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ORIGINAL ARTICLE

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Histological grading in the deep invasive front of T1 and T2 glottic squamous cell carcinomas has high prognostic value

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Abstract The characteristics of the deep invasive front area of squamous cell carcinomas may reflect tumour prognosis better than other parts of the tumour. Consequently, the authors have recently developed a simple malignancy grading system based solely on the characteristics of the deep invasive front area of oral squamous cell carcinomas, which has great prognostic value. Our previous materials were somewhat heterogeneous, and the prognostic value of our system needed to be confirmed in homogeneous patient material. In the present study of 95 T1–2/N0 glottic carcinomas all treated by radiation, the high prognostic value for invasive front grading of biopsy specimens is confirmed. The grading significantly predicted local recurrence, i.e. treatment failure (P=0.001). Histological characteristics of the deep invasive front proved to be a better indicator of prognosis than the T-category (size of tumour), and our findings may be of value in the selection of treatment. Of the individual variables in the grading system (pattern of invasion, degree of keratinization, nuclear polymorphism and host response), pattern of invasion and degree of keratinization were the strongest prognosticators in the multivariate analyses. Invasive front characteristics may also prove to be of prognostic value in other cancers.

Key words Malignancy grading · Invasive front characteristics · Head and neck · Glottic carcinomas · Prognosis

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Introduction

The invasive edges of head and neck squamous cell carcinomas often display different morphological and molecular characteristics than more superficial parts of the same tumours [4, 5, 7, 11, 13, 14, 17, 19]. We have recently proposed that cellular characteristics of this deep invasive front of oral squamous cell carcinomas provide the most prognostic information [4, 5, 7, 8]. On the basis of this hypothesis we developed a new, simple histological malignancy grading system focusing exclusively on the deep invasive front of the tumour. This grading, which proved to have an acceptable interobserver reproducibility before and after calibration by pathologists (kappa>0.6) [6, 7], correlated more accurately with prognosis than the previously used Broders grade [5, 7]. Our previous material, on the basis of which this hypothesis was proposed, was heterogeneous in stage and treatment, however. It was therefore desirable that the prognostic value of our system be confirmed in more homogeneous cases, and this has recently been done by Odell et al. [17], who studied a group of small, localized lingual cancers, confirming the high prognostic value of invasive front grading.

This study comprises 95 T1–2/N0 glottic squamous cell carcinomas treated with similar doses of radiation. The invasive front malignancy grading on the biopsy specimens is confirmed to be of high prognostic value; a high malignancy score correlated with a poor response to radiotherapy.

Materials and methods

Patients

From a total of 280 patients with T1 and T2 glottic squamous cell carcinomas admitted to the Department of Otolaryngology, National Hospital, University of Oslo, Norway from 1983 through 1991, we randomly selected 40 cases with local recurrences and twice as many nonrecurring tumours. Twenty-five patients were excluded: 2 who could not be treated because of other illnesses, 5

who developed either distant or regional metastases, 7 for whom the sections could not be obtained and 11 for whom the histological material was too sparse for complete histopathological grading. The material thus comprises 95 patients, 34 with local recurrence and 61 with no recurrence. The clinical findings, treatment and results of follow up have been recorded prospectively. There were 89 male and 6 female patients, ranging in age from 39 to 86 years (mean 64 years). The tumours were classified according to the UICC TNM classification of 1987. All patients received high-energy radiation therapy (1.2–6 MeV) with a field size of 6×6 cm, 2 Gy daily 5 days a week to a total dose of 66–70 Gy. If a recurrence occurred, either total or partial laryngectomy was performed.

The length of follow up ranged from 3 to 11 years (mean 5.8 years), and 46 patients were alive after follow up with no evidence of disease. Of the 34 patients with recurrences, 8 died of the disease, 22 were alive and 4 died of intercurrent diseases with no evidence of laryngeal cancer. None of the patients was lost to follow up. Death certificates were obtained and autopsy records were reviewed when available. Owing to the high rate of salvage for T1 and T2 glottic carcinomas we have chosen to evaluate the recurrence rate rather than the disease-free survival. A minimum of 3 years' follow up was considered sufficient, as only 13% of recurrences occur later than 3 years after treatment [3].

Histopathology

The histopathological grading was performed on routinely processed biopsy specimens stained with haematoxylin and eosin. Malignancy grading of the deep invasive front [7] was done without knowledge of clinical outcome by one of the authors (M.Br.). Patients whose biopsy specimens did not include obvious invasive areas were omitted from the study. For each tumour the degree of keratinization, nuclear polymorphism, pattern of invasion and host response (degree of leucocyte infiltration) was graded and given scores between 1 and 4 according to Bryne at al. [7] (Table 1). Tumour cell characteristics were graded exclusively within the least differentiated parts of the most invasive, approximately 4–6, cell layers at the invasive front of tumours. An average of at least three different high-power fields was recorded as the score for each parameter. The tumour areas considered for grading are presented in Fig 1. More details regarding criteria for each variable are given in Anneroth et al. [1]. Previously we included the num-

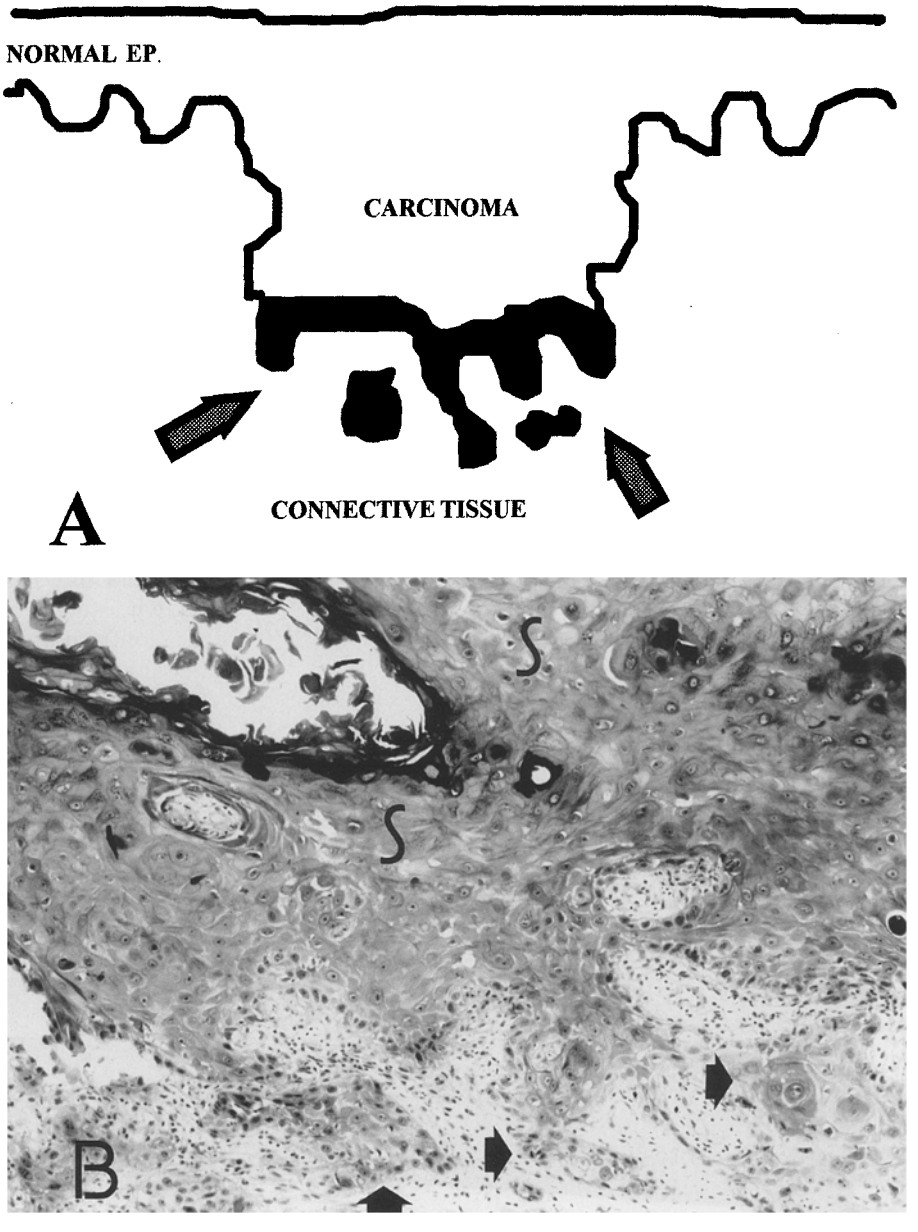

Fig. 1A, B Invasive front grading. **A** Schematic drawing of the areas within a squamous cell carcinoma that were considered for grading (marked with *black* and *two arrows*). The other areas of the tumours were disregarded. **B** Microphotograph of a glottic carcinoma (original magnification ×100, HE). Examples of the invasive front area are marked with *three black arrows*. Note the histological differences between the more superficial parts of the carcinoma (*S*) and the invasive front area. Our working hypothesis is that molecular and cellular characteristics at the invasive front area are most important for prediction of prognosis

Table 1 Histological malignancy grading system used in the present study. The most invasive parts of the tumours only were graded. For further details, see refs. [1, 5, 7]. The score of each variable is added into a total malignancy score

Morphologic feature	Score			
	1	2	3	4
Degree of keratinization	Highly keratinized (>50% of the cells)	Moderately keratinized (20–50% of the cells)	Minimal keratinization (5–20% of the cells)	No keratinization (0–5% of the cells)
Nuclear polymorphism	Little nuclear polymorphism (>75% mature cells)	Moderately abundant nuclear polymorphism (50–75% mature cells)	Abundant nuclear polymorphism (25–50% mature cells)	Extreme nuclear polymorphism (0–25% mature cells)
Pattern of invasion	Pushing, well delineated infiltrating borders	Infiltrating, solid cordss, bands and/or strands	Small groups or cords of infiltrating cells (n>15)	Marked and widespread cellular dissociation in small groups and/or in single cells (n<15)
Host response (infiltration of leukocytes)	Marked	Moderate	Slight	None

ber of mitoses, but in this study we omitted this variable, as suggested elsewhere [7]. The time needed for grading of each section was 1–3 min.

Statistics

SAS 6.06 software (SAS Institute, Cary, N.C.) was used to store and analyse the data. After selection of cut-off levels, the prognostic impact of single and combinations of variables were tested by the log-rank method. Time to local recurrence from the date of initial treatment was defined as the dependent variable. A case was treated as censored if there was no evidence of disease at the last follow-up consultation or if deaths occurred from unrelated diseases. Prognostic powerful single and combinations of variables were tested in the multivariate proportional hazard regression model (Cox method) and analysed by the backward elimination procedure. Log(-log) plots were used to check the assumption of proportional hazard rates in the Cox model. Survival curves were obtained by the Kaplan-Meyer method. The accepted limit of significance was P<0.05.

Table 2 Number and percentage (in parenthesis) of 61 non-recurring and 34 recurring glottic T_1 and T_2 squamous cell carcinomas according to grading of histopathological parameters

	Score			
	1	2	3	4
Keratinization				
Non-recurring	16 (27)	38 (62)	6 (10)	1 (2)
Recurring	5 (15)	18 (53)	8 (23)	3 (9)
Nuclear polymorphism				
Non-recurring	12 (20)	30 (49)	17 (28)	2 (3)
Recurring	4 (12)	16 (47)	13 (38)	1 (3)
Pattern of invasion				
Non-recurring	5 (8)	31 (51)	21 (34)	4 (7)
Recurring	4 (12)	11 (32)	10 (29)	9 (27)
Host response				
Non-recurring	4 (7)	25 (41)	24 (39)	8 (13)
Recurring	2 (6)	9 (27)	10 (29)	13 (38)

Results

For 11 (9%) of the total of 120 patients, too little biopsy material was available for the criteria given for our invasive front grading, and these patients were therefore excluded. Table 2 summarizes the various histopathological variables evaluated in relation to T-status, nonrecurrences and recurrences. After selection of cut-off levels, single parameter analysis by means of log-rank revealed significant results for the degree of keratinization, pat-

Table 3 Association between various parameters and local recurrence of small glottic carcinomas. Single parameters and combination of parameters analysed by means of log-rank and proportional hazards model

	Cut-off levels	No. of cases	Log-rank p-value	Cox regression p-value	Risk ratio
Age	<64 vs.>64	46/49	0.9647		
T	T1 vs. T2	46/49	0.0190	0.0695	
Sex	Male vs. female	89/6	0.4086		
Keratinization	≤2 vs.≥3	77/18	0.0083	0.0472	2.1
Nuclear polymorphism	≤2 vs. ≥3	62/33	0.2926		
Pattern of invasion	≤3 vs. 4	82/13	0.0004	0.0047	3.1
Host response	≤3 vs. 4	74/21	0.0014	0.0695	
All histological parameters	≤12 vs. ≥13	76/19	0.0010	0.1578	
Keratinization, pattern of invasion and host response	≤8 vs. ≥9	80/15	0.0004	0.2393	
Keratinisation and pattern of invasion	≤6 vs. ≥7	77/18	0.0019	0.2981	

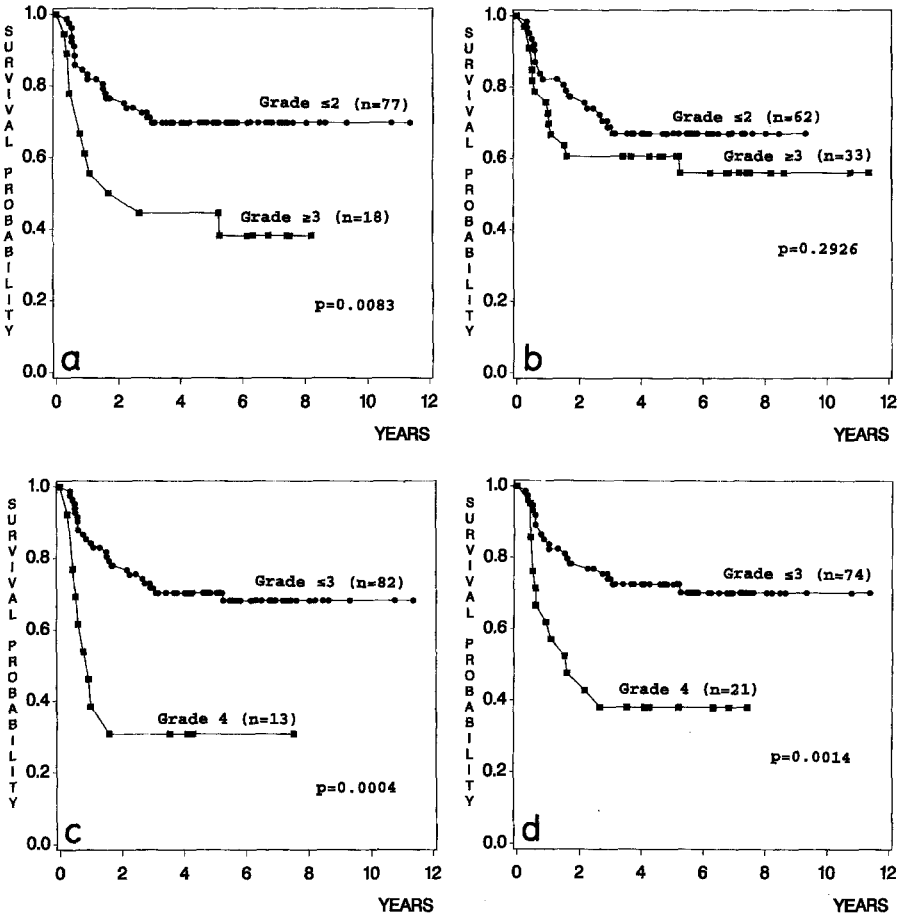

Fig. 2a–d Kaplan-Meyer plots of time to recurrence for small glottic carcinomas. The plots for the histological parameters degree of keratinization (**a**), nuclear pleomorphism (**b**), pattern of invasion (**c**) and host response (**d**) are shown

tern of invasion and host response, but not for the degree of nuclear polymorphism (Table 3). Both the total malignancy score and other combinations of variables revealed significant results on univariate analyses (Table 3). The Cox multivariate analysis showed that the pattern of invasion (P=0.0047) and degree of keratinization (P=0.047) were the only significant prognosticators (Table 3). Figure 2 shows the Kaplan-Meyer plots for all histological variables.

Discussion

With few exceptions, in previous studies dealing with histopathological scoring systems for squamous cell carcinomas of the head and neck, the patient populations analysed have been heterogeneous with respect to site, TNM stage or treatment (reviewed by Odell et al. [17] and Bryne et al. [7]). In conformity with Odell et al. [17], this study benefits from a homogeneous sample, as only T1 and T2 glottic carcinomas treated with similar doses of radiation were included.

The previously reported high prognostic value of our malignancy grading system [5, 7, 17] is confirmed in this study. The main difference between this grading and other systems is that only characteristics at the deep invasive front of the tumour are considered [1, 4, 7]. Although glottic biopsies are generally very small, only 9% of the routine biopsies did not admit of evaluation by our method. In addition, although some of these biopsies may be somewhat unrepresentative, a high prognostic significance was found. The implication of this demonstrated powerful prognostic importance of the deep invasive front is that clinicians should be encouraged to take biopsy specimens that include the deep part of the tumour area so that this grading, or possible expression of various tumour markers, can be performed [2, 7, 8, 13, 17, 19]. Our grading is probably less time consuming than other systems, because large parts of the tumour can be disregarded [7]. Grading for one tumour takes less than 3 min after some training, and the reproducibility of the system has been shown to be acceptable for clinical use (kappa >0.6) [4, 7]. We believe our grading or variants thereof may be useful for various other carcinomas.

Since mitotic counts proved difficult to standardize [7, 18], this variable was omitted in the present study. Of the four histological variables, only the degree of keratinization and, in particular, the pattern of invasion appeared significant in the multivariate analyses. These single variables turned out to be slightly better prognosticators than the total malignancy score and combinations of variables for these small glottic cancers. Our findings are in accordance with the results of a recent study on small lingual cancers [17]. The present results suggest that the malignancy grading could be further simplified, so that an even higher degree of reproducibility may be obtained [6].

Although cells located more superficially or centrally in the tumour may have the capacity for invasion and metastasis, their localization within the tumour prevents them from expressing these harmful properties. Tumour heterogeneity is well established [16] and has frequently been observed as a gradual dedifferentiation of cells toward the invasive front of oral squamous cell carcinomas [4, 7, 10], suggesting biological differences in various parts of tumours. In addition, alterations in the biosynthesis of various structures, such as oncogene mRNAs [19], proliferation markers [13], adhesion molecules [2, 14], blood group antigens [8] and others [11] in invasive front cells compared with other parts of the various tumours have been reported. These reports support our hypothesis that possible keys for increased understanding of growth, invasion and metastatic potential may reside in the invasive front area of tumours.

Despite radiotherapy doses of 66–70 Gy, approximately 12% of T1 and 33% of T2 tumours give rise to local recurrences. With salvage surgery the 5-year survival for these tumours is 90% [21]. Several studies have been performed in efforts to find prognostic factors, such as DNA content, proliferation markers and nuclear volume, that can predict treatment failure of glottic cancers [15, 20], but none has yet found clinical application. However, the present results suggest that patients with tumours characterized by marked cellular dissociation and little keratinization in the invasive front area should be offered primary surgical treatment. Alternatively, such patients might be allocated to new experimental strategies that enhance the tumour cell response to radiation [9, 12].

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References

1. Anneroth G, Batsakis J, Luna M (1987) Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. Scand J Dent Res 95: 229–249
2. Aruffo A, Dietsch MT, Wan H, Hellstrom KE, Hellstrom I (1992) Granule membrane protein 140 (GMP140) binds to carcinomas and carcinoma-derived cell lines. Proc Natl Acad Sci USA 89: 2292–2296
3. Boysen M, Løvdal O, Tausjø J, Winther F (1992) The value of follow-up in patients treated for squamous cell carcinoma of the head and neck. Eur J Cancer 28: 4264–4230
4. Bryne M (1991) Prognostic value of various molecular and cellular features in oral squamous cell carcinomas: a review. J Oral Pathol Med 20: 413–420
5. Bryne M, Koppang HS, Lilleng R, Stene T, Bang G, Dabelsteen E (1989) New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. J Oral Pathol Med 18: 432–437
6. Bryne M, Nielsen K, Koppang HS, Dabelsteen E (1991) Reproducibility of two malignancy grading systems with reportedly prognostic value for oral cancer patients. J Oral Pathol Med 20: 369–372
7. Bryne M, Koppang HS, Lilleng R, Kjaerheim A (1992) Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol (Lond) 166: 375–381
8. Bryne M, Thrane PS, Dabelsteen E (1991) Loss of expression of blood group antigen H is associated with cellular invasion and spread of oral squamous cell carcinomas. Cancer 67: 613–618
9. Denekamp J (1992) Inadequate vasculature in solid tumours: consequences for cancer research strategies. BJR Suppl 24: 111–117
10. Hambraeus GM, Mercke CE, Willen R, Ranstam J, Samuelsson L, Lamm IL, Landberg T (1988) Prognostic factors influencing survival in combined radiotherapy and surgery of squamous cell carcinoma of the esophagus with special reference to a histopathologic grading system. Cancer 62: 895–904
11. Heyden A, Thrane PS, Brandtzaeg P (1992) Loss of epithelial L1 expression is associated with cellular invasion of oral squamous cell carcinomas. J Oral Pathol Med 21: 330–335
12. Hockel M, Schlenger K, Doctrow S, Kissel T, Vaupel P (1993) Therapeutic angiogenesis. Arch Surg 128: 423–429
13. Kearsley JH, Furlong KL, Cooke RA, Waters MJ (1990) An immunohistochemical assessment of cellular proliferation markers in head and neck squamous cell cancers. Br J Cancer 61: 821–827
14. Mattijssen V, Peters HM, Schalkwijk L, Manni JJ, Grootenboer B van 't Hof, Mulder PH de, Ruiter DJ (1993) E-cadherin expression in head and neck squamous-cell carcinoma is associated with clinical outcome. Int J Cancer 55: 580–585
15. Munck-Wikland E, Fernberg J, Kuylenstierna R, Lindholm J, Auer G (1993) Proliferating cell nuclear antigen (PCNA) expression and nuclear DNA content in predicting recurrence after radiotherapy of early glottic cancer. Eur J Cancer B Oral Oncol 29: 75–79
16. Nicolson GL (1987) Tumor cell instability, diversification, and progression to the metastatic phenotype: from oncogene to oncofetal expression. Cancer Res 47: 1473–1487
17. Odell EW, Jani P, Sherriff M, Ahluwalia SM, Hibbert J, Levison DA, Morgan PR (1994) The prognostic value of individual histologic grading parameters in small lingual squamous cell carcinomas. The importance of the pattern of invasion. Cancer 74:789–794
18. Quinn CM, Wright NA (1990) The clinical assessment of proliferation and growth in human tumours: evaluation of methods and application as prognostic variables. J Pathol 160:93–102
19. Riviere A, Wilckens C, Loning T (1990) Expression of c-erbB2 and c-myc in squamous epithelia and squamous cell carcinomas of the head and neck and the lower female genital tract. J Oral Pathol Med 19: 408–413
20. Tausø J, Stenersen TC, Reitan J, Clausen OP, Moen E, Boysen M (1995) Nuclear volume is a prognostic factor in carcinoma of the larynx. Clin Otolaryngol (in press)
21. Vermund H, Boysen M, Brandenburg JH, Evensen J, Jacobsen AB, Kaalhus O, Tausø J, Thorud E, Wiley AL, Winther F (1990) Primary irradiation, surgery or combined therapy in squamous cell carcinoma of the larynx. A comparison of treatment results from two centers. Acta Oncol 29: 489–503