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# The Prognostic Value of Cytometric DNA Analysis in Early Stage Tongue Cancer

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**In spite of a small size and seemingly localised properties T1 tongue cancer does recur after surgical treatment, locally and/or in regional lymph-nodes, in 30–40% of the patients, and 50% of patients with recurrent disease die because of their cancer. If these patients could be identified by analysis of relevant parameters on the primary biopsy reflecting the biological properties of the tumours more extensive treatment regimes could be given selectively. In 47 primary biopsy specimens from patients with T1N0M0 squamous cell carcinoma of the mobile tongue the aberration in cellular DNA content was significantly higher in the group of tumours which recurred after surgical treatment compared with the non-recurrent group. Tumours in females recurred more frequently than in males. No significant correlation between recurrence and grade of histological differentiation or tumour thickness could be found. Image cytometry DNA analysis provides an objective and reproducible assessment of the nuclear DNA content which could facilitate selection of adequate treatment strategies.**
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## INTRODUCTION

ALTHOUGH THE prognosis for patients with squamous cell carcinoma of the tongue largely depends on stage, patients with tumours of comparable stages frequently show divergent clinical courses. The concept of histological differentiation generally practiced by pathologists is subjective and not clearly defined. In order to increase the prognostic value of histopathologic morphology for head and neck cancer, different grading systems have been applied. These systems, relating morphological findings to clinical outcome, provide additional information, but remain subjective and the reproducibility is still unsatisfactory.

The nuclear DNA content of a tumour cell population offers an objective and quantitative parameter which can indicate the biologic behaviour of the tumour. Numerous investigators report a relationship between the DNA content of tumour cells and prognosis. With few exceptions a more pronounced DNA aberration implies a worse prognosis for solid tumours. The prognostic value of nuclear DNA content of tumour cells in squamous cell carcinoma of the head and neck has been discussed previously [1–6]. The reason for the divergent conclusions may be that most investigations comprised a heterogenous population of patients with tumours from different head and neck sites and of different stages. Moreover, a number of divergent therapy regimes were employed. Finally, different methods for analysis and classification were applied.

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The present investigation was undertaken in order to determine the impact of DNA aberration on risk for local or regional recurrence in small (T1N0M0) tongue cancers without metastases all treated by partial glossectomy only.

## MATERIALS AND METHODS

Records from 58 previously untreated patients registered as T1N0M0 squamous cell carcinoma of the mobile tongue were reviewed. The patients had been treated at 14 different university or county hospitals in Sweden between 1974 and 1986. All patients had been treated with resection of the visible tumour including a margin of at least 1 cm of surrounding macroscopically uninvolved tissue. 11 patients were excluded from the study, either because resection had not been histopathologically radical (2 patients), radiotherapy had been given in addition to surgery (3 patients), immunosupressive treatment had been given due to kidney transplantation (1 patient), previous treatment for oral cancer (2 patients), missing specimen (2 patients), or non-invasive cancer *in situ* (1 patient). The study comprised 21 (45%) men and 26 (55%) women who ranged in age from 36 to 84 years (median age = 70).

All patients were followed-up until their death or at least 36 months after the initial treatment. The median follow-up time was 46 months. The tumours had been classified as highly ($n=22$), moderately ($n=20$), or poorly ($n=5$) differentiated squamous cell carcinoma. A clinical macroscopical estimation about tumour thickness by the surgeon was available in 40 patients.

For the cytometric DNA analysis two sections, 4 μm thick, were cut from the paraffin embedded tumour tissues. The first section was stained with haematoxylin–eosin and the second according to the Feulgen method [7]. The DNA measurements were performed directly on the slides by means of a densitometrical device (Ahrens Image System, Bargteheide/Hamburg, Germany). The investigator had no knowledge as

to the outcome of the disease. There were 70–100 nuclei per specimen analysed, and lymphocytes and/or granulocytes were used as control cells to establish the normal diploid 2c value. DNA content between 2c and 4c is seen in pre-mitotic cell nuclei, but cell nuclei with DNA values exceeding 5c are not normally found in healthy squamous cell epithelium and thus reflect DNA aberration. The number of tumour cells with nuclear DNA content exceeding 5c was expressed in per cent and referred to as 5c exceeding rate (5cER). A cut off level of 10% 5cER was used as a threshold for pronounced aneuploidy.

*Statistical methods*

The student's t-test was used to determine any significant: (a) difference in DNA aberration (5cER) between the recurrent and the non-recurrent group; (b) difference in tumour thickness between recurrent and non-recurrent tumours.

Fisher's exact test was used to determine if: (a) tumours displaying more than 10% 5cER recurred more frequently than tumours with less than 10% 5cER; (b) any sex showed a higher rate of recurrence; (c) poorly differentiated tumours recurred more frequently than well and moderately differentiated tumours.

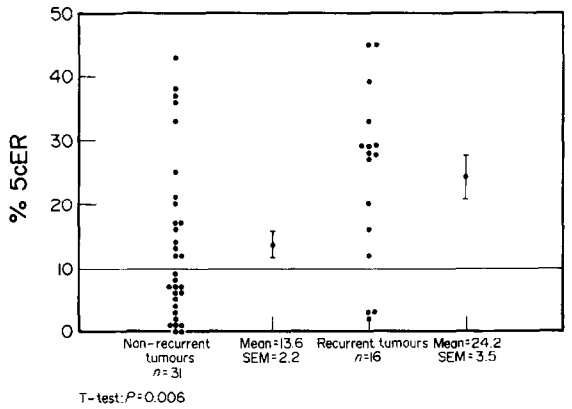


**Fig. 1. The group of tumours which later recurred showed a more pronounced aberration in nuclear DNA content (%5cER) as compared with the tumours that were cured by local resection. Thirteen of 28 tumours (46%) with more than 10% 5cER recurred, while 3 of 19 (16%) with less than 10% 5cER recurred ($P = 0.029$).**

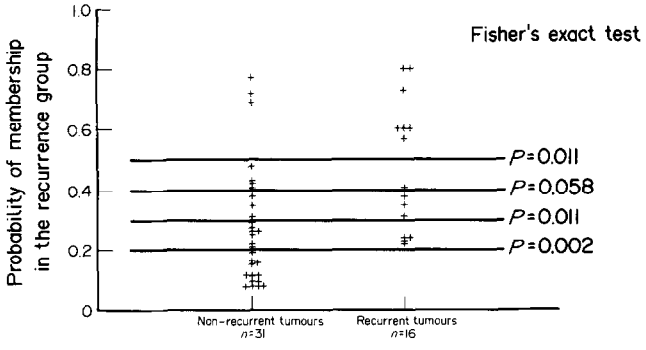


**Fig. 2. When sex and %5cER were combined in a discriminant function the probability of membership in the relapse group could be estimated and graphically displayed. The power to discriminate between the relapse and the cured group by a combination of these two parameters was tested at different levels of probability of membership in the relapse group.**

The Spearman rank correlation was used to investigate any significant: (a) correlation between grade of histological differentiation and risk for recurrence; (b) correlation between grade of histological differentiation and 5cER.

Multivariate stepwise linear discriminant analysis of %5cER, sex, grade of histological differentiation and tumour thickness was used to establish a discriminatory function assigning every case the probability of membership in the relapse/cured group. The discriminatory power was tested in Fisher's exact test at different threshold levels.

## RESULTS

16 of 47 patients (34%) had a recurrence either locally (6 patients), in regional lymph nodes (8 patients), or both (2 patients). Recurrences occurred between 3 and 67 months after primary treatment, median time to recurrence was 10.5 months. 8 patients succumbed to their recurrent cancer disease (2/6 with local recurrence, 5/8 with regional recurrence, 1/2 with local and regional recurrence). Recurrence occurred more frequently in women (12/26 = 46%) than in men (4/21 = 19%) ($P = 0.049$).

The group of tumours which recurred after local resection showed a more pronounced aberration in nuclear DNA content (i.e. higher 5cER) as compared with the tumours that were cured ($P = 0.006$, Fig. 1). Tumours with more than 10% 5cER recurred in 46% (13/28), while tumours with less than 10% 5cER recurred in 16% (3/19) ($P = 0.029$). The multivariate stepwise linear discriminant analysis defined %5cER and sex as the two strongest variables. Neither grade of histological differentiation nor tumour thickness added any significant discriminatory power. Sex and %5cER were combined in a discriminant function and the probability of membership in the relapse group is graphically displayed (Fig. 2). The power of the discriminatory function was tested at different thresholds. If the threshold level was set at 0.2, 13/31 (42%) of the non-recurrent tumours were correctly classified as non-recurrent, and all recurrent tumours were correctly classified ($P = 0.002$, Fig. 2).

Recurrence occurred in 6 out of 22 patients (27%) with well differentiated squamous cell carcinomas, 6 out of 20 patients (30%) with moderately differentiated tumours, and 4 out of 5 patients (80%) with poorly differentiated tongue cancers. No statistically significant correlation between degree of histological differentiation and risk for recurrence could be revealed. However when well and moderately differentiated tumours were compared with poorly differentiated tumours it was found that recurrence occured more frequently in poorly differentiated tumours ($P = 0.040$). Information concerning tumour thickness was obtained in 40 patients. Mean tumour thickness was 5.3 mm ranging from 2 to 15 mm. Tumour thickness did not yield any significant correlation to risk for recurrence.

No significant correlation between degree of histological differentiation and DNA aberration (%5cER) was found.

## DISCUSSION

Carcinoma of the tongue presents serious therapeutical problems. Patients with advanced primary tumours and metastatic neck disease are rarely cured with current treatment modalities [8]. In small, localised tumours a possible decrease in recurrence rate, and improved cure rate, must be weighed against the side effects of extensive surgery or irradiation. In the present material, 79% of the patients had no occult neck

disease, and consequently elective neck therapy would not have been of benefit for the majority. Thus, careful selection of treatment is essential in order to avoid unnecessary morbidity. Since neither T-staging system nor histopathological malignancy grading accurately predict which patients are most likely to have later metastasis [9], efficient and reproducible methods for characterising the tumours, including their tendency to recur, locally or in regional lymph nodes, are needed in order to individualise and optimise the treatment. Measurement of nuclear DNA content represents an objective and reproducible method of grading genetic instability possibly related to tumour malignancy.

The rate of aneuploidy in head and neck cancer may vary according to tumour site, method of analysis and classification criteria used. Kaplan found 41% of these tumours to be aneuploid using flow cytometric technique [10], while Holm (Feulgen DNA single cell [3]) and Johnson (flow [11]) found that 78–86% of the head and neck tumours were aneuploid. Oral cavity cancers seem to be diploid more frequently than pharyngeal and laryngeal tumours [3, 10]. Kokal found patients with aneuploid tumours to have more advanced stage carcinomas, lymph node metastasis, perineural invasion and extracapsular lymph node invasion [5]. Kearsly, on the other hand, could not confirm any significant association between ploidy and either tumour size or degree of nodal involvement [4].

Several investigators found a correlation between DNA ploidy and degree of histological differentiation, i.e. an increase of aneuploid tumours with a decreasing degree of histological differentiation [3, 4, 12]. Kaplan refutes this finding and states that nuclear DNA content is a variable unrelated to histological grade [10]. The present study could not confirm any statistically significant correlation between degree of differentiation and DNA aberration (5cER). Only when well and moderately differentiated tumours were grouped together and compared with poorly differentiated tumours could a correlation between recurrence and degree of differentiation be revealed. This may partly be due to the limited number of tumours analysed, but also reflects the problem of reproducibility in grading histological differentiation. There seems to be a correlation between morphological differentiation and prognosis, but the differences are small and the power of morphological grading may be lost due to reproducibility problems. With the help of computerised image analysis, morphological criterias could be objectively assessed with a higher degree of reproducibility and possibly give a better correlation to prognosis.

Earlier investigations have found a correlation between tumour thickness and recurrence rate [9, 13, 14]. Here, this finding could not be confirmed. This indicates the difficulties in estimating tumour size and thickness without a more refined measuring technique. Tumour size and depth of cancer infiltration should preferably be measured by means of a measuring stick graded in millimeters which was not performed in this material.

The reason for the higher recurrence rate in females than in males is open to discussion. There was no age difference between the two groups. Could differences in hormone levels, mucosal resistance or low serum iron levels, as seen in Plummer Vinson patients who have an increased rate of hypopharyngeal tumours, be of any significance?

The prognostic value of quantitative DNA analysis on squamous cell carcinoma of the head and neck has been validated by several investigations [4–6] but rejected by others [1]. Possibly, different tumour sites and stages should be investigated separately. Goldsmith [15] found that patients with laryngeal cancer and hypopharyngeal cancer aneuploid tumours had better prognosis than patients with diploid tumours, while the opposite was true for oral cancer. In a preliminary study on T1-T3 N0 tongue cancers, using the flow technique on archival material, Farrar *et al.* first showed that DNA ploidy had promising prognostic value. Later in a larger series of tongue cancer, this finding could not be confirmed [1]. In this latter study, only 30% of the tongue cancers were considered aneuploid. From our experience, when dealing with squamous epithelial cancer, the use of flow technique is hampered by the risk of a too large portion of non-tumorous cells in the samples. This is why we prefer the single cell image cytometry method, where solely tumour cells are measured, for DNA assessment in these tumours.

Kokal found that the DNA content of the squamous cell carcinoma of the head and neck was the single most important prognostic variable and independent of all other clinical and pathological variables examined. Furthermore, recurrence rate for patients with aneuploid tumours stage I and II was essentially similar to that of patients with aneuploid stage III and IV carcinomas, being 78% (7/9) and 69% (27/39), respectively. He concludes, although on the basis of a limited material, that DNA content may be more important than stage as a predictor in squamous cell carcinoma of the oral cavity, larynx and pharynx [5].

The present investigation showed a higher degree of DNA aberration in tumours which recurred after primary surgical treatment as compared with the non-recurrent tumours. Although we studied only small, localised tongue cancers the results indicate that nuclear DNA content is a parameter which enables a more accurate assessment of the biological aggressiveness of the tumour than can be obtained from standard clinical or pathological parameters. In conclusion, DNA analysis can be used as an additional prognostic tool alongside clinical staging and histopathological grading for evaluating squamous cell carcinoma of the tongue and be included as one important parameter when selecting treatment strategies.

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