Prognostic Value of Histopathological Response to Radiotherapy and Microvessel Density in Oral Squamous Cell Carcinomas

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The prognostic value of histopathological response to preoperative radiotherapy (50 Gy) in radically resected oral carcinomas was studied in 39 consecutive patients. Microvessel density (MVD) was evaluated for relation to radioresponse and outcome. Resected tumour tissue was examined histopathologically and response to radiotherapy was scored according to induced morphological changes. Pretreatment biopsies were stained with antibodies to von Willebrand factor to evaluate MVD in hot-spot regions, in stromal tissue and in tumour epithelial tissue. Histopathological response to radiotherapy was highly prognostic of local failures and survival ($p = 0.002$), though microscopic surgical radicality was obtained. In good responders to preoperative radiotherapy, the 5-year survival rate was 68% compared with 24% in poor responders. In 12 patients with local recurrence after radical surgery, 11 had poor histopathological radiotherapy responses. In univariate analysis, a high MVD score in tumour epithelium was associated with poor clinical outcome but MVD did not correlate with histopathological radiotherapy response.

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Head and neck cancer patients are much more likely to succumb to locoregional failures than to distant metastases and prognosis largely depends on local tumour control.

In the radical treatment of locally advanced squamous cell carcinoma of the head and neck, surgery is usually combined with radiotherapy given either pre- or postoperatively. From a surgical point of view, postoperative radiotherapy is generally preferred, in order to avoid tissue damage prior to surgery. The arguments for preoperative radiotherapy are radiobiological. First, radiotherapy is likely to be more effective in otherwise unaffected and well-oxygenated tissue (1–3). Second, the reduction of tumour volume by radiotherapy gives better options for subsequent radical surgery.

It is well established that a selected subgroup of cancer patients, including patients with bladder carcinomas (4) and rectal carcinomas (5, 6), benefit from radiotherapy given prior to surgery. Identifying these patients at an early stage of treatment would make it possible to optimize individual therapy.

Although tissue oxygenation is consequential for the response to radiotherapy, the connection between tumour microvessel density (MVD) and tumour oxygen levels has still to be established. Studies of cancer of the head and neck, uterine cervix and prostate indicate that high MVD scores may be associated with risk of local recurrence and spread of disease (7–10), though some investigators disagree (11).

In contrast with reports of tumour vascularity being associated with spread of disease, Zätterström et al. (12) found that in 23 patients with head and neck cancer treated with radiotherapy, tumours with high MVD scores measured in hot spots (the most densely stained areas) had better response and survival rates. The proposed explanation was that more microvessels around the tumours would lead to higher oxygen levels and more effective radiotherapy. In the same study it was noted that prognosis was in fact determined by the response to radiotherapy rather than by subsequent surgical procedures.

The main purpose of the present study was to further investigate to what extent histopathological response to

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Table 1
Tumour site vs. gender, tumour stage, histological grade and treatment with chemotherapy

Tumour site	n	Male	Female	T-stage				Stage UICC			Grade*			Chemo-therapy
				1	2	3	4	II	III	IV	High	Medium	Poor	
Tongue	16	8	8	0	13	3	0	8	6	2	9	6	1	0
Gingiva	10	6	4	1	3	1	5	3	2	5	5	4	1	1
Floor of mouth	8	8	0	0	5	2	1	4	2	2	0	4	4	2
Bucca	5	4	1	0	1	1	3	0	1	4	2	1	2	1
Total	39	26	13	1	22	7	9	15	11	13	16	15	8	4

* one tumour was not graded

irradiation correlated with prognosis and vascularization in resectable tumours of the oral cavity. After resection, the preirradiated tumour tissue was evaluated with a modified histological scoring system originally designed to evaluate microscopic response to radiotherapy in squamous cell carcinoma of the oesophagus (13). Untreated diagnostic biopsies from the same patients were analysed for microvessel density in the search for a possible correlation between tumour vascularity and histopathological response to radiotherapy.

MATERIAL AND METHODS

Forty-two consecutive patients with squamous cell carcinomas of the oral cavity were referred to the Department of Oncology, Lund University Hospital, for preoperative radiotherapy, during the period 1990–1994.

Surgery was not performed in 3 patients owing to cardiovascular deaths in two cases and a second malignancy in the third patient. Thus, 39 patients underwent surgery after radiotherapy, as planned, and form the basis of the present study.

Age at diagnosis ranged from 22 to 79 years, median 61 years. Gender, tumour site, tumour stage (UICC 1987) and tumour grade are presented in Table 1.

Median preirradiation haemoglobin levels were 14.0 g/L (range 9.7–16.7) and 13.1 g/L (range 11.6–15.4) for males and females, respectively. Lower normal haemoglobin limits were 13 g/L for males and 11 g/L for females. Endpoints of the study were overall survival, disease-specific survival and locoregional control with a follow-up of at least 4 years.

Combined treatmentt

Computed tomography- (CT) based treatment planning preceded radiotherapy in all but one of the 39 patients. Only one patient received preoperative radiotherapy without a CT treatment plan. Radiotherapy was administered with a Cobalt 60 unit ($n = 8$) or 4–6 MV photons ($n = 31$), 50 Gy with 2 Gy per fraction, in one course, with an average treatment time of 36 days (range 31–43). Three patients received hyperfractionated radiotherapy, 1.6 Gy b.i.d., with a treatment time of 22 days (range 21–23).

Tumour and metastases were included in the target volume in all but one case, where the dose to the lymph node metastasis was 40 Gy. Thirty-four of the 39 patients were immobilized, with a cast, during radiotherapy.

Prior to radiotherapy, four patients received neoadjuvant chemotherapy, consisting of three courses of cisplatin (100 mg/m^2) and a subsequent 120-h infusion of 5-fluorouracil (1 000 mg/m^2 per 24 h) repeated every 3 weeks.

Surgery was performed at the Department of Otorhinolaryngology, Lund University Hospital. The median time to surgery was 41 days (range 15–111) after completion of radiotherapy. For the patient operated on after 111 days, surgery had to be postponed because of a venous thrombosis. All patients underwent grossly radical surgery. In 3 patients reresection was necessary. Microscopic radicality was finally obtained in all but one patient, according to the patho-anatomical report following surgery. This patient received an additional 20 Gy postoperatively.

Evaluation of radiation therapy

The surgical specimens were examined histologically and classified according to a modified scoring system originally designed to evaluate microscopic response to radiotherapy in squamous cell carcinoma of the oesophagus (13). Effect of radiotherapy was graded in four levels, from no effect ($-$) to excellent effect ($+++$), i.e. no signs of viable tumour remaining (see Table 2).

All specimens were retrospectively graded by a senior pathologist (R.W.) who had no information about individual treatment or outcome.

Microvessel density

Biopsies for diagnostic purposes were stained for MVD with a monoclonal antibody to the factor Vlll-related von Willebrand factor (DAKO A082, dilution 1 : 200) in a Streptavidin-Biotin-HRP-DAB system. Staining was done in a conventional way, using the staining machine Teckmate 500; 1–3 slides were prepared for each patient, depending on the amount of tissue available. In three cases biopsy material was not available and in two cases the staining failed, leaving 34 cases for this part of the study. Microvessel density was studied in the hot-spot region of the specimen, i.e. the most densely stained area within the tumour, in three consecutive microscopic fields of view, at

Table 2

The scoring grades of radiotherapy effect

Rating		Effect
Excellent	+++	Destruction, negative mitosis and disappearance of nuclei. Severe degeneration of cytoplasm. Complete destruction of the cancer nestle
Good	++	Negative mitosis. Pyknotic change of nuclei. Vacuolization of the cytoplasm. Moderate destruction and transformation of the cancer nestle.
Some	+	Positive mitosis. Basophilic change of cytotoplasm. Polymorphic cells. Nuclei present. Proliferation of cancer nestle
No effect	−	−

Table slightly modified from Akakura et al. (25).

a magnification of × 200, and a median value was calculated. As a second step, MVD was studied in the tumour stroma area as well as in the epithelial tumour tissue region and scored as follows: absence of microvessels (0), moderately (1) or abundantly (2) vascularized tissue. Intermediate scores, i.e. some microvessels but less than moderately vascularized (0–1), and more than moderately, but not abundantly vascularized (1–2) were also allowed. The absence of microvessels, only seen in epithelial tumour tissue, was defined as tumour fields exceeding 15 cells in width without any vessels. All slides were characterized on two different occasions, by the same investigator (E.B.), with no significant difference in the results.

Statistical methods

Survival curves were estimated with the Kaplan–Meier technique. Tests for differences in overall survival and disease-specific death hazards were done using the logrank test. Fisher's exact test was applied to test for independence between category variables.

RESULTS

Treatment outcome and overall survival

With a follow-up time of more than 4 years after surgery, 18 patients were alive without any evidence of disease. Estimated overall 5-year survival was 46%, (95% CI 30–61%). Of the 21 deceased patients, 15 deaths were disease specific, whereas 6 patients died of intercurrent diseases. Out of a total of 12 patients with local recurrence, 11 died of the disease. Four patients developed distant metastases.

The proportion of disease-specific deaths after 3 years was twice as high for node-positive than for node-negative patients, 57% and 28%, respectively ($p = 0.07$). Surprisingly, there was no significant correlation between overall survival and stage of disease. Nor was there any correlation between tumour grade or patient age and prognosis. The lack of correlation between clinical stage and survival may be explained by the distribution of tumour subsites in our material (Table 1). Thus, the subgroup of patients with gingival tumours ($n = 10$) had better survival rates compared with those with other tumour localizations ($n = 29$), ($p = 0.09$).

Furthermore, patients with high pretreatment haemoglobin values (> 13.6 g/L) tended to have somewhat better survival rates, although these were not statistically significant.

Time factors, i.e. the time required to complete radiation therapy and the amount of time elapsing between radiotherapy and surgery, did not correlate with survival.

Survival and response to preoperative radiotherapy

There was an association between the histopathological response score to radiotherapy in the surgical specimens and treatment outcome (Fig. 1a and Table 3). Excellent and good responses (+ + + and + +) were grouped together as were poor and no responders (+ and −) because of the small number of cases. There was a strong relationship between overall survival and histopathological response to radiotherapy when comparing excellent and good response to poor or no response, with a 5-year survival of 68% and 24%, respectively ($p = 0.002$). The association between response to radiotherapy and survival was even stronger in younger patients (< 60 years) ($p = 0.0002$) (Fig. 1b). The radiotherapy response score was also highly predictive of locoregional control. Out of 13 relapses, 11 patients had no or poor response to radiotherapy (− or +) ($p = 0.006$). It is noteworthy that 12 of the 13 recurrences were local, despite surgically free margins.

The histopathological response to radiotherapy did not correlate with patient age, tumour stage, histologic grade or pretreatment haemoglobin levels. Gingival tumours had slightly better histologic response scores and survival rates than other tumour sites. There was no association between the distribution of the histologic response score and time to completed radiotherapy, nor to time between radiotherapy and surgery.

Therapy outcome and microvessel density

The predominant histological pattern in our specimens of head and neck squamous cell carcinomas was a richly

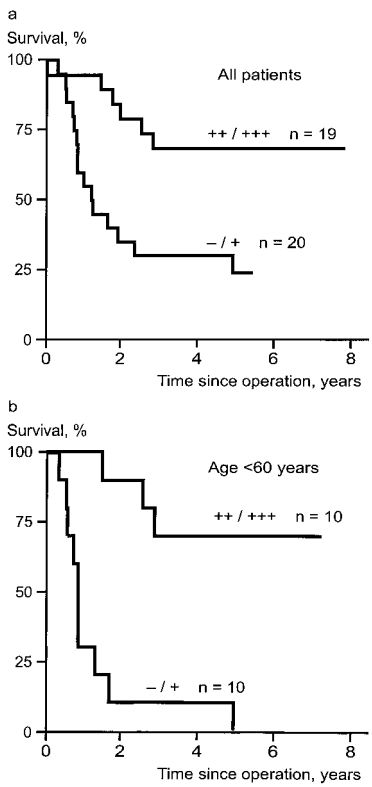


Fig. 1. Overall survival and histopathological response to preoperative radiotherapy, good and excellent (+ + / + + +) or none and poor (– / +). a) All patients. b) Patients less than 60 years of age.

vascularized stromal tissue, clearly separated from epithelial tumour tissue, containing few or no vessels (Fig. 2a). In a few biopsies, tumour and stromal tissues were mixed, without clear boundaries, with microvessels evenly represented, although stromal vessels were often predominant (Fig. 2b).

The distribution of radiotherapy response scores did not correlate with MVD when evaluated in hot spots, stroma and epithelial tumour tissue.

Disease-specific death hazard was greater for patients with a high microvessel epithelial tumour score (2 or 3) ($p = 0.01$), while no such association was found in relation to the stromal vessel score ($p = 0.71$) (Table 4). There was no correlation between prognosis and MVD in hot spots and hot-spot MVD was not associated with pretreatment haemoglobin count.

A low microvessel score in epithelial tumour tissue was associated, though not significantly, with better overall survival. A low microvessel score in tumour epithelial tissue was observed in both patients with node-negative disease and patients with no evidence of recurrence during follow-up.

DISCUSSION

The present study evaluated the predictive value of histopathological response to preoperative radiotherapy in relation to locoregional control and survival in squamous cell carcinoma of the head and neck. Overall 5-year survival in patients with histopathologically complete (= excellent) or good response (grades + + + or + +) was 68%, as compared with 24% for poor or non-responders (+ or –). The latter group included 11 of 13 patients with relapses. Importantly, among the 13 recurrences, there were 12 local failures and 11 of these had free histological margins at the time of surgery. The strong association between histopathological response to radiotherapy and survival suggests that in selected cases, when there is complete histological response after preoperative radiation of 50 Gy, radical radiotherapy of 70 Gy could be evaluated as a single treatment instead of a combination with surgery. Identifying these patients at an early phase of therapeutic planning would enable us to individualize therapy and optimize the use of treatment resources.

The findings in our study are in accordance with studies of bladder carcinoma (4) and rectal carcinoma (5, 6), where a positive correlation has been reported between prognosis and histopathological response to preoperative radiotherapy.

In a previous study of head and neck squamous cell carcinoma, we found that high microvessel density in hot spots (MVD) in tumours was associated with response to radiotherapy and better survival (12). Based on these findings, it was proposed that high MVD could lead to improved oxygenation, thereby making radiotherapy more effective.

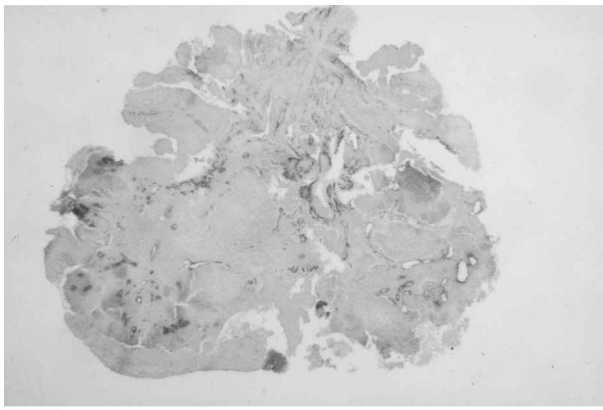
In the present study, MVD did not correlate with response to radiotherapy. Instead, high MVD in epithelial tumour tissue correlated with poor locoregional control and poorer disease-specific survival (Table 4). These findings are in accordance with other studies of head and neck cancer (8) as well as studies of other tumour types, including cancer of the breast (14), prostate (15), lung (16) and stomach (17). A possible explanation for the divergence in results between this and the previous study (12) may be either differences in techniques when evaluating MVD or differences in the distribution of tumour subsites.

Another explanation may be that MVD and radiosensitivity are two independent prognostic factors for head and

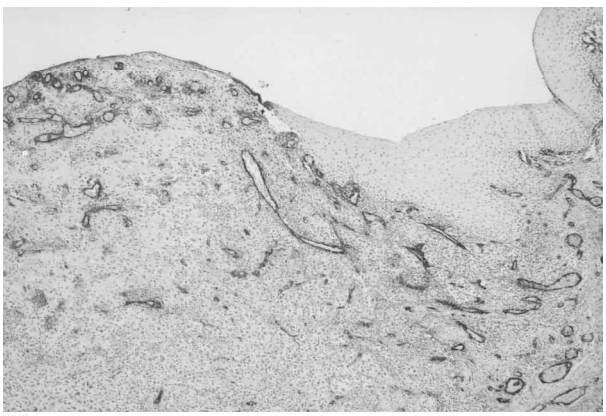
Table 3

Radiotherapy response score, no (–) or poor (+) response and good (++) or excellent (+++) response versus locoregional tumour control

	Score:no or poor response (– and +)	Score: good or excellent response (++ or +++)
Locoregional control	9	17
Locoregional failure	11	2
Total	20	19



(a)



(b)

Fig. 2. a) Vascularized tumour stroma separated from tumour epithelial tissue, with few microvessels. b) Epithelial tumour tissue and stromal tissue mixed, microvessels evenly distributed.

neck cancer. Cooper (18) investigated the relationship between the intrinsic radiosensitivity and vascularity in carcinoma of the cervix. It was found that patients with both radioresistant and highly vascularized tumours had a 5-year survival rate of 18% compared with 77% for patients with radiosensitive and poorly vascularized tumours.

The correlation between oxygenation status in tumours and response to radiotherapy, as originally described by Thomlinson & Gray in 1955 (19), stipulates that tumour cells close to blood vessels have higher oxygen levels than cell populations at a greater distance from the microcirculation. Indeed, large hypoxic fractions measured in vivo in head and neck tumours have been found to correlate with poorer survival after radiotherapy (2, 3). The same has been found for squamous cell carcinomas of the uterine cervix (20).

On the other hand, when squamous cell carcinomas and adenocarcinomas of the cervix were examined for vascularization and oxygenation, the adenocarcinomas were significantly better oxygenated than the squamous cell carcinomas, although there was no difference in vascular density between the two histologically different types (21). In a study of oxygenation during radiotherapy of head and neck cancer, the O_2 levels remained unchanged after 10–15 Gy, demonstrating that reoxygenation could occur during therapy, as, otherwise, oxygenation status was expected to drop when well-oxygenated cells were killed off (22).

It is clear that oxygen transport, important for the success of radiotherapy, may be affected by numerous other factors that are not related to MVD per se but to changes in blood flow, rheology, pH, oxygen consumption and demand, as discussed by Dewhirst (23). It is also known that tumour hypoxia induces changes with upregulation of several genes, which may have an effect on prognosis (24).

In conclusion, the histological response after preoperative radiotherapy was the strongest predictor of survival in head and neck cancer. The response to radiotherapy did not correlate with the density of microvessels in the tumour. It is suggested that tumour angiogenesis and tumour cell radiosensitivity in head and neck cancer may be independent prognostic factors, as previously described for cancer of the uterine cervix (18).

Table 4

Therapy outcome versus microvessel density. Relation between epithelial tumour microvessel density score and a) node-positive or -negative disease; b) recurrence; and c) disease-specific death

	Tumour score 0 and 1, low microvessel density	Tumour score 2 and 3, high microvessel density
Node positive/negative disease	(n = 18)	(n = 16)
Node-negative disease	17	7
Node-positive disease	1	9
Recurrence	(n = 18)	(n = 16)
No evidence of disease during follow-up	15	10
Recurrence	3	6
Disease-specific death	(n = 8)	(n = 9)
Intercurrent deaths	5	1
Disease-specific deaths	3	8

Further studies with a larger group of patients are required in order to determine the relationship between radiosensitivity and microvascularity in squamous cell carcinomas of the head and neck.

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