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MEASUREMENT OF HUMAN TUMOUR OXYGENATION STATUS BY A POLAROGRAPHIC NEEDLE ELECTRODE

An analysis of inter- and intratumour heterogeneity

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Tumour oxygenation status was measured by a polarographic needle electrode in 31 patients with lymph node metastasis of squamous cell carcinoma of the head and neck and 18 patients with primary soft tissue sarcoma. Two oxygenation parameters, the median pO_2 and the proportion of measured values less than 5 mm Hg, were used in comparing the inter- and intrasubject heterogeneity in tumour and subcutaneous tissue. Results of the analysis may be summarized as follows: 1) the variation in oxygenation between tumours was significantly greater than that within tumours, 2) the variation in oxygenation of subcutaneous tissue between patients was significantly greater than the variation within patients, 3) oxygenation of tumour was significantly lower than that of subcutaneous tissue, 4) no significant difference in the distribution of the oxygenation parameters in the two tumour types, and 5) both oxygenation parameters correlated. In conclusion, measurements by oxygen electrodes were able to distinguish intratumour heterogeneity from intertumour heterogeneity provided that several electrode tracks were done. The method therefore appears to be feasible for differentiation of tumour oxygenation clinically.

It is well known that hypoxic tumour cells are relatively resistant to radiotherapy (1, 2). Hypoxic regions have been demonstrated in human tumours already in the sixties by use of glass-sealed polarographic oxygen needle electrodes (3). Recently, a technically more advanced polarographic oxygen electrode system, developed for clinical application, has become commercially available (4, 5). Using this assay oxygen status has been assessed in a number of histologically different human tumours (5–10). Several different oxygenation parameters have been employed in these studies: mean pO_2, median pO_2, the proportion of measured values less than 2.0 mm Hg, less than 2.5 mm Hg, less than 5 mm Hg, or less than 10 mm Hg. The choice of parameter has been made on purely empirical grounds or the biological rationale that a certain threshold value represents tumour hypoxia being indicative of reduced radiosensitivity (5–10). Furthermore, preliminary results indicate that tumour oxygenation determined by oxygen electrodes may be a predictor of radiation response (3, 11, 12). Still this needs to be documented in larger clinical trials of different tumour types.

We have measured tumour oxygenation status by this new polarographic oxygen electrode system in node metastases from squamous cell carcinoma of the head and neck and in primary soft tissue sarcomas. In this report the method is analyzed with respect to inter- and intratumour heterogeneity, using two oxygenation parameters: median pO_2 and the proportion of measured pO_2 values below 5 mm Hg.

The aims of the present study were: 1) to compare the variation of the two oxygenation parameters between tu-

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mours with the variation of the same parameters within each tumour. This analysis was made for both head and neck nodes and soft tissue sarcomas; 2) to compare the variation of subcutaneous tissue oxygenation within the same patient; 3) to compare tumour oxygenation with subcutaneous tissue oxygenation; 4) to compare oxygenation of head and neck nodes with oxygenation of soft tissue sarcomas and 5) to compare the two oxygenation parameters.

A preliminary report on the experience with oxygen measurements in lymph node metastases from head and neck cancer has been presented elsewhere (13).

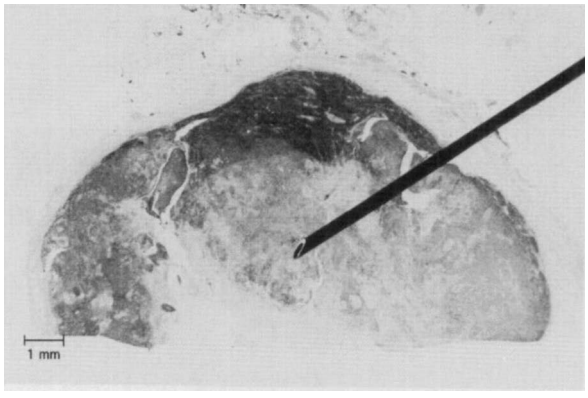

Fig. 1. An oxygen needle electrode superimposed upon a histological section of a neck node metastasis. The probe is 0.35 mm in diameter. An average of partial oxygen pressure available in the intercellular environment around the electrode tip is measured. The electrode is moved automatically through the tissue and pO_2 recalled every 0.7 mm. Subsequently the oxygenation profile of a tissue is a result of several measurements obtained by more electrode tracks, as each track represents a number of pO_2 measurements.

Material and Methods

Soft tissue sarcoma patients. Tumour oxygenation status was measured in 18 patients with primary soft tissue sarcoma, all with superficial extension in order to be accessible for pO_2 measurements. The patients were referred from the Sarcoma Tumour Center at Aarhus University Hospital. Patients' age ranged between 30 and 87 years, the median being 63 years. Ten were female and 8 male. Prior to any treatment tumour oxygenation status was measured. A diagnostic biopsy was subsequently taken from the same region and characterized histopathologically. Three tumours were localized to the proximal extremities, 9 to the distal extremities, 3 to the truncus, and 3 to the abdomen.

Lymph node metastases from squamous cell carcinoma of head and neck. Tumour oxygenation status was measured in node metastasis of 31 patients with head and neck cancer, referred to the Department of Oncology, Aarhus University Hospital. Twenty-six of these patients had primary disease and 5 recurrent disease but only one patient had a node recurrence in a previously irradiated field. Patients' age ranged between 22 and 77 years, the median being 63 years. Nineteen were male and 12 female. All tumours were histopathologically classified as squamous cell carcinoma of head and neck. TNM staging was done according to the UICC recommendations (edition 1987). Nodes ranged from N1 to N3.

The study was approved by the local ethical committee and informed consent according to the Helsinki Declaration II was obtained from all patients.

Tumour oxygenation. Measurements of tumour oxygenation were done using a polarographic needle electrode (the KIMOC-6650 pO_2-histograph, Eppendorf, Hamburg, Germany). Each measurement represents the average partial oxygen pressure of the intercellular environment in the sampling range of the probe. This may include necrotic as well as viable tissue. Fig. 1 shows the size of the oxygen needle probe superimposed upon a histological section of a head and neck node.

Practical procedure of pO_2 measurements. After local anaesthesia (0.5 ml lidocaine hydrochloride 10 mg without addition of a vasoconstrictive agent) a sterile Venflon 2 (17G) was inserted 2 mm into the skin (insertion). The trochar was withdrawn, a window cut in the plastic tube and the oxygen needle electrode guided through here into the tissue of interest. Initially the oxygen electrode was allowed a few minutes of adaptation to the tissue environment. Thereafter measurements of subcutaneous tissue overlying the tumour were done followed by the tumour measurements. Single measurements were collected consecutively over a predetermined, tumour size-dependent distance (track). The length of each track was maximized in order to gain optimal representation of oxygenation within the tumour. The oxygen electrode moved automatically in a stepwise pattern: 1 mm forward, followed by 0.3 mm retraction, giving a net step length of 0.7 mm between measurements. Each pO_2 measurement was done within less than 500 ms. Artifacts due to compression of vessels and to oxygen consumption of the probe were diminished by the fast response time and the special movement pattern (14).

After the oxygen probe was removed, tumour temperature measurements were done through this same plastic trocar using a 0.8 mm needle probe with a thermocouple connected to a digital thermometer (Ellab, DK). In general, the whole procedure described lasted 10–20 min and was well tolerated by all patients.

In all 49 tumours, pO_2 measurements were sampled as follows. One to three insertions were done per tumour. Between one and three tracks were achieved through one insertion, the median number of tracks per tumour being three (range 2–7). In 3 patients only 2 tracks were done. The median number of measurements per track was 28 (range 9–65) (equivalent to track lengths from 6.3 mm to 45.5 mm) and the median number of pO_2 measurements

per tumour was 129 (range 36–279). Measurements of subcutaneous tissue were available in 14 patients of whom 10 had neck nodes and 4 soft tissue sarcoma. Three tracks from the same insertion were achieved in each patient. The median number of measurements per track was 18 (range 12–36) (equivalent to track lengths from 8.3 mm to 25.2 mm) and the median number of mesurements per patient was 51 (range 36–84). The pO_2 measurements were corrected for air pressure and tumour tissue temperature and automatically stored on the pO_2-histograph software. To facilitate further analysis, data were transferred to a LOTUS 123 spreadsheet.

Technical information. The oxygen probe had a stainless steel shaft, 350 μm in diameter, and a lancet shaped tip, containing a membranized polarographic recessed microcathode—a gold wire, 12 μm in diameter. In 37°C isotonic NaCl solution at pH = 7.4, the oxygen probe sensitivity was 6 ± 3.0 pA/mm Hg pO_2, when polarized against a Ag/AgCl reference electrode at −700 mV. During tissue mesurements the reference electrode was placed on the skin close to the tumour. Oxygen probes were formalin gas sterilized. Furthermore the probes were calibrated before and after each measurement in a physiological 0.9% NaCl solution though which nitrogen or air flowed alternately. The probe drift was corrected by a time-dependent linear interpolation between pre- and recalibration. A temperature effect of 2.4%/°C on partial oxygen pressure was taken into consideration. O_2 current drift was accepted up to 0.6%/min.

Analysis of variance. Variability between and within tumours was estimated using two different oxygenation parameters, the median pO_2 and the proportion of pO_2 measurements less than or equal to 5 mm Hg. The variability within tumours was determined by equivalent oxygenation parameters calculated in each individual track in a tumour. This led to the following hypotheses to be tested: 1) The variation of oxygenation from tumour to tumour is equal to the variation between individual tracks within a tumour; 2) The variation of subcutaneous tissue oxygenation from patient to patient is equal to the variation between tracks within the patient. A mixed model of variance (15) was used to test hypothesis 1 and 2 with a significance level of 5%. This type of model may accommodate a different number of tracks per tumour. In the model the value of a specific oxygenation parameter for a given track, m, was decomposed as

$$m = \mu + \alpha_{tumour} + \epsilon_{track}$$

where μ is the overall mean, α_{tumour} is the deviation from the overall mean for this particular tumour, and ϵ_{track} is the error term representing a (hypothetical) random biological variability between tracks and measurement error.

It follows under this assumption that the variance estimate is

$$\sigma^2_{tot} = \sigma^2_{tumour} + \sigma^2_{track}$$

where σ^2_{tumour} denotes the intertumour variability, and σ^2_{track} denotes the intratumour, or track-to-track, variability.

Comparison of distributions of oxygen parameters. The distributions of the oxygenation parameters (median pO_2 and % $pO_2 \leqslant 5$ mm Hg) in neck nodes, soft tissue sarcoma and subcutaneous tissue were compared and the following hypotheses were advanced: 3) The oxygenation status of tumour is correlated to that of subcutis; 4) The distribution of the oxygenation status of neck nodes is equal to that of soft sarcoma. Hypothesis 3 was tested using a linear regression analysis and hypothesis 4 by a Wilcoxon rank-sum test. Finally, the distributions of median pO_2 and % $pO_2 \leqslant 5$ mm Hg of both tumour types were compared using Spearman's rank correlation coefficient. In all cases a significance level of 5% was used.

Results

Oxygenation within tumours and between tumours. The general impression when measuring pO_2 in both tumour and subcutaneous tissue was large variation of pO_2 measurements. This is illustrated by representative examples in Fig. 2a and b, where points are single measurements along separate tracks. In Fig. 2a, data are obtained from a lymph node metastasis in a patient with squamous cell carcinoma of the larynx. The oxygen partial pressure varies in both tracks though most pronounced in track two. Fig. 2b is an example of measurements along three tracks in subcutaneous tissue. Also here the variation of pO_2 measurements is evident. The very same data was illustrated as histograms—Fig. 3a from the neck node and 3b from subcutaneous tissue. Most values measured in the tumour are extremely low, the median pO_2 being only 5 mm Hg, whereas the histogram of pO_2 values in subcutaneous tissue is shifted to the right, with a median pO_2 of 38 mm Hg and no values below 20 mm Hg.

The oxygenation is not only heterogeneous within tumours and within subcutaneous tissue. Also the variability of oxygenation between tumours from head and neck nodes and soft tissue sarcomas and between oxygenation status of subcutaneous tissue is pronounced. This is illustrated in Fig. 4a and b, where median pO_2 and the proportion of pO_2 values less than 5 mm Hg respectively are given for every patient. As an example the proportion of pO_2 values less than 5 mm Hg for neck nodes ranges from 0 to 100%, whereas such low values are not present in subcutaneous tissue.

It was decided to evaluate within-tumour heterogeneity as the variability of oxygenation parameters from one track to another. This was done as it was not possible to achieve exactly repeated single measures due to the invasive character of the method. The variability of oxygenation between tracks may be the result of biological intratumour heterogeneity as well as methodological error.

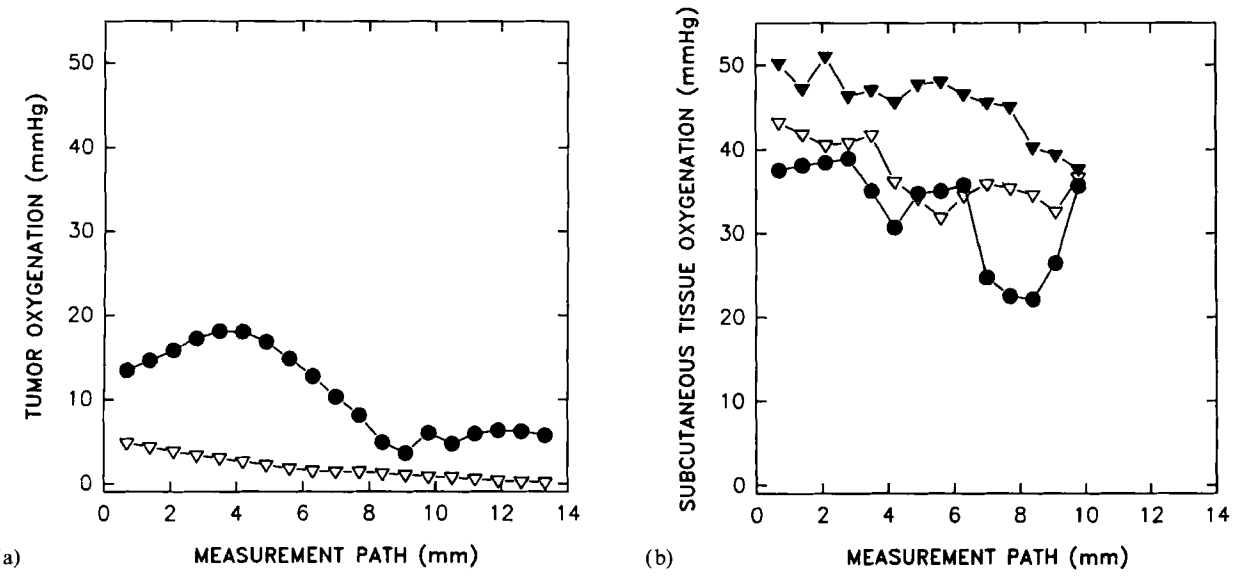

Fig. 2. a) Representative examples of pO_2 measurements from two oxygen electrode tracks in a node metastasis from a laryngeal cancer, ● = track 1; ▽ track 2. Points are single measurements separated by a distance of 0.7 mm. b) Results of pO_2 measurements in subcutaneous tissue from the same patient as a). Points are single measurements separated by a distance of 0.7 mm. ● = track 1; ▽ = track 2; ▼ = track 3.

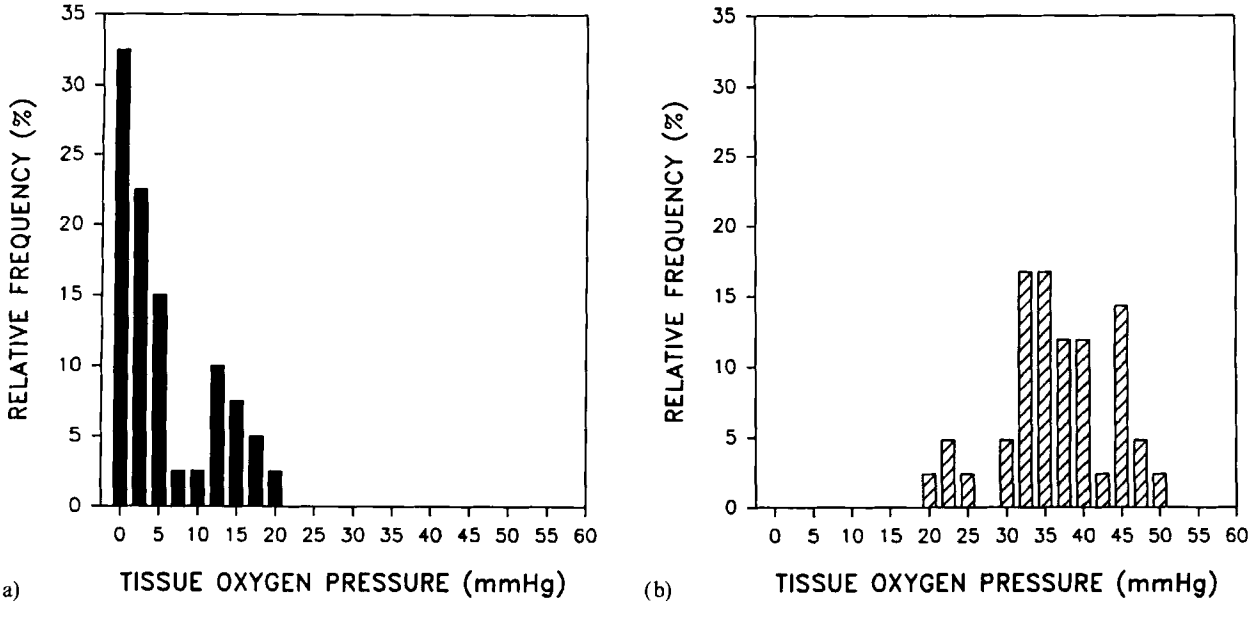

Fig. 3. a) The relative distribution of pO_2 measurements from a neck node in the patient shown in Fig. 2a. Median pO_2 was 5 mm Hg and the proportion of $pO_2 \leq 5$ mm Hg = 55%. b) Histogram showing the distribution of pO_2 measurements in subcutaneous tissue. The tissue was well oxygenated, median pO_2 being 38 mm Hg and no measurements below 20 mm Hg.

The variance component estimates are listed in the Table and it is seen that the variance components are higher between tracks than between tumours. Therefore it is advisable to measure more than one track per tumour to reduce the effects of the methodological error and biological intratumour heterogeneity. In our material several tracks per tumour were made and thereby the contribution of the overall variability of the oxygenation parameters was reduced. Furthermore, the analysis of variance shows that variation between tumours is significantly greater than the variation of the oxygenation status within tracks in tumours when both oxygenation parameters were analyzed (Table).

Oxygenation of subcutaneous tissue between and within patients. In Fig. 4a it is seen that median pO_2 in the subcutis ranges from 30 mm Hg to 60 mm Hg in 14 pa-

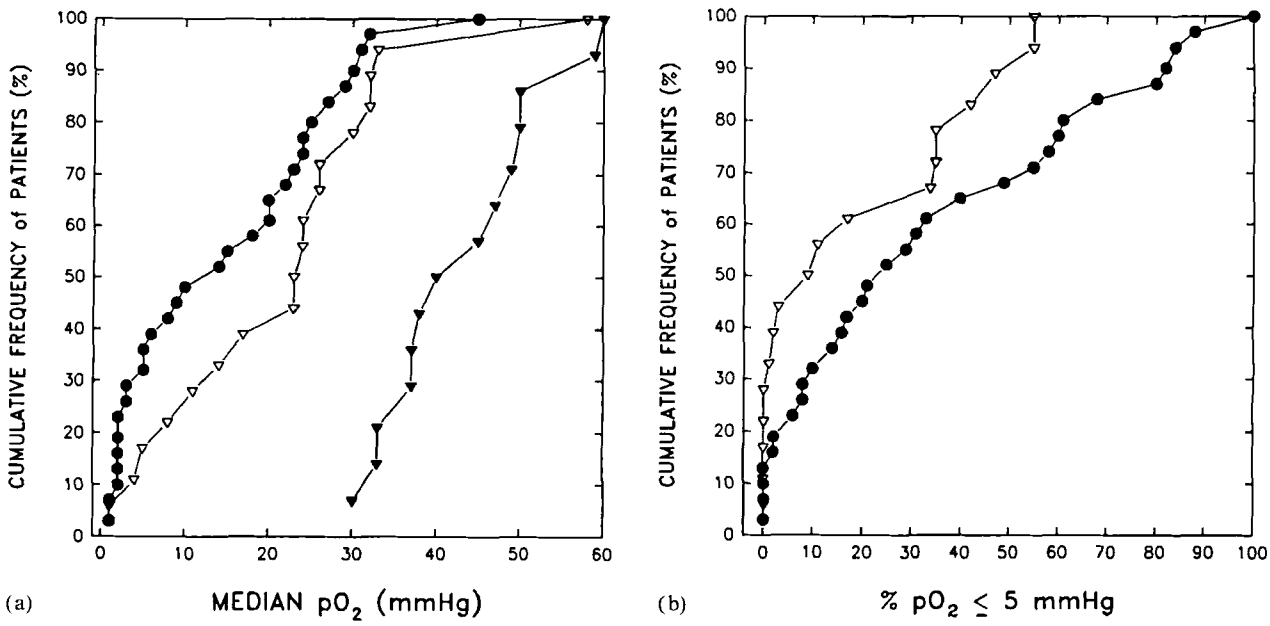

Fig. 4. a) The cumulative frequency of median pO_2 values in 31 head and neck nodes, 18 soft tissue sarcomas, and in the subcutaneous tissue in 14 patients where this was measured. ● = head & neck node; ▽ = soft tissue sarcoma; ▼ = subcutaneous tissue. b) The cumulative frequency of patients as a function of proportion of pO_2 values ≥ 5 mm Hg is presented here for neck nodes and soft tissue sarcomas. The variation between tumours in both types is evident and most pronounced for nodes of head and neck where the full spectrum from 0–100% is seen. ● = head & neck node; ▽ = soft tissue sarcoma.

Table

Variance component estimates from mixed analysis of variance

	Median pO_2 (mm Hg)	Proportion of $pO_2 \leq 5$ mm Hg
Head and neck nodes (n = 31)		
σ^2_{tumour}	64	692
σ^2_{track}	197	734
p-value	0.013	7×10^{-7}
Soft tissue sarcomas (n = 18)		
σ^2_{tumour}	104	314
σ^2_{track}	228	553
p-values	0.001	5×10^{-5}
Subcutaneous tissue (n = 14)		
$\sigma^2_{patient}$	70	–
σ^2_{track}	65	–
p-value	0.001	–

The p-value indicates the probability that the variability between tumours (or patients) is equal to the variability within tumours (or patients).

tients. Median pO_2 was the only oxygenation parameter in question for the analysis of variance in subcutaneous tissue as no values below 10 mm Hg were measured. Interestingly, the variation between patients was significantly greater than the variation of median pO_2 within tracks in patients (Table).

Oxygenation status of tumour compared to subcutaneous tissue. Expectedly, there was a marked difference between the oxygenation status of subcutaneous tissue and tumour as shown in Figs 2 and 3. The distribution of median pO_2 in neck nodes and soft tissue sarcoma was chosen and was uniformly found to be significantly lower than the distribution of median pO_2 in subcutaneous tissue measured in the same patient ($p = 0.001$).

As illustrated in Fig. 5 no correlation was found between the distribution of median pO_2 in tumour and subcutaneous tissue in the same patient ($r = 0.25$ by linear regression, 95% confidence limits $(-0.32, 0.69)$).

Oxygenation of head and neck nodes compared to oxygenation of soft tissue sarcoma. Both primary soft tissue sarcoma and nodes of head and neck were poorly oxygenated. From Fig. 4a and 4b nodes of head and neck seemed slightly more hypoxic than primary soft tissue sarcomas, but the difference was not significant neither for median pO_2 nor % $pO_2 \leq$ 5 mm Hg, $p = 0.5$ and $p = 0.16$, respectively.

Comparing median pO_2 and % $pO_2 \leq 5$ mm Hg. Finally, the median pO_2 and % $pO_2 \leq 5$ mm Hg were compared in neck nodes and in soft tissue sarcoma. As shown in Fig. 6, the two oxygenation parameters correlated significantly, although the correlations were not very strong being 0.8 for both head and neck nodes and soft tissue sarcomas. Moreover, the two parameters resulted in a different ranking of the tumours with respect to oxygenation status. This was quantified by Spearman's rank correlation coefficient which was 0.84 and 0.77 for neck nodes and soft tissue sarcomas, respectively.

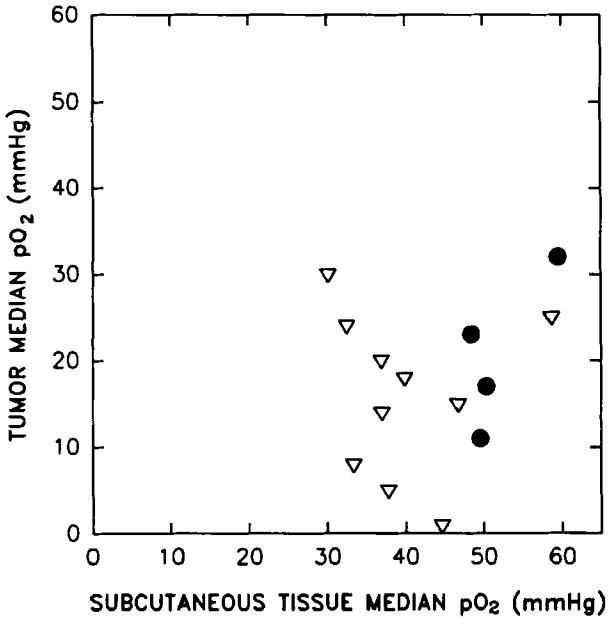

Fig. 5. Relationship between median pO_2 of tumour and median pO_2 of subcutaneous tissue measured in the same patient. ● = nodes of head and neck (n = 10). ▽ = soft tissue sarcomas (n = 4). No significant correlation was found between the oxygenation status of tumour and subcutaneous tissue, r = 0.25 by linear regression, p > 0.1.

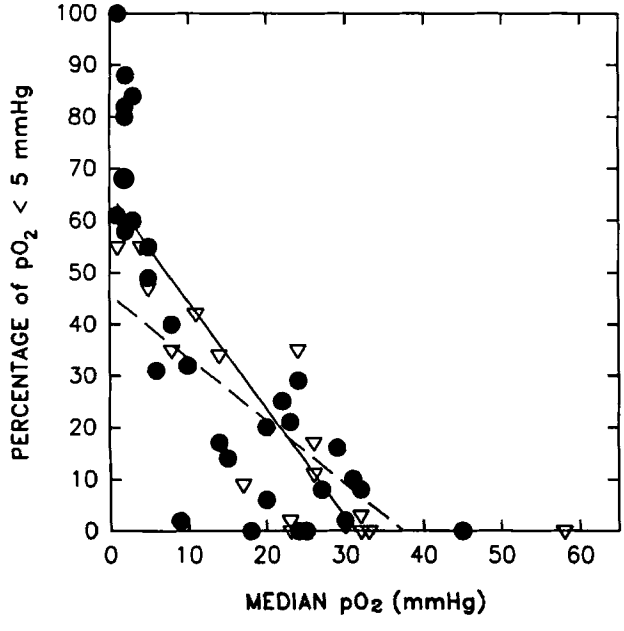

Fig. 6. Corresponding values of the proportion of pO_2 values below 5 mm Hg and median pO_2 in the two tumour types studied here. The correlation was significant ($p < 10^{-4}$) when using Spearman's rank correlation in neck nodes ($\rho = 0.84$) and soft tissue sarcomas ($\rho = 0.77$) respectively. -●- = head & neck node. -▽- = soft tissue sarcoma.

Discussion

A number of studies have shown that oxygenation status of human tumours evaluated by oxygen electrodes varies considerably from tumour to tumour (5–12). However, it is not clear to what extent this could simply reflect a large experimental noise in the assay. To evaluate the feasibility of the method for clinical application it was reasonable to test whether measurements by the oxygen electrodes were able to resolve a significant tumour-to-tumour variability in oxygenation status of both primary soft tissue sarcomas and node metastases of head and neck.

Results of the present analysis of variance clearly showed that it is possible to discriminate intertumour heterogeneity from intratumour heterogeneity by polarographic oxygen electrodes. However, the analysis also demonstrated that conclusions should not be based on a single electrode track due to a substantial variability between tracks. More than one track was needed to overcome the variation between tumours and a minimum of 3 tracks are recommendable. This variability between tracks can be explained as the combined effect of methodological error and biological intratumour heterogeneity. Also, it was found that the first track sampled was not significantly different from the subsequent tracks (data not shown). This is in agreement with reports on normal muscle tissue (16). The analysis of variance was not made by means of single pO_2 measurements as the invasive nature of the method made it impossible to do exact repeated measures.

Interestingly the variability of oxygenation in subcutaneous tissue varied substantially both from track to track in a specific patient and between patients. Also here the analysis of variance showed that the variation between patients was significant. Furthermore, we found that normal tissue oxygenation was substantially higher than the oxygenation status of tumours. This is in agreement with earlier reports (5, 7, 17). Finally, oxygenation status of primary soft tissue sarcomas was found not to be different from oxygenation status in node metastasis of head and neck cancer. Unfortunately, no measurements of primary head and neck tumours were available for comparison and it remains to be clarified whether the oxygenation status of primary tumour is similar to that of the metastatic lesions.

The use of clinical pO_2 data in oncology has so far mainly been devoted to the pursuit of radioresistant hypoxic clonogenic cells (2, 5, 8–12, 16). Radioresistance is predominant at low pO_2 values < 3 mm Hg and therefore pO_2 values above 10 mm Hg seem to be of less interest (18). Even though animal experiments have shown a correlation between radiobiological hypoxia and direct estimates of tumour oxygenation (19), it is well known that the oxygen electrode does not only measure clonogenic hypoxic cells. Therefore a clear correlation needs to be demonstrated between electrode derived oxygenation parameters and a valid measure of clonogenic hypoxic cells in human tumours. However, the ideal oxygenation parameter may not necessarily be a conversion of the O_2 reduction current into oxygen partial pressure as the calibration is not absolute. This indicates that a threshold of tumour oxygenation in mm Hg in one situation may not

be exactly the same in another caused by factors related to physical qualities of the electrodes, chemical properties of the tissue and temperature gradient in the tissue (20).

Analyses in the present study are based on oxygenation parameters chosen on a radiobiological rationale. The two oxygenation parameters we used did correlate though the correlation was not strong. This suggests that using the two parameters may not necessarily produce the same conclusion. The clinical merit of the two parameters needs to be clarified. However, testing the statistical significance of more than one measure of oxygenation status at a nominal 5% significance level, will result in a greater than 5% chance of one of the tests coming out significantly by chance.

In conclusion, measurements by the oxygen electrodes were able to distinguish intratumour heterogeneity from intertumour heterogeneity provided that several electrode tracks were done. Thus, the method seems feasible for evaluation of oxygenation status in human tumours. Two oxygenation parameters, median pO_2 and the proportion of values less than 5 mm Hg, were recommended for evaluation of the clinical data.

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