Oxygenation status of primary and recurrent squamous cell carcinomas of the vulva

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Summary

Introduction: Cancers of the vulva are relatively rare and, therefore, little is known about the pathophysiological role of tumor oxygenation in this entity.

Methods: Data are presented on the oxygenation status of primary (n = 15) and recurrent (n = 19) cancers of the vulva, as measured by the Eppendorf pO_2 histography system.

Results: Contrary to other tumor entities, no significant differences in the oxygenation status between primary (median pO_2 = 13 mmHg; hypoxic fraction ≤ 5 mmHg = 37%) and recurrent (median pO_2 = 11 mmHg; hypoxic fraction ≤ 5 mmHg = 45%) tumors were found. Oxygenation was significantly lower in cancers of the vulva than in the subcutis. Anemic patients had significantly poorer tumor oxygenation compared with patients whose cHb values were within the normal range (p = 0.02).

Conclusions: The oxygenation of vulvar cancers is similar to other tumor entities, but does not show more severe hypoxia in recurrent cases. Anemia is associated with a poorer oxygenation status in vulvar cancers, whereas in the normal tissue no impact of cHb values on the median pO_2 was observed.

Key words: Vulvar cancer; Tumor hypoxia; Tumor Oxygenation; Oxygen measurements; Anemia.

Introduction

The occurrence of hypoxic tissue areas is a characteristic trait of solid malignancies which is important for many aspects of cancer pathophysiology/tumor biology and treatment. Hypoxia induces proteome changes, mainly through the activity of hypoxia-inducible factor 1 (HIF-1). Currently, over 70 target genes of HIF-1 which are capable of influencing various central aspects of malignant growth are known [1] (e.g., cell survival and proliferation [2, 3], angiogenesis [4], cancer cell invasiveness [5], metastasis [6]), and resistance to therapy [7, 8]. Hypoxia also contributes to the genetic instability of cancer cells ("mutator phenotype", [9]) by promoting point mutations [10], chromosomal aberrations [11], gene amplification [12] and microsatellite instability [13]. In addition to the direct induction or promotion of mutations, tumor hypoxia leads to the selection of mutations that are advantageous to cancer cell growth, as has been strikingly demonstrated for p53 [14]. These processes can lead to a more aggressive phenotype in hypoxic tumor cells, causing poor prognosis, which has been demonstrated for several tumor entities [15-22].

FIGO stage is the strongest prognostic factor in cancers of the vulva [23] and includes the assessment of the largest superficial tumor diameter, invasion depth and lymph node metastasis. In a recent study of squamous cell cancers of the vulva published in this journal, Stone and co-workers examined possible correlations of the oxygenation status with lymph node metastasis in 20 women [24]. These authors stated that, "to date, there are no published reports assessing tumor oxygenation in vulvar cancer using the oxygen electrode". However, data on primary and recurrent vulvar cancer were already published by our group in 2002 [25]. The latter, complemented by new data, are presented here.

Patients and Methods

The evaluation of intratumoral pO_2 distributions was performed in primary (n = 12, FIGO II–IV) and locally recurrent (n = 17) squamous cell carcinomas of the vulva at the Department of Obstetrics and Gynecology, University of Mainz, between July 1989 and January 1993. Additionally, pO_2 measurements were carried out in three cases of primary and two cases of recurrent squamous cell carcinomas of the vulva at the Department of Obstetrics and Gynecology, University of Leipzig, between November 2001 and November 2004. Oxygen tension measurements were performed under direct visual control, and the pO_2 data presented are based on at least two electrode tracks within each tumor. The study design was approved by the local ethics committee, and all patients enrolled gave written informed consent. Pretreatment pO_2 measurements were performed using the pO_2 histography system following a standard procedure which has been described in detail earlier [16, 26]. All pO_2 readings were made exclusively in vital cancer tissue. Before vulvar pO_2 measurements were performed, oxygen readings in the subcutis of the pubic mount were obtained. Measurements in tissue without malignant cells were excluded (verification via needle core biopsies of approximately 2 mm in diameter and 20 mm in length with subsequent histological processing and histopathologic examination). For the description of the oxygenation status of the cancer of the vulva, the median pO_2 and the fraction of pO_2 readings ≤ 2.5 mmHg (HF 2.5) and ≤ 5 mmHg (HF 5) are stated. Results are expressed as means ± standard error of mean (SEM), unless stated otherwise. Differences between groups were assessed by the Wilcoxon test. The significance level was set at α = 5% for all comparisons.

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Results

In the group of patients undergoing pO_2 measurements in Mainz (n = 29), hypoxic tissue areas ($pO_2 \leq 2.5$ mmHg) were detected in 50% of locally advanced primary cancers of the vulva and in approximately 56% of locally recurrent tumors, respectively. The median pO_2 was 11 mmHg in primary squamous cell carcinomas and 12 mmHg in recurrent malignancies (median pO_2 in the subcutaneous tissue of the mons pubis = 52 mmHg, and of normal vulvar tissue = 63 mmHg). The fraction of pO_2 values ≤ 2.5 mmHg was 29% in primary tumors and 24% in local recurrences (p = 0.79). The fraction of pO_2 values ≤ 5 mmHg was 35% in the primary tumors and 44% in the recurrent vulvar cancers (p = 0.47).

When recent data from measurements (n = 5) at the Department of Gynecology and Obstetrics, University of Leipzig, were included, hypoxic tissue areas ($pO_2 \leq 2.5$ mmHg) were found in 53% of primary vulvar cancers and in approximately 42% of recurrent tumors, respectively. The updated data representing the pooled patient group (n = 34) are shown in Table 1. Table 2 shows clinical and pathological tumor stages, where available. In Figure 1, representative pO_2 histograms for subcutaneous tissue of the mons pubis and of invasive vulvar cancer are shown for a primary and a recurrent carcinoma, respectively. The mean patient age was 69 (± 11.2) years, with no difference between the Mainz and Leipzig patient groups (p = 0.39). The oxygenation of vulvar cancer specimens was significantly lower than oxygenation of normal tissue (p = 0.0001, p = 0.001 and p = 0.0001 for median pO_2, HF 2.5 and HF 5, respectively). Oxygenation data (median pO_2, HF 2.5 and HF 5) showed no statistically significant differences between primary and recurrent cancers (Figure 2).

Table 1. — *Oxygenation status of normal subcutis, primary and recurrent squamous cell carcinomas of the vulva.*

	Mons pubis	Primary	Recurrent*
N	13	15	19
n	527	1063	1514
median pO_2 (mmHg)	55.7 (± 13.0)	12.9 (± 14.9)	11.3 (± 14.5)
mean pO_2 (mmHg)	57.8 (± 13.1)	16.4 (± 14.4)	15.7 (± 13.4)
HF 2.5 (%)	0	25.1 (± 32.8)	24.7 (± 30.0)
HF 5 (%)	0	37.1 (± 36.4)	44.8 (± 34.0)

N = Number of women; n = number of pO_2 measurements; values are means ± SEM; *The oxygenation status showed no significant differences between primary and recurrent squamous cell carcinomas of the vulva.

Table 2. — *Patient age, FIGO and TNM stages of primary squamous cell carcinomas of the vulva.*

Patient number	Age (years)	FIGO stage	TNM stage
1	64	n.a.	T2 N0 MX
2	52	3	T3 N0 MX
3	79	4	T3 N3 M0
4	58	3	T2 NX MX
5	54	3	n.a.
6	68	3	n.a.
7	71	3	T2 NX MX
8	54	4	n.a.
9	82	4	n.a.
10	83	3	T2 N0 MX
11	84	1	T1 N0 MX
12	69	3	T2 N2 MX
13	57	4	T2 N2 MX
14	62	3	T2 N2 M1
15	62	3	T2 N1 MX

n.a. = not available.

Analysis of the oxygenation status was performed as a function of pretreatment hemoglobin concentration (cHb). This analysis was carried out for the entire patient group (including the new data). There were no differences in cHb between the patients with primary or recurrent cancer. One patient was not included in this analysis, because cHb data were not documented. Patients were divided into three groups based on "high" (above median cHb; median cHb of age-matched healthy women = 13.95 g/dl [27]), "intermediate" cHb values (12 g/dl < cHb < 13.95 g/dl) and anemic patients (cHb < 12 g/dl [28]). Based on this separation, a correlation between Hb levels and median pO_2 values, as shown in Figure 3, was obtained in tumor tissue.

In anemic patients, the median pO_2 in cancers of the vulva was 5.4 mmHg (cHb = 11.1 ± 0.75 g/dl). At a mean Hb level of 13.15 ± 0.69 g/dl (representing the "normal" range of cHb), the median pO_2 was significantly (p < 0.02) higher (18.1 mmHg) and declined at greater cHb values (14.5 ± 0.71 g/dl) to a median pO_2 of 12.7 mmHg. From these data, it can be concluded that an optimal Hb level with regard to the oxygenation status is to be expected at cHb values of between 12 and 14 g/dl. In this group, the median pO_2 values ranged from 2 to 58 mmHg. No differences were found in the oxygenation status of normal tissue (mons pubis, n = 13) between the three groups of cHb values (Figure 3).

Discussion

In the present study, no differences in the oxygenation status between primary and recurrent cancers of the vulva were found. This is in contrast to data obtained previously in cancers of the uterine cervix, where both groups showed significant differences (p = 0.0013), with a median pO_2 of 12.1 (± 1.0) mmHg in primary and of 7.1 (± 1.1) mmHg in recurrent cases [29]. A recent study of experimental rat tumors exhibited a lower microvessel density in post-irradiation recurrences compared with primary tumors [30], indicating that the lower pO_2 in recurrent tumors may be associated with prior radiotherapy. However, in our former study [29], the oxygenation status in recurrences of cervix cancers was independent of the treatment modality. The small number of cases and heterogeneous treatment protocols prevented an analysis of the influence of the treatment modality on the oxygenation status of recurrent vulvar tumors in the present study. Additionally, recurrences of vulvar cancer may often in fact be "secondary" primary tumors (field cancerization due to "high-risk" HPV) rather than true recurrences of the original tumor, which may arise in part due to the hypoxic milieu of the surgical scar [31]. These

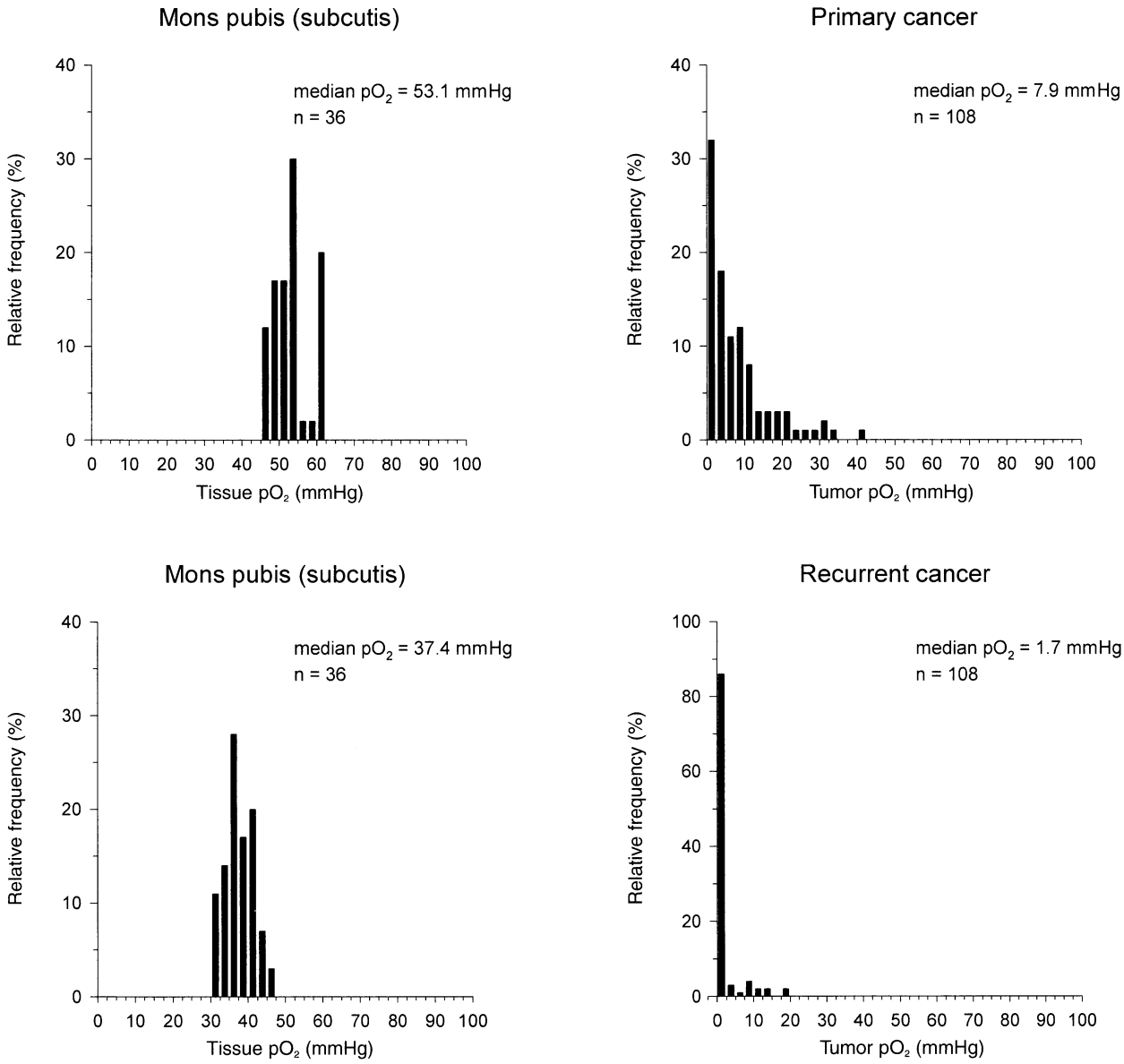

Figure 1. — Representative pO_2 histograms of normal subcutis (mons pubis, left) and vulvar cancers of two patients (right). Upper panels: primary tumor, lower panels: recurrent tumor. Note the different scaling in the lower right panel. n = number of pO_2 measurements.

"secondary" primary tumors are not expected to exhibit lower oxygenation. Indeed, the prognosis of local relapses located at the vulvectomy scar was significantly poorer compared to relapses at sites of the vulva more distant from the operation field [32].

Comparison of our data with those of Stone and co-workers [24] shows lower values in our patient group. While the grand median pO_2 values (data not shown) in our patient cohort was approximately 5 mmHg and, therefore, comparable with the median value in the lymph-node positive group in the study of Stone and co-workers (4.8 mmHg), the median pO_2 in the lymph-node negative patients in the study of Stone *et al.* [24] was 11.9 mmHg. Accordingly, the median HF 5 was ≈ 52.5% in the lymph-node positive group as reported by Stone and co-workers [24] and 50% in our study. Contrary to this, the median HF 5 in the lymph-node negative patients in the study of Stone *et al.* was only ≈ 16.4%. However, the differences described in their study were not statistically significant.

In an earlier assessment of 29 of the 34 cases of vulvar cancer presented here, we found a significantly lower

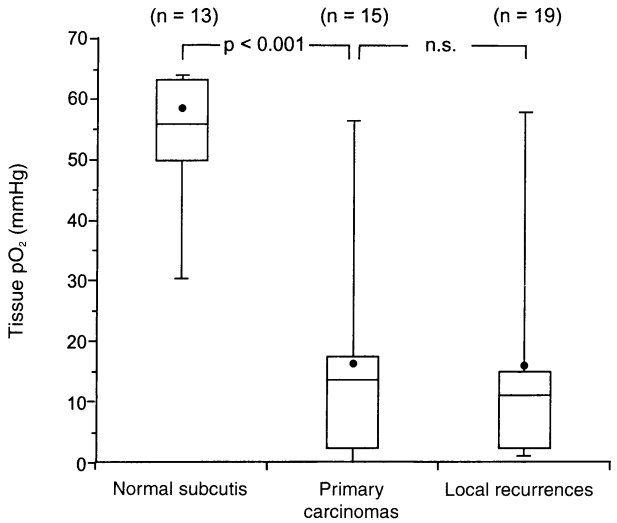

Figure 2. — Oxygenation status of normal subcutis, primary and recurrent cancer of the vulva represented in Box-and-Whisker-plots which display the median pO_2 (line), the mean pO_2 (dot), the 1st and 3rd quartile and the range of pO_2 values measured.

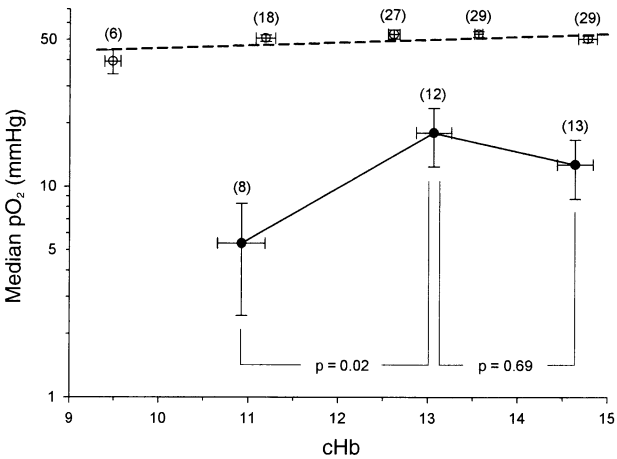

Figure 3. — Median pO_2 values in primary and recurrent cancers of the vulva (lower curve) and within the normal subcutis (mons pubis, upper line) as a function of hemoglobin concentration (cHb). Patients are divided into three groups based on cHb values: above median of age-matched healthy women (cHb ≥ 13.95 g/dl), below median (12 g/dl < cHb < 13.95 g/dl) and anemic patients (cHb < 12 g/dl). Values are means ± SEM; number of patients investigated in brackets.

tumor oxygenation in the group of anemic patients (i.e., cHb < 12 g/dl; [25]). This correlation is corroborated in the present study. Anemic patients had a median pO_2 of 5.4 mmHg compared with 18.1 mmHg in the group with a normal cHb (12 g/dl < cHb < 13.95 g/dl). The poorer tumor oxygenation at low cHb values (< 12 g/dl) is most probably a result of the reduced oxygen transport capacity of the blood under these conditions. Conversely, the reduced oxygenation values at higher hemoglobin concentrations are probably caused by a substantial rise in the viscous resistance to flow (i.e., a deterioration of the blood's rheological properties) within the chaotic and highly permeable, leaky tumor microvasculature which would in turn lead to a net reduction in oxygen supply. The restricted O_2 supply at higher cHb values is thus primarily caused by hyperviscosity in tortuous, elongated, dilated and functionally abnormal tumor microvessels, which counteracts and finally may overweigh the higher O_2 transport capacity which might have been expected in this range [25, 33]. The non-linear relationship between Hb level and tumor pO_2 values clearly mirrors the opposing effects of increasing the blood's O_2-carrying capacity (cHb < 14 g/dl) and rising viscous resistance to flow (hyperviscosity) at the upper edge of the cHb scale (cHb ≥ 14 g/dl). Contrary to this, no significant differences in the oxygenation status of normal tissue (mons pubis) were found over the whole cHb range reported. That the oxygenation status in the normal tissue remains constant down to cHb values of ≈ 8 g/dl indicates a physiological compensation in anemic patients most probably related to an increase in tissue perfusion [34]. A similar effective adaptation is hardly possible in locally advanced solid tumors. Both findings are therefore similar to data obtained in breast cancers and corresponding normal tissue [35]. In contrast to this, Stone and co-workers [24] did not describe a correlation between the oxygenation status and cHb. Their analysis, however, was performed in only 17 cases, compared to 33 in the present study.

In conclusion, analyses of Eppendorf measurements in vulvar cancers showed no differences in primary and recurrent tumors. The oxygenation status of primary cancers of the vulva is comparable to cancers of the uterine cervix. Anemic patients have a significantly lower pO_2 than patients with normal cHb levels.

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