−0.45	0.028
Age, HSV	0.43	0.041
Age, MCV	−0.51	0.012
Stage, tumor volume	0.57	0.004
Stage, HSV	0.55	0.005

Table 5. Intratumoral median pO_2 during fractionated radiation therapy.

Tabelle 5. Mediane pO_2-Werte der gemessenen Tumoren während fraktionierter Bestrahlung.

Patient	Median pO_2 (mmHg) at radiation dose (Gy)																				
	0	3	6	7	8	9	12	15	16	17.5	18	21	24	27	30	31.5	33	38.5	39	45	45.5	52.5
4	0.00						7.20															
5	0.80						3.10								3.70							
6	1.20		0.60				13.70				3.00		1.10									
7	0.00		2.40				0.00				0.80		0.00									
8	29.95		21.70				10.45				3.50		2.55									
9	11.45									3.00						32.80		15.50			2.05	
10	14.00		23.70				0.75				2.25		2.20									
11	0.15		1.05				0.90				0.10		23.00									
13	36.00							0.80				4.25		27.70								
14	2.50		9.60				21.10				13.10		2.80									
16	4.90									9.60						6.10		1.50			5.05	7.60
17	0.00				2.00				1.10													
19	29.40	35.70				25.75						1.65		21.10			2.30			41.70	5.10	
20	5.30			14.00						7.65								55.70			9.60	
21	0.00			30.35						33.90						33.60		5.50			2.70	
22	23.30			45.60																		
23	0.00		38.40				1.75				29.10		21.15									
25	2.50		1.85				0.00				3.55		2.05									
26	–		1.50				14.70				18.25		28.40									

tistical significance. The course of median pO_2 differences to pretreatment pO_2 is shown in Figure 3. These data suggest that normoxic tumors tended to become more hypoxic, while hypoxic tumors tended to remain hypoxic.

The dose per fraction (high [6–8 Gy] = palliative; low [3–3.5 Gy] = curative) had no influence on tumor oxygenation. There was no statistical difference. Further, the influence of the histology on tumor oxygenation was analyzed. However, the small number per histology precluded a statistical analysis. Tumors were then divided into a sarcoma group and a non-sarcoma group. No statistical difference was found between these two groups.

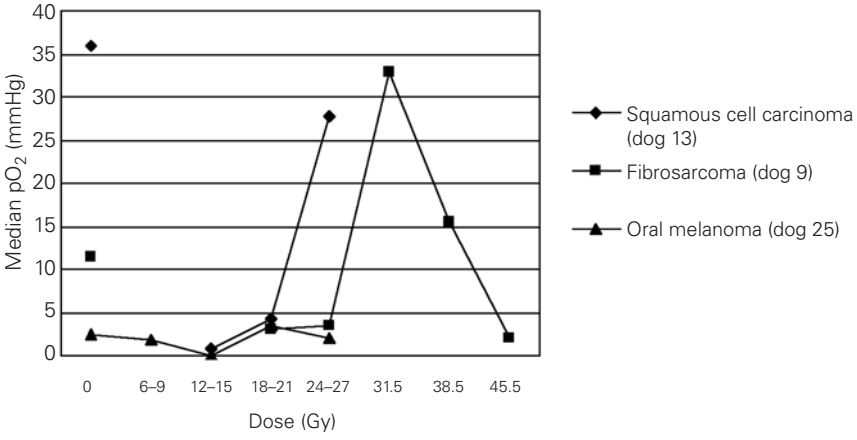

Figure 1. Oxygenation status of a fibrosarcoma, squamous cell carcinoma and oral melanoma during fractionated radiotherapy. Measurements of the squamous cell carcinoma could not be completed due to tumor shrinkage.

Abbildung 1. Oxygenierungsstatus eines Fibrosarkoms, Plattenepithelkarzinoms und oralen Melanoms unter fraktionierter Radiotherapie. Die Messungen im Plattenepithelkarzinom konnten nicht bis zum Ende der Radiotherapie durchgeführt werden, da der Tumor unter der Therapie makroskopisch nicht mehr nachweisbar war.

Discussion

It is well established that tumor oxygenation has an impact on tumor growth, tumor recurrence and development of metastases. The phenomenon of reoxygenation plays a major role in clinical radiotherapy, because it increases the cell kill of initially hypoxic tumor cells during fractionated radiotherapy [24]. However, there are few data describing oxygenation of tumors while the patient is undergoing radiation therapy [11, 17, 29]. Primarily based on laboratory work [19], one assumes reoxygenation does also take place in human patients [31]. Therefore, we wanted to investigate first the pretreatment oxygenation status in spontaneous canine tumors and then obtain serial oxygen measurements in these tumor in order to describe the oxygen changes over the course of radiation therapy.

Before the beginning of radiation therapy, 15 of 26 canine tumors were severely hypoxic. 57% of all oxygen levels recorded in the 26 tumors were

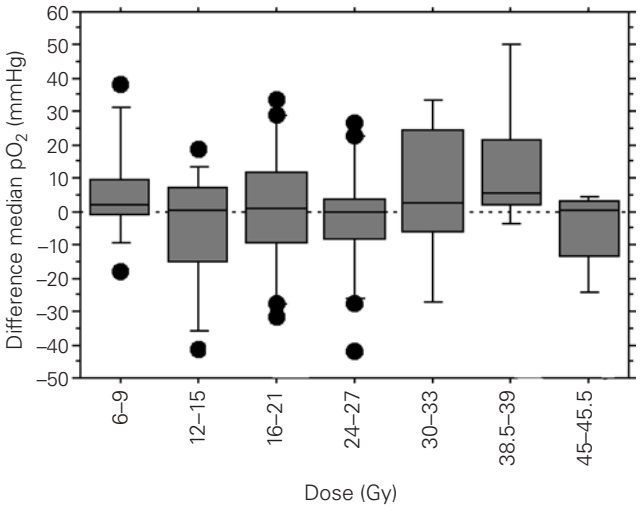

Figure 2. Difference of median pO_2 to pretreatment median pO_2 at different dose intervals indicated on the x-axis. The box plots show the median, the 25% and 75% inter-quantile range and the 10% and 90% quantile. The dots are values beyond the 10% and 90% quantile. Number of dogs per dose interval: 6–9 Gy (n = 14), 12–15 Gy (n = 12), 16–21 Gy (n = 16), 24–27 Gy (n = 11), 30–33, 38.5–39 and 45–45.5 Gy (each n = 5). There was no significant change during fractionated radiotherapy.

Abbildung 2. Differenz der medianen pO_2-Werte zu verschiedenen Dosisintervallen zum prätherapeutischen medianen pO_2-Wert. Die Box-Plots beschreiben den Median, den 25%- bis 75%-Quantilenbereich sowie die 10%- und 90%-Quantilen. Die Punkte sind Werte außerhalb der 10%- und 90%-Quantile. Anzahl der Hunde pro Interval: 6–9 Gy (n = 14), 12–15 Gy (n = 12), 16–21 Gy (n = 16), 24–27 Gy (n = 11), 30–33, 38,5–39 und 45–45,5 Gy (jeweils n = 5). Es war kein signifikanter Unterschied unter fraktionierter Radiotherapie nachweisbar.

< 10 mmHg. The high percentage of hypoxia in these tumors could, in part, be explained by the fact, that 20 of the 26 tumors analyzed were of soft tissue origin. From human medicine it is known that sarcomas tend to be severely hypoxic [8, 9]. It is probable that canine and human sarcomas are alike.

When baseline oxygen values were correlated to the red blood cell parameter, there was no correlation found for Ec, PCV, and Hb. Only a negative correlation between the hypoxic fraction and MCV was observed.This negative correlation between the MCV and the hypoxic fraction found in this study has already been reported previously [1]. Four out of 24 tested blood samples had an MCV below normal (64–73 fl). Microcytic red blood cells are commonly found in patients with blood loss anemia caused by different cancer types [37, 39]. In human patients, a hemoglobin level between 12 and 14 g/dl was found to be optimal for tumor oxygenation [46].

Repeated pO_2 measurements were done very carefully. Regularly, up to eight measurements during fractionated radiation therapy were obtained, which is, to our knowledge, unique. Also, the described guidance of the polarographic needle with ultrasonography supports the high quality of the pO_2 measurements.

Oxygen levels in individual tumors were seen to increase, decrease or undulate over the course of radiation therapy. This is in agreement with a study on human head and neck carcinomas, where a significant increase as well as a decrease in oxygenation were found [31]. pO_2 changes seemed to be correlated to changes in tumor cell density, but interestingly not to changes in vascular density. Other studies reported either an increase or a decrease of pO_2 in head and neck or cervical carcinoma [11, 17, 29]. However, these studies included only two measurements, one at beginning and one at the end of therapy. Braun et al. [6] and Dewhirst et al. [12] measured blood flow and pO_2 over a certain period of time in a rat mammary adenocarcinoma model. Interestingly, a periodicity of the blood flow as well as of the pO_2 (range: 1.3–38.5 mmHg) of four to seven cycles in 1 h was found. So, tumor oxygenation is not on-

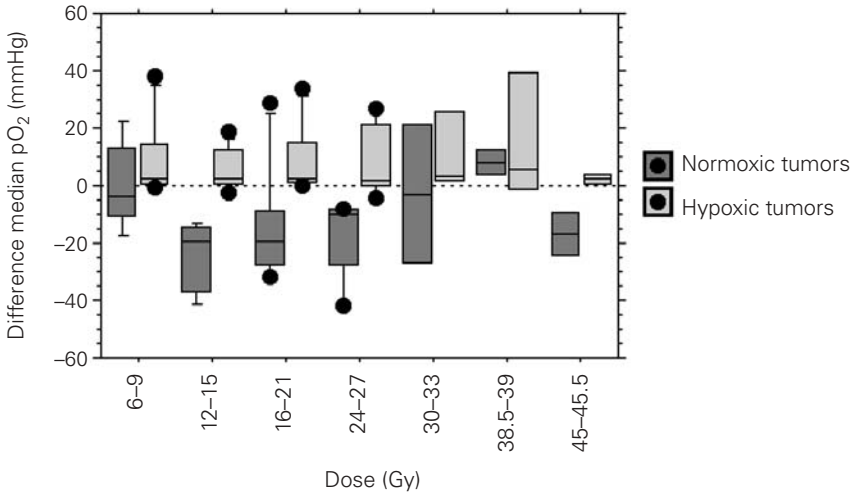

Figure 3. Difference of median pO_2 to pretreatment median pO_2 in normoxic and hypoxic (pretreatment median $pO_2 \leq 10$ mmHg) tumors. The same box plots are indicated as described in Figure 2. Number of dogs per dose interval: 6–9 Gy (normoxic n = 4/hypoxic: n = 10), 12–15 Gy (n = 3/9), 16–21 Gy (n = 5/11), 24–27 Gy (n = 4/7), 30–33, 38.5–39 and 45–45.5 Gy (each n = 2/3). The oxygenation of initially hypoxic tumors did not change during fractionated radiotherapy, whereas normoxic tumors became more hypoxic.

Abbildung 3. Darstellung derselben Box-Plots wie in Abbildung 2, jedoch aufgeteilt in hypoxische (prätherapeutischer medianer $pO_2 \leq 10$ mmHg) und normoxische Tumoren. Anzahl der Hunde pro Interval: 6–9 Gy (normoxisch n = 4/hypoxisch: n = 10), 12–15 Gy (n = 3/9), 16–21 Gy (n = 5/11), 24–27 Gy (n = 4/7), 30–33, 38,5–39 und 45–45,5 Gy (jeweils n = 2/3). Der Oxygenierungsstatus der hypoxischen Tumoren änderte sich unter Radiotherapie nicht, während die normoxischen Tumoren stärkere Hypoxie zeigten.

ly heterogeneous within tumor tissue, it also fluctuates over time.

When the group of initially hypoxic tumors was compared to the one with normal oxygen levels, a significant difference was observed after a dose of 24–27 Gy. Oxygen levels remained unchanged in the hypoxic tumor group, while the oxygen content decreased in normally oxygenated tumors. In head and neck squamous cell carcinoma of human patients, Brizel et al. [7] found also no significant change of pO_2 after 10–15 Gy (8.3 mmHg: pretreatment vs. 10.1 mmHg: after 10–15 Gy). By contrast, initially normoxic tumors seemed to become more hypoxic during the first part of fractionated radiotherapy. This could be interpreted by the phenomenon that a large proportion of well-oxygenated cells were killed in these tumors.

In transplanted rat rhabdomyosarcoma, Zywietz et al. [48] reported a significant decrease of tumor oxygenation with total radiation dose > 45 Gy. It has been suggested that with high radiation doses vascular damage may result in hypoxia [14, 16, 23].

One way to repeatedly evaluate tumor vascularity might be color and power Doppler ultrasound. Correlations between pretreatment pO_2 measurements and tumor vascularity measured with Doppler ultrasonography were performed previously. This preliminary study showed a moderate to high correlation [30, 38].

No significant difference in pO_2 was found between patients treated with a curative versus a palliative protocol. This means, the dose per fraction did not influence the oxygen status of these canine tumors during irradiation. However, when a single dose of 40 Gy versus 20 Gy was applied in a C3H mouse mammary carcinoma, tumors reoxgenated, but it took 12 rather than 4 h [18]. Kallman & Dorie [27] obtained similar results in the RIF-1 sarcoma. We assume the time between fractions (1 day) was sufficient to allow for reoxygenation to take place.

Conclusion

This study demonstrated that pO_2 values appeared to be quite variable in individual tumors during fractionated radiation therapy. The pO_2 of initially hypoxic tumors (pretreatment median $pO_2 \leq 10$ mmHg) remained unchanged during fractionated radiotherapy, whereas in initially normoxic tumors the pO_2 decreased. Based on this study, we think canine tumors could serve as an excellent model to study new treatment modalities such as hypoxic cell toxins and antivascular drugs. In the future, a larger series of patients will be examined to further support our data. The canine population presented here is being evaluated for tumor control and survival in relation to tumor oxygenation. However, at this moment in time, the median for Kaplan-Meier survival analysis has not been reached.

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Address for Correspondence
Prof. Dr. med. vet. Barbara Kaser-Hotz
Section of Diagnostic Imaging and Radiation Oncology
Veterinary School
University of Zurich
Winterthurerstraße 260
8057 Zürich
Switzerland
Phone (+41/1) 635-8449, Fax -8940
e-mail: bkaser@vetclinics.unizh.ch