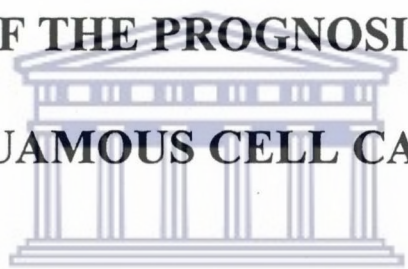
AN INVESTIGATION INTO THE ROLE OF HISTOLOGICAL PARAMETERS IN THE PREDICTION OF THE PROGNOSIS FOR T1 AND T2 ORAL SQUAMOUS CELL CARCINOMAS



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CONTENTS

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I. DECLARATION

I,..... declare that "An investigation into the role of histological parameters in the prediction of the prognosis for T1 and T2 oral squamous cell carcinomas" is my own work and that all the sources I have quoted have been shown and acknowledged by means of references.

Signed: .....

II. DEDICATION

This dissertation is dedicated to:

My beloved children, Carla Anne and Mahesh Michael that unknowingly, have sacrificed many hours of their short lives and has made every moment worthwhile.

My parents, Cecil and Elaine whose love, support and guidance are irreplaceable.

My best friends- Tracy and Hayley, Jerome and Sarel who have supported me throughout my career - thank you for your encouragement and help.

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1. SUMMARY

T1 and T2 squamous cell carcinomas of the head and neck have an unpredictable prognosis that often pose therapeutic problems. Sophisticated methods such as cytometric DNA analysis, immunocytochemistry and detection of cellular growth factors, have been applied with varying success rates for predicting recurrences, metastatic rates and overall prognoses. However, with the general lack of resources in Africa, devising a simple, reliable, reproducible and cost-effective method of predicting tumour behaviour to aid optimal treatment planning is imperative. Surgical excision specimens of forty-eight primary T1 and T2 squamous cell carcinomas of the floor of the mouth and tongue were histologically evaluated by two individual pathologists (double-blinded study) who had no prior knowledge of clinical course or outcome. The following morphological criteria were assessed: degrees of keratinisation, nuclear aberration, mitoses, patterns of an invasion, stage of an invasion, depth from surgical excision margin, tumour presentation, vascular invasion, perineural involvement and degree of inflammation. A histological score between 1 and 4 was allocated to individual parameters. The inter- and intraobserver agreement was tested using the Kappa statistical method. The overall survival times, time till nodal metastases and time till local recurrence were estimated using Kaplan-Meier estimates. The relationship between the individual parameters and survival, nodal recurrence and local recurrence was investigated using Cox's Proportional Hazard Regression. The initial results showed a reliable level of agreement between observers that improved markedly after reevaluation and clearer definition of each parameter. The intraobserver agreement levels of the experienced examiner were markedly better than that of the inexperienced examiner.

Of the 48 patients in the sample, 6 patients died of carcinoma and 2 of unrelated causes. The

overall 2-year survival rate was 84%. The mean time to death was approximately 2 years. The 2-year survival probability rate was 92% that decreased to 73% after 5-year. Twenty patients developed regional lymph nodes metastasis. The mean time to nodal metastasis was 3.2 years. The probability of remaining free of nodal metastasis after 2-years was 65% that decreased to 46% after 5 years. Four patients developed recurrent tumours at the primary site. Two of these patients subsequently developed nodal metastasis. The mean time to local recurrence was 1.5 years. The probability of remaining free of local recurrence after 1 year is 92%. Seventy-five percent of the primary recurrence and 80% of the nodal metastasis developed within 2 years. The pattern of invasion correlated significantly to both local recurrence and nodal metastasis. There was a strong association between depth from surgical excision margin in relation to nodal metastastasis and degree of keratinisation in relation to local recurrence.

This study has shown that attaining a significant level of agreement between examiners is possible after discussion and an increased understanding of the criteria of the proposed grading system. The reproducibility of such a grading system improves with increased use. Inexperienced examiners should repeatedly use the grading system to increase their familiarity with the grading system.

Our results show a high 2-year survival rate for this patient cohort possibly because only T1 and T2 sized tumours were included in the study. These positive results are overshadowed by the high percentage of patients developing primary and nodal recurrences, which contribute to the high morbidity rates with these lesions. Clinically, tumours that recur, develop nodal metastasis or result in death are regarded as treatment failures.

OPSOMMING

T1 en T2 plaveisel karsinome van die kop en nek het 'n onvoorspelbare prognose en dit skep dikwels terapeutiese probleme. Gevorderde metodes soos sitometriese DNA analise, immunohistochemie, en die identifisering van sellulêre groei faktore word met 'n wisselende mate van sukses toegepas om herhalings, metastatiese potensiaal en tumor prognose te voorspel en dus om optimale behandeling te beplan. Agt en veertig chirurgiese monsters van T1 en T2 plaveisel karsinome van die mondvloer en tong was onafhanklik deur twee patoloë ondersoek. Die ondersoekers het geen vorige kennis van die kliniese verloop of uiteindelike gevolg vna die toestande gehad nie. Die volgende morfologiese kriteria (parameters) was evaluee: graad van keratinisasie, kern abnormaliteite, mitotiese aktiwiteit, patroon van infiltrasie, stadium van infiltrasie, diepte van die tumorvanaf die chirurgiese eksisie rand, tumor presentasie, vaskulêre infiltrasie, perineurale betrokkenheid, en graad van ontsteking. 'n Histologiese waarde tussen 1 en 4 was aan die afsonderlike parameters toegeken. Inter- en intraondersoeker ooreenkoms is met behulp van die Kappa statistiese metode getoets. Die algehele oorlewingstye, tyd tot nodale metastase en lokale herhaling was geskat met behulp van die Kaplan-Meier skattings. Die verwantskap tussen die individuele parameters en oorlewing, nodale metastase en lokale herhaling was met behulp van die Cox Proportional Hazard Progression ondersoek. Die inisiele resultate het 'n betroubare vlak van ooreenstemming tussen ondersoekers getoon. Die vlak van ooreenstemming het egter merkbaar verbeter na herevaluasie en duideliker definisie van elke parameter. Die intraondersoeker ooreenkoms van die ervare ondersoeker was aansienlik beter as die van die onervare ondersoeker.

Van die 48 pasiente in die studie het 6 gesterf as gevolg ban karsinoom en 2 as gevolg van

onverwante oorsake. Die 2 jaar oorlewing syfer van die groep was gevolglik 84%. Die gemiddelde tyd van die pasiente at gesterf het na behandeling was 2 jaar. Die waarskynlikheid om 2 jaar te oorleef was 92% maar dit het na 5 jaar tot 73% gedaal. Twintig van die pasiente het metastatiese letsels ontwikkel. Die gemiddelde tyd voor die tumore gemetastaseer het was 3.2 jaar. Die waarskynlikheid om na behandeling nie metastatiese letsels vir 2 jaar te ontwekkel nie was 65% en dit het na 5 jaar verminder na 46%. Vier pasiente het herhalings ontwekkel in die area van die primêre en 2 van hulle het metastatiese letsels na die limfnodes ontwikkel. Die mediane typd tot herhaling was 1.5 jaar. Die waarskynlikheid om vry van lokale herhalings te bly was 92% na 1 jaar. Vyf-en-sewentig persent van herhalings in die area van die oorspronklike tumor en 80% van nodale uitsaaiings het binne 2 jaar ontwikkel. Die patroon van infiltrasie van die primêre tumor het betekinisvolle ooreenkomste getoon met beide lokale herhaling en nodale uitsaaiings. Daar was 'n sterk verband tussen die diepte van die tumor vanaf die eksisie lyn en die moontlkikheid om nodale uitsaaiings te ontwikkel sowel as die graad van keratinisasie en die waarskynlikheid om lokale herhalings te ontwikkel.

Die studie toon dat dit moontlik is om 'n betekenisvolle hoë vlak van ooreenkoms tussen ondersoekers te kry as die kriteria van die voorgestelde gradering sisteem voldoende bespreek word en durt die ondersoekers verstaan word. Die herhaalbaarheid van so 'n gradering sisteem verbeter ook met gebruik. Onervare ondersoeders moet voortdurend van die gradering sisteem gebruik maak sodat hulle met die sisteem vertroud kan raak.

,n Hoë 2 jaar oorlewing syfer was vir die groep pasiente gerapporteer. Dit kan toegeskryf word aan die feit dat slegs T1 en T2 tumore by die studie ingesluit was. Dit moet egter nie as 'n algehele sukses met betrekking tot behandeling beskou word nie aangesien die morbiditeit, wat

deur pasiente wat met veral nodale uitsaaiings gepresenteer het, hoog was.

2. INTRODUCTION

Despite the introduction of many anti-tobacco campaigns, the incidence of oral squamous cell carcinoma is still surprisingly high. It is the fourth most common malignancy occurring in South African males (Hille et al, 1996). Oral squamous cell carcinomas have an unfavourable prognosis (Platz et al, 1983; Tytor, 1990). The recorded five - year survival rates are between 35 - 50% (Wildt et al, 1989). The unchanged survival rates and accompanying high incidence of squamous cell carcinomas have resulted in a significant increase in mortality (Hille et al, 1996). The UICC TNM classification is a popular clinical staging system that allows for the comparison of data from different institutions and aids in treatment planning. "T" refers to the size of the primary tumour, "N" is the measure of regional lymph node metastasis and "M" indicates distant metastases (see Appendix1 for details of UICC TNM classification). According to this system, smaller lesions without regional or distant metastasis has a good prognosis (Willen et al, 1975; Lund et al, 1975; Lund et al, 1976; Holm et al, 1982; Yamamoto et al, 1984). Its role in predicting the outcome of disease, however, remains controversial. Many studies have illustrated that even small oral squamous cell carcinomas have high recurrence rates and eventually cause the death of patients (Anneroth et al, 1984; Cunningham et al, 1986; Spiro et al, 1986; Jones et al, 1992). Irrespective of whether these recurrences are local or to regional lymph nodes, they represent treatment failure. One can therefore deduce that clinical staging of squamous cell carcinomas of the oral cavity per se is not a reliable indicator of prognosis. This is especially true in small lesions without metastases. At the Head and Neck Oncology Unit at Groote Schuur Hospital, Cape Town, oral squamous cell carcinomas are treated with surgery, radiotherapy or both. Chemotherapy is administered in special cases. The high rate of treatment failure and in particular of T1 lesions, often poses therapeutic problems. The identification of potentially aggressive lesions is therefore

important to plan optimal treatment. Both the clinical and the biological properties of malignant lesions should therefore be considered (Nicholson, 1986) when planning treatment and predicting outcome.

Since Broders published his first histological grading system in 1921, many alternative systems have been proposed, but none are widely used. The reproducibility and complexity of these systems could account for this failure. Recently, sophisticated methods are being employed to predict prognosis. These include cytometric DNA analysis (Munck-Wikland et al, 1992; Balsara et al, 1994), immunocytochemistry with emphasis being placed recently on the detection of mutant p53 (Gluckman, 1994; Yeudall et al, 1995) and various cellular growth factors (Storkel, 1993) Results from these studies appear promising. However, these methods are costly and with the recent decrease in the health budget in South Africa, their use will prove economically ineffective. A reliable, reproducible, cost-effective method of predicting the outcome of squamous cell carcinomas of the oral cavity is needed to assess tumour behaviour, aid treatment, predict prognosis and ultimately prevent recurrences. International studies have been performed on heterogeneous study populations with regard to site and clinical size. The current study is unique as it is the first study of a South African population and involves only T1 and T2 squamous cell carcinomas of the tongue and floor of the mouth.

The study compared various histological parameters in grading squamous cell carcinomas of the tongue and floor of the mouth to evaluate its reproducibility and establish its value in predicting prognosis. It is also the first grading system to evaluate the significance of tumour presentation in predicting survival.

3.LITERATURE REVIEW

3.1 Prognosis

The prognoses of oral squamous cell carcinomas are influenced by early diagnosis and treatment. However, patients usually present for treatment when lesions are advanced. Reasons for the late presentation include: the lack of early warning signals (like pain or lack of function), failure to recognise the seriousness of oral lesions, late referral of patients suffering from chronic lesions, inaccessibility to treatment centres, patients' attitudes, especially denial and their fear of cancer and the lack of accessibility to the tumours' site. (Phillips, 1989).

The diagnosis of carcinomas of the posterior and lateral borders of the tongue are complicated by their inaccessible sites and the absence of warning signals and are detected when they are more advanced. When diagnosed, these lesions are often accompanied by regional metastasis to either the submandibular or digastric lymph nodes (Phillips, 1989).

Squamous cell carcinomas at other intraoral sites are more easily accessible. Lip lesions only invade deeply late during the disease. Carcinomas of the buccal mucosa and gingiva and, in particularly a variant of a squamous cell carcinoma called verrucous carcinoma, is slow-growing and never metastasises. Palatal lesions usually involve the soft palate and can extend posteriorly to involve faucial tissue.

The indicators of prognosis are complete cure, local recurrence, regional metastasis and death. Prognosis is measured at two or five year intervals. Over the past decades many authors have

attempted to design a reliable, reproducible histological grading system to predict the clinical outcome of squamous cell carcinomas (Broders, 1921; Jakobsson et al 1973; Lund et al 1975). Although many disagreed on the value of individual parameters, a consensus was reached on the significance of both tumour and host factors.

3.2 The evolution of a grading system

3.2.1 Tumour factors

Inspired by the work of Hanseman who coined the term "anaplasia" in the late 19th century, Broders in 1920 published the first grading system for cancer. His series of 537 squamous cell carcinomas or "epitheliomas"of the lip was the first large clinical study of cancer. The aim of the investigation was to correlate histological grades with a prognosis. The observations were purely histological. There was no prior knowledge of the clinical histories or outcome of the cases. The study sample was divided into four groups according to their differentiation. Grade one tumours showed normal differentiation in three quarters of its epithelial structure. Grade two neoplasms consisted of equal proportions of differentiated and undifferentiated epithelial components; grade three lesions contained more undifferentiated epithelial cells and grade four tumours lacked differentiation completely. In 1925, Broders added two additional parameters to his classification. These were "mitotic" figures and "prominent nucleoli" which he included into the fourth category of lesions. The term "self-control" was adopted as an alternative to "differentiation". The grading system proved successful in determining the incidence of metastasis. Broders later expanded the scope of his research and evaluated carcinomas of the genitourinary organs (1921), skin (1921),

internal organs (1940) and the rectum (1940). Even at this early stage in the evolution of a grading system, its significance in establishing prognoses and its value as an aid in treatment planning was apparent. Although many authors reported Broders' classification to be of limited prognostic value (Bethman et al, 1965; Arthur and Fenner, 1966; Stottard, 1966; Krishnamurthi et al, 1971), Arthur and Farr (1972) reported that the histological grade, based on Broders' criteria, reflected tumour aggression. The latter authors studied squamous cell carcinomas of the mouth and oropharynx and reported a significant relationship between the grade of a tumour, clinical stage, cure rate and incidence of metastases. A major shortcoming of Broders' grading system was that it lacked a precise definition of the "degree of differentiation". Further problems arose when the "degree of differentiation" varied from site to site in the same tumour (Cade and Lee, 1957).

McGraven and co-workers (1961) reported a high incidence of regional lymph node metastases in poorly differentiated primary squamous cell carcinomas of the larynx. Enroth and co-workers (1972) attempted to clarify the "degree of differentiation". They assigned the term "well differentiated" to tumours composed of neoplastic epithelial cells arranged in well-defined cords and strands and to those that produce keratin. Neoplasms with diffuse growth patterns and those that did not produce keratin were considered as poorly differentiated. The latter group of lesions behaves more aggressively (Ragson, 1989).

Using their clearly defined criteria Enroth and co-workers (1973) studied 123 primary squamous cell carcinomas of the palate. They reported a higher rate of metastastasis and a lower survival rate in patients with poorly differentiated carcinomas compared to patients with well-differentiated lesions. Mendelson and co-workers (1976), Frierson and Cooper (1986) and Ragson (1989)

reported similar results. Odell and co-workers (1994) reported a significant correlation between Broders' grade and survival.

Hambraeus et al (1988) failed to establish a significant relationship between the differentiation of squamous cell carcinomas of the esophagus and survival.

At present, well-differentiated tumours comprises of neoplastic cells that appear squamous with obvious intercellular bridges, arranged in cords and strands and produce keratin. Poorly differentiated neoplasms show extreme pleomorphism, a diffuse growth pattern and produce little or no keratin. Moderately differentiated varieties are somewhere between. Anaplastic lesions have no resemblance at all to their tissue of origin.

3.2.2 Tumour-Host Factors

Jakobsson and co-workers (1973) added a new dimension into the grading of oral squamous cell carcinomas. They incorporated the "mode" and "stage" of an invasion, vascular involvement and lymphoplasmacytic infiltrate as estimating parameters of the host-tumour relationship. In doing so they identified the biological effects malignancies have on their immediate environment. The modified system took cognisance not only of epithelial changes, but also emphasised the importance of tumour-host interaction. Jakobsson and co-workers validated their findings in a study of glottic carcinomas and showed a statistically significant correlation between malignancy grading, recurrence and survival rates. Nuclear cell pleomorphism and mode of an invasion were important prognostic factors for recurrence in that particular study.

Many authors have applied Jakobsson's multifactorial grading system both in its original and modified forms (Lund et al 1975; Willen et al 1975; Lund et al, 1975; Lund et al, 1976; Helweg-Larsen et al 1978; Holm et al 1982; Yamamoto et al, 1984; Anneroth et al, 1984; Crissman et al 1986; Anneroth et al, 1986). The aims of all modifications were to refine and simplify the system to reduce subjectivity and improve prognostic accuracy.

3.3 Histological parameters

3.3.1 Degree of keratinisation

Normal squamous epithelial cells produce keratin. The amount of keratin produced by neoplastic cells may indicate the degree to which these cells resemble their normal counterparts. One can therefore conclude that the amount of keratin produced by neoplastic cells reflects the degree of differentiation. Although "tendency to keratinisation" is commonly used for deciding the grade of tumours, its prognostic value is seldom mentioned in the literature (Crissman et al, 1984). Anneroth and co-workers (1984) used this term to reflect the differentiation of neoplasms in their study of oral squamous cell carcinomas. These authors failed to show its prognostic value in predicting survival.

Crissman et al (1984), applied a modified version of Jakobsson's classification to study 77 squamous cell carcinomas of the oropharynx. The authors failed to show a significant relationship between the degree of keratinisation and survival or lymph node metastasis. Sarbia et al (1995) and Overholt et al (1996) in their study of 159 consecutive oral squamous cell carcinomas,

reported similar findings. Odell and co-workers (1994) used Broders' classification to evaluate small lingual squamous cell carcinomas and reported a significant correlation between degree of keratinisation at the invasive front and local recurrence of tumours.

3.3.2 Pattern of invasion / Growth pattern / Cohesiveness

The ability of some tumours to remain cohesive during invasion is an inherent property. Large bulky tumours with well-demarcated borders generally do not metastasize. In contrast, neoplastic lesions invading in single cells or small groups of cells have a higher frequency of lymph node involvement. It would appear that single neoplastic cells or small groups of cells have easier access to small blood vessels and lymphatics in the stroma (Crissman, 1986).

McGraven and co-workers (1961) identified two types of growth patterns in their study of squamous cell carcinomas of the larynx. The first pattern had a "pushing" and the second an "infiltrative" margin. In this series of laryngeal carcinomas these authors showed a significant correlation between the type of growth pattern and the frequency of a metastasis. Twenty-five of the thirty-five tumours with infiltrative margins exhibited nodal metastases as opposed to six of fifty tumours with pushing margins. In the same study the authors also reported that poorly differentiated lesions invaded with infiltrative margins and better differentiated tumours infiltrated with pushing margins.

Crissman and co-workers (1980) coined the term "pattern of an invasion" which encompassed "vascular invasion", "structure" and "mode of invasion". This parameter concentrated on the

cohesiveness of neoplastic cells or their ability to remain together. This phenomenon therefore illustrated the effect invading neoplastic cells had on the underlying stroma. These authors retrospectively studied 77 squamous cell carcinomas of the oropharynx and reported "differentiated" neoplasms to be more cohesive and invade with well defined "pushing" margins. These tumours had a better prognosis than "less-differentiated" groups where cellular adhesion was poor or absent and infiltration occurred in groups or single cells. The authors omitted "stage of invasion" from this study as they believed that only advanced tumours penetrated deep into the underlying stroma.

Yamamoto et al (1984) investigated the relationship between the " mode of invasion" of primary neoplasms and lymph node metastases. They assessed the deepest part of primary lesions using a modified version of Jakobsson's classification and subdivided grade four lesions into two separate grades. Grade 4C lesions showed cord-like infiltration patterns and grade 4D lesions infiltrated diffusely in single cells and/or small groups. Using their modified grading system, the authors evaluated 102 squamous cell carcinomas of the oral cavity. The results of their study showed that lesions with diffuse invasion patterns resulted in a high frequency of metastases and a poor prognosis. Thus, diffuse invasive margins suggest a poor prognosis in terms of nodal metastasis.

Odell and co-workers (1994), concurred with these results. In a series of 47 small carcinomas of the tongue, they reported a statistically significant correlation between the pattern of invasion and both local recurrence and lymph node metastasis. Woolgar and co-workers (1995) emphasised the importance of the pattern of invasion and described it as a reflection of the biological behaviour of tumours. Close et al (1989) and Ragson et al (1989), was unable to establish the

prognostic significance of the pattern of invasion in their respective studies.

3.3.3 Tumour thickness

The use of tumour thickness in predicting prognosis in melanomas was first described by Breslow (1970). Although the characteristics of oral squamous cell carcinomas vary from site to site, many authors have found tumour thickness to be a significant prognostic indicator.

Spiro and co-workers (1986) initiated the use of Breslow's criteria to evaluate 105 squamous cell carcinomas of the floor of mouth and tongue. They found 58% of T1 and 25% of T2 and T3 tumours that were clinically free of nodal metastases, to measure 2 mm or less in thickness. The 2-year survival rate for patients with these lesions was 86%. These authors suggested that tumours measuring more than 2mm were at risk of developing nodal disease. These results showed that pathologically thin tumours (2mm or less) have a good prognosis irrespective of the clinical stage. Mohit-Tabatabai and co-workers (1986) reviewed 84 stage I squamous cell carcinomas of the floor of the mouth. The authors reported recurrence rates of less than 2% in tumours measuring less than or equal to1.5mm. In contrast, the metastatic rate of tumours measuring more than 3.5mm in thickness, was up to 60%. They also found the incidence of metastases to increase with an increase in tumour thickness. Based on these findings they suggested that tumours measuring more than 1.5mm thickness are at a higher risk of developing cervical metastases and these patients should be treated with elective neck dissections. Frierson and Cooper (1986) suggested a critical tumour thickness of 6mm. The authors studied 186 squamous cell carcinomas of the lower lip and reported a statistically significant correlation between primary tumour thickness and lymph node metastases.

Ragson and co-workers (1989) documented a significant increase in cervical metastases in tumours measuring more than 5mm.

Ambrosch and co-workers (1995) distinguished between "tumour thickness" and "depth of invasion". They defined "depth of invasion" as the extent to which the tumours grow into the tissue below the epithelial surface. "Tumour thickness" on the other hand, is a measurement of the vertical bulk of a tumour. In their study of 128 squamous cell carcinomas of the upper aerodigestive tract, the authors reported exophytic tumours with little invasion to behave oncologically similarly to "thin" tumours. They also described a strong correlation between "depth of invasion" and nodal disease and suggested a depth of invasion of 4mm to be a critical measure in the occurrence of lymph node metastases.

Fukano and co-workers (1997) reported similar results in their study of 34 squamous cell carcinomas of the tongue. They recorded a total metastatic rate of 35.3 %. Sixty-four percent of the primary lesions that resulted in metastasises extended 5mm or more into the underlying connective tissue. In contrast, only 5.9% of the primary tumours measuring less than 5mm had developed subsequent metastasises. Of interest was the high incidence of clinically negative neck nodes that were histologically positive (30%). More than 88% of these cases had histological tumour depths exceeding 5mm. Based on these findings, the authors suggested elective neck therapy for tumours exceeding 5mm in depth. Morton and coworkers (1994) failed to show a significant relationship between tumour thickness and either nodal metastasis or survival.

3.3.4 Stage of invasion

Authors such as Crissman and co-workers (1984) excluded the stage of invasion from their

grading system as they believed that only advanced tumours penetrated deep into the underlying stroma. Hambraeus and co-workers (1988) studied 66 squamous cell carcinomas of the esophagus and reported a significant correlation between the stage of invasion and survival.

3.3.5 Mitoses

Deciding mitotic rates are tedious often resulting in large inaccuracies (Frierson and Cooper, 1986). The role of the mitoses as a significant parameter in the evolving grading system has always been controversial. Bryne and co-workers (1991) suggested that the difficulties encountered in standardising mitotic counts often affected results. Mitoses were first introduced into the grading system by Broders' in his revised classification in 1925. Initially their presence showed an extreme loss of differentiation (Broders, 1941). Although this parameter was used quantitatively in many grading systems (Jakobsson et al, 1973; Willen et al, 1975; Holm, 1981), its prognostic significance was seldom documented in the literature.

Frierson and Cooper (1986) noted that mitotic rates varied directly with the cytological grade and concluded that determination of mitotic rates was not prognostically significant. Bryne and co-workers (1991) reported that the omission of mitotic counts from their grading system improved the system's reproducibility without compromising its prognostic value. Crissman and co-workers included the frequency of mitosis as a prognostic parameter in their study of squamous cell carcinomas of the floor of the mouth (1980) and oropharynx (1984). These authors reported mitoses to be of significance in predicting survival. In contrast authors such as Willen and co-workers (1975), Frierson and co-workers (1986) and Woolgar and co-workers (1994) failed to do so.

3.3.6 Nuclear aberration

Nuclear polymorphism refers to changes in nuclear size, number of nucleoli and the presence of multinucleated anaplastic nuclei. Neither Crissman and co-workers (1980,1984), Frierson and Cooper (1986) nor Woolgar and co-workers (1995) could establish a significant correlation between nuclear aberration and lymph node metastases.

3.3.7 Perineural involvement

Perineural invasion is an indication of tumour aggression and is associated with a poor prognosis (Ballantyn et al, 1963). The perineural space is a tissue plane of least resistance, offering easy access to invasive, neoplastic cells and is a route of spread for various types of cancer (Goepfert et al, 1984). Neural invasion has been found to correlate with tumour recurrence and poor survival in colorectal cancer (Krasna et al 1988; Horn et al, 1990) and carcinoma of the pancreas (Nagakawa et al, 1992). Several authors have reported on the predisposition of squamous cell carcinomas and neoplastic salivary gland tumours of the head and neck to spread via nerves. Frierson and Cooper (1986), studied 20 patients with squamous cell carcinomas of the lip that exhibited perineural involvement. Cervical lymph node metastases developed in 60% of the cases. The authors noted that the likelihood of metastases increased as involvement became more extensive. They also reported perineural involvement to be more common in deeply invasive tumours, but stressed the importance of an extensive neural invasion in small tumours. Woolgar and co-workers (1995) studied 45 surgical specimens from different oral sites and identified

perineural invasion in 70% of cases that developed metastases. In contrast, only 28% of the cases without perineural invasion developed nodal metastasis. Umeda and co-workers (1986) failed to establish this relationship.

3.3.8 Vascular invasion

Sugarbaker (1979) and Batsakis (1984) stated that when neoplastic cells enter the circulation, metastases to regional lymph nodes or distant sites are more likely to occur. Although Jakobsson and co-workers (1973) included vascular invasion in the multifactorial grading system, authors such as Willen and co-workers (1975) and Anneroth and coworkers (1986)excluded this parameter from their modified systems. In 1978 Van Nagel and co-workers reported tumour recurrence in 34% of cervical carcinomas demonstrating vascular invasion. In contrast, tumour recurrence occurred in only 6% of cervical carcinomas without vascular invasion. They also reported a survival rate of 73% in cases showing vascular involvement compared to a 93% survival rate in cases without vascular involvement. Crissman and co-workers (1984) recorded similar findings in their study of 77 oropharyngeal carcinomas.

Vascular invasion significantly correlates with lymph node metastasis from tumours of the thyroid and kidney. Spiro and co-workers (1974), showed a statistically significant correlation between vascular invasion and cervical lymph node metastasis in a study of squamous cell carcinomas of the oral cavity and oropharynx.

Close and co-workers (1989) reported a statistically significant correlation between vascular

invasion and survival. In a retrospective study of 43 untreated stage II and greater squamous cell carcinomas of the oral cavity and oropharynx, the authors reported the presence of vascular invasion 81% of the study sample. Regional lymph node metastases developed in 77% of these cases (p=0.09). These results were similar to those obtained in their previous study (Close et al, 1987). Sarbia and co-workers (1995) studied 161 consecutive squamous cell carcinomas of the oesophagus and reported a clear correlation between lymphatic vessel involvement and regional metastases as well as between blood vessel and lymphatic involvement and distant metastases. Martinez-Gimeno in that same year reported vascular invasion to be the most significant independent indicator of neck metastases. McGraven and co-workers (1961) and Frierson and Cooper (1986) failed to establish these findings in their respective studies.

3.3.9 Inflammatory infiltrate

Inflammatory cells and in particular lymphocytes in the tissue subjacent to squamous cell carcinomas, are part of a stromal response to malignant tumours (Vose, 1985). It signifies the interaction between the host immune system and the tumour. The cell population is composed of B and T lymphocytes, natural killer cells and macrophages. Wolf and co-workers (1986) and Crissman (1986) reported a significant correlation between the density of the inflammatory cell infiltrate and the survival of patients with cancer.

Van Nagel and co-workers (1978), in their study of cervical carcinomas reported the presence of nodal metastasis in 25% of the cases with a scant inflammatory response. In contrast, only 9% of the cases with a marked inflammatory cell response developed nodal metastasis. A marked

inflammatory response significantly correlated with a low incidence of metastatic disease.

Mohit-Tabatabai and co-workers (1986), Frierson and Cooper (1986), Ragson and co-workers (1989) and Martinez-Gimeno (1995) reported similar findings in their respective studies at different intraoral sites. Woolgar and co-workers (1995) were unable to show the significance of inflammatory response as an independent prognostic indicator.

3.3.10 Depth from surgical excision margins

Scholl and co-workers (1986) reviewed T1 and T2 squamous cell carcinomas of the tongue and reported a correlation between positive surgical margins and local recurrence. Local recurrences occurred irrespectively of whether the residual positive margins were immediately reexcised but failed to develop when patients received either preoperative or postoperative radiation therapy. These authors also reported an increase in the rate of recurrence (11%) when positive surgical margins were present. Jones et al (1992) in their study of 49 T1 and T2 squamous cell carcinomas of the oral cavity, reported an overall recurrence rate of 41%. Of the total recurrences, 45% recurred locally, 40% spread to regional lymph nodes and 15% recurred locally and also metastasised to the neck. All recurrences occurred within two-years. Positive surgical margins were present in 20% of the cases developing recurrences. The majority of the positive margins were previously diagnosed as histologically negative or close to the surgical excision margins. Unlike Scholl and co-workers, these authors failed to show a difference in recurrence rates between the group of patients receiving a combined therapeutic regime and those who did not. Based on these findings, they emphasised the importance of complete excision of even small T1 and T2 squamous cell carcinomas of the oral cavity.

Unsatisfactory deep excision margins resulted in extensive deep-seated recurrences (Woolgar and co-workers, 1995).

3.3.11 Tumour presentation

To date none of the grading systems published in the literature has evaluated tumour presentation as a possible prognostic factor of oral squamous cell carcinomas.

3.3 Lymph node metastases

Lymph node metastasis is an important clinical prognostic factor in evaluating survival at initial presentation. After primary treatment is complete, the presence of clinically positive lymph nodes represents recurrent disease (Hibbert et al, 1983). Diseased lymph nodes per se is an indicator of poor prognosis (Norris, 1963; Teichgraeber et al, 1984). The reported incidence of lymph node metastases varies between 40% and 74% (Hiroko et al, 1994). The lymphatic drainage of the oral cavity is rich and often anastomosing (Jones et al, 1994). Consequently, bilateral and contralateral nodal involvement is common. Infection, surgery or radiotherapy alters normal lymphatic flow, making contralateral lymph node involvement possible.

Primary oral squamous cell carcinomas usually spread to various lymph nodes depending on their anatomical sites. It is well-known that head and neck tumours produce relatively consistent patterns of lymph node invasion. The most common site of metastases is the submandibular lymph nodes. The relationship between nodal metastases and decreased survival rates is well

established (Norris, 1963; Grandi et al, 1985). Woolgar and co-workers (1995) reported on the outcome of 123 patients with squamous cell carcinomas of the oral cavity / oropharynx. The authors confirmed reduced survival rates of patients with cervical lymph node metastasis. A large percentage of patients with metastatic disease also developed recurrent carcinomas at the initial site of the primary lesion.

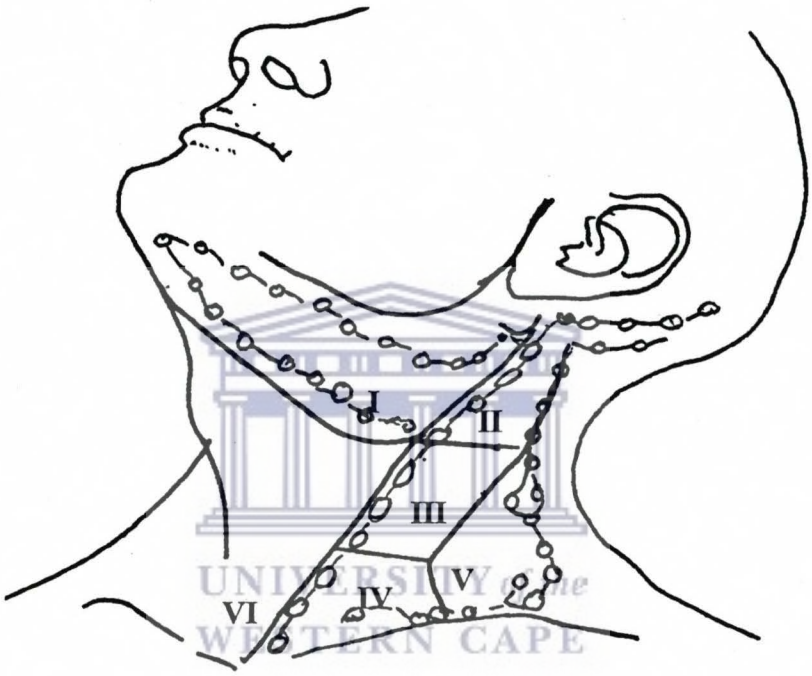
Patients presenting with lymph node metastasis lower in the neck have a worse prognosis compared to those who have nodal involvement higher up (Stell, 1983; Grandi et al, 1985). Shah and co-workers (1990) reviewed 501 patients with squamous cell carcinomas that underwent radical neck dissections and concluded that patients with squamous cell carcinomas of the oral cavity without clinically positive lymph nodes were at risk of developing metastasis to levels I, II and III. Clinically positive lymph nodes were commonly located in level IV and level V. Cerezo and co-workers (1992) and Jones and co-workers (1994) concurred with these findings in their series of squamous cell carcinomas of the head and neck. Jones and co-workers in a series of 492 patients with nodal metastases reported a significant correlation between the level of metastases and survival. In this series of head and neck carcinomas, the 5-year survival rates decreased from 38% for involvement of level I lymph nodes to 21% at level IV.

The accepted clinical classification is as follows (Shah et al, 1990-figure 1):

Level I neck nodes	Submental and Submandibular triangle
Level II neck nodes	Upper Jugular group
	Base of the skull to Carotid bifurcation/Hyoid bone
Level III neck nodes	Middle jugular group
	Carotid bifurcation to Omohyoid muscle
Level IV neck nodes	Lower Jugular group

	Omohyoid muscle to clavicle
LevelV neck nodes	Posterior triangle group
Level VI neck nodes	Anterior compartment group
	Paratracheal, perithyroid, pre-cricoid nodes

Figure 1: Lymph node levels



Umeda and co-workers (1992) reviewed 60 oral squamous cell carcinomas and reported a significant correlation between histological grade of malignancy and the level of neck metastasis. Grade I and II lesions showed limited involvement of level I and II nodes, whereas grade III and IV lesions often had metastases extending beyond level III. These observations were true irrespective of the clinical stage of the neoplasm. The prevalence of neck metastasis did not significantly correlate with the site.

3.2.1 Extracapsular spread

An important prognostic indicator is the spread of malignant cells from the lymph node beyond its connective tissue capsule, i.e. extracapsular spread. Not much research has been done to classify this phenomenon. Kalnins and co-workers (1977) reported a decrease in the 5-year survival rates of patients with metastatic disease penetrating the surrounding soft tissue. Woolgar and co-workers (1995) reported that lesions showing extension into the lymph node capsule, irrespective of whether involvement was microscopic or widespread, resulted in a poor prognosis. Their series of squamous cell carcinomas, 9 patients showed extracapsular nodal involvement. The lesions were all postoperatively treated with radiotherapy. At the end of the study only 1 patient survived. Based on these findings, the authors concluded that any form of extracapsular spread, irrespective of treatment, results in death. They reported that the number of nodes involved, the anatomical site and the number of anatomical levels containing neoplastic disease was of high prognostic significance. The poor prognosis of the patients with advanced metastatic disease could result from the late onset of postoperative radiotherapy.

Umeda and co-workers (1992) reported the incidence of second neck metastases in patient who initially presented without clinically involved neck nodes to be between 15 - 46%.

3.3 Scoring systems

Jakobsson (1973) added another dimension to the grading system by assigning numerical values to each parameter. The sum of the individual scores called the" total malignancy point value".

In a study of squamous cell carcinomas of the larynx, he and fellow authors reported that a high malignancy point score reflected a high grade of malignancy. They were also able to prove a significant correlation between malignancy scores and survival. High malignancy point values correlated with poor survival.

Enroth and Moberger (1973), using Jakobsson's original grading system, studied squamous cell carcinomas of the palate and reported a low death rate in patients with low point values. Similarly, there was a high death rate in the cases with high point values. Using these results they confirmed the reliability of the system. Statistical significant relationships exist between malignancy values, disease control and survival for squamous cell carcinomas of the tongue, the floor of the mouth and oropharynx (Holm et al, 1982; Anneroth et al, 1986; Crissman et al, 1986). Hambreus and co-workers (1988) reported the tumour-host value to be the most significant prognostic indicator of survival. Subsequent studies evaluated the significance of individual histologic parameters of the primary lesion on the prognosis (Spiro, 1974; Platz et al, 1983).

Lund and co-workers (1976) refined the system further when they assigned a clear definition to each parameter of Jakobsson' classification. They also introduced a histological score consisting of the total sum of points divided by the number of parameters used. Using this system in a study of squamous cell carcinomas of the lip and T1 and T2 squamous cell carcinomas of the tongue, they reported a statistically significant correlation between the microscopic score and local recurrence, regional lymph node metastasis and death. Helweg-Larsen (1978) used Lund's grading system to evaluate 52 squamous cell carcinomas of the larynx, but failed to show a significant correlation between histological score and clinical stage. These authors reported the reproducibility of the system too poor for predicting outcome. Willen and co-workers (1973,

1975) in a study of 124 squamous cell carcinomas of the gingiva concurred with the findings of the previous authors but reported a significant relationship between histological score and metastases. Lesions with high histological scores corresponded with a high incidence of metastases, whereas low scores correlated significantly with no metastases. Primary lesions with high histological scores (and high metastatic rates) were less differentiated, showed extensive pleomorphism, had a diffuse growth pattern, showed advanced invasion and had a moderate cellular response. Lesions with low histological scores were without metastases, well differentiated, had low mitotic rates, infiltrated in solid cords with well-defined margins and had an intense inflammatory response.

One can then only conclude that Willen showed a significant correlation between histological score, metastases, cellular differentiation, the degree of pleomorphism, mitotic rates, inflammatory response and pattern of invasion.

Holm and co-workers (1982), studied 95 squamous cell carcinomas of the anterior two-thirds of the tongue. These authors reported that lesions with low malignancy scores had a five-year survival rate of 85% compared to the 44% recorded in patients with higher scores. These authors also recorded a statistically significant correlation between total malignancy scores and lymph node metastases. Unlike previous authors however, they found a statistically significant correlation between total malignancy scores and clinical status at the time of initial diagnosis.

Anneroth and co-workers (1984) unified many published scoring systems and formulated a grading system based on the degree of keratinisation, nuclear pleomorphism, pattern of invasion, inflammatory response, stage of invasion and mitotic activity. They eliminating "vascular

invasion" from their series of squamous cell carcinomas of the floor of the mouth and tongue. The authors showed a statistically significant correlation between mean total malignancy, tumour cell population, host-tumour relationship and mean scores for most morphological parameters. These findings were validated in a subsequent study of 89 squamous cell carcinomas in the floor of the mouth. The authors showed a statistically significant correlation between mean total malignancy scores, clinical stage, frequency of recurrence and death from first primary lesions. Apart from having the best prognostic value in areas of least differentiation viz. the deep invasive fronts, these criteria have proved to be reproducible between observers.

3.4 Invasive grading

Many tumours are well differentiated at the surface while areas deep in lamina propria are poorly differentiated. These deep areas are the most invasive and show an aggressive growth pattern (Jakobsson et al, 1975; Anneroth et al, 1984). Nicolson (1987) proposed that histologically most invasive parts of a tumour may contain cells that are most likely to metastasize. For these reasons, many authors advocate invasive grading of malignant lesions (Bryne et al, 1990, 1991).

Several authors have reported that malignancies to be less differentiated at the base of tumours than in more superficial regions(Frierson and Cooper, 1986; Bryne et al, 1991; Saywell et al, 1996). Saywell and co-workers (1996) reported a case in which a patient with a grade I squamous cell carcinoma died of a metastatic disease 5 years after complete surgical excision of the primary lesion. Retrospective examination of the histology revealed that the lesion showed grade I changes in the superficial layers, but showed grade IV changes in the base. They concluded that superficial biopsies of squamous cell carcinomas may provide false assessments

of the lesions and grading of these lesions are contraindicated.

3.5 Survival

Odell and co-workers (1994) reported local recurrence to correlate significantly with Broders' grade, keratinisation at the invasive front and pattern of invasion. In the same study, regional metastases correlated significantly with Broders' grade, pattern of invasion and invasive grading total score. The following year Overholt (1996) reviewed 155 squamous cell carcinomas of the gingiva, failed to establish a significant correlation between tumours "ability to differentiate" and survival.

3.6 Grading systems

There still exists a great controversy regarding the value of different grading systems and individual histological grading parameters in predicting local recurrence, metastasis and survival. The correlation between the degree of differentiation and regional lymph node metastasis was confirmed by authors like Arthur and Farr (1972). These authors evaluated squamous cell carcinomas of the mouth and pharynx and showed a significant relationship between tumour grade, cure rate, stage of disease, metastatic involvement and survival. However, many other authors were not in agreement and published conflicting results (Bethman et al, 1965; Stoddart 1966; Kishnamurthi et al, 1971; Lund et al, 1975; Willen et al, 1975; Holm et al 1982). Bryne (1989) published an article in the Broders' grading system is compared to the multifactorial grading system proposed by Anneroth and co-workers (1987). Anneroth's system was superior

and a highly significant prognostic in a multivariate survival analysis. Broders' system on the other hand, did not prove to be a significant prognostic factor for survival.

3.7 Reproducibility

As perceptions differ from individual to individual, and within the individuals, depending on various factors such as stress, time of the day, hyper or hypoglycaemia, etc., so do the concepts of histologic grading of tumours vary. The reliability of any grading system depends on the reproducibility of the criteria used. The Federation Dentaire Internationale suggested that the most reliable unit of measure in clinical trials should be associated with the least diagnostic variability. To obtain reproducibility, it is imperative that each criterion be explained and understood. Minimum standards should be set and standard methods used. In this manner uniform interpretation and application of the criteria within a grading system can be achieved (Eklund et al, 1993). Authors like Pitts (1985) questioned the inter and intra examiner reproducibility of the radiographic diagnosis of caries. They suggested that errors in examiner observation could give a distorted picture of true progression rates. Graem and co-workers (1980) questioned the reproducibility of histologic grading systems for laryngeal carcinomas. After six pathologists graded 22 laryngeal squamous cell carcinomas, he reported a significant bias regarding pathologist-patient interrelationship. Another concern the bias introduced by a pathologist's therapeutic philosophy. Pathologists favouring a specific treatment modality may overestimate or underestimate scores to favour preferred treatment. Preconception of the ultimate score was questioned and concerns expressed regarding the method pathologists use for grading - do they systematically evaluate each parameter or do they first form a general opinion

about how malignant the tumour is? The authors studied the interobserver variability of squamous cell carcinomas of the larynx (using Lund's grading system) and reported the level of disagreement regarding mitoses and vascular involvement to be significant.

Levels of agreement are measured in terms of Kappa values. Anneroth and co-workers (1984) and Bryne and co-workers (1991) each using modifications of Jakobsson's grading systems reported low Kappa values for measuring interobserver variability - 0.33 and 0.44 respectively. If a Kappa value of one means total agreement and that of zero measures agreement obtained purely by chance, these values represent very low levels of reproducibility. The clinical value of a diagnostic system will increase with improved agreement (Bryne et al, 1991). The simplification of a system and clearly defining grading parameters can accomplish improved agreement(Wulff, 1981; Svanholm et al, 1989). Eliminating criteria from a grading system for the sake of simplification may compromise its prognostic value (Bryne et al, 1991). Similarly, reducing the number of scores may also fail to improve the prognostic value. Bichell and co-workers (1991) in a comparative study of cervical cancers, found the level of agreement to increase after calibration of the criteria. The authors concluded that the clinical value of a grading system increases when pathologists are familiar with the system and its criteria.

3.8 Biopsy size

Fisher and co-workers (1975) noted that grading larger surgical specimens gave a better prognosis than corresponding biopsy specimens. Because most neoplasms are composed of a heterogeneous cell population, small biopsies may not contain cells with the ability to metastasize (Nicolson,

1987; Anneroth et al, 1987; Heim et al, 1988; Bryne et al, 1989). Because the treatment of squamous cell carcinomas partially depend on malignancy grading, the grading of non-representative biopsies should be avoided (Bryne et al, 1989).

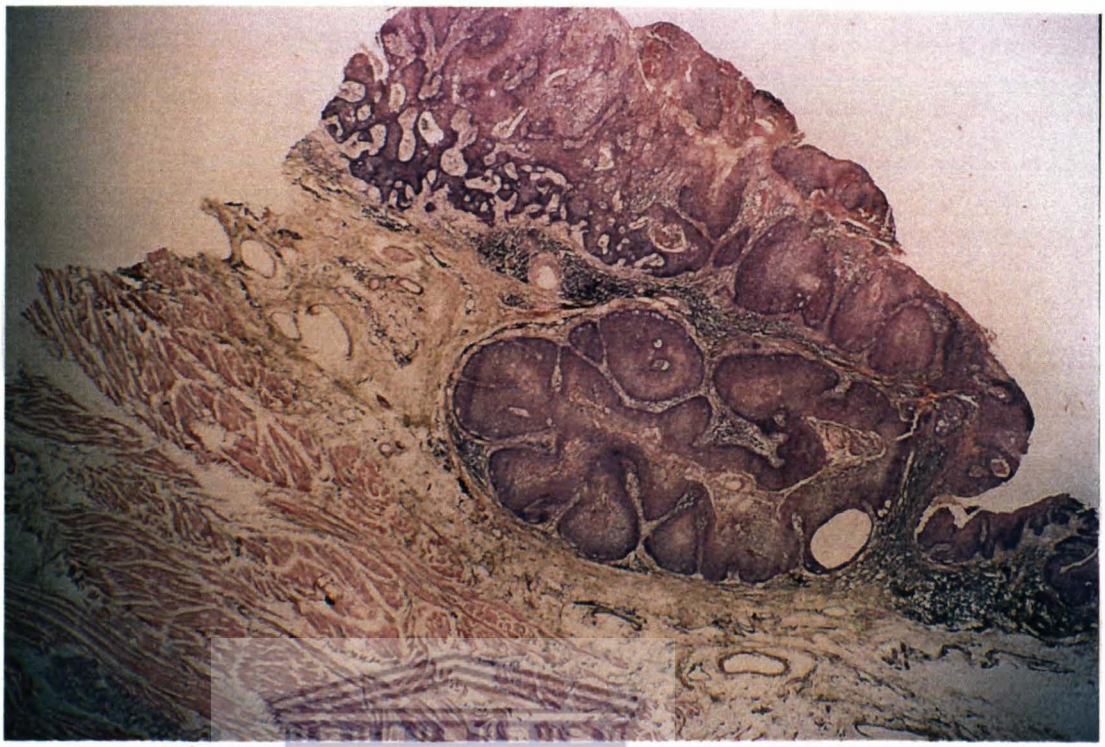


Figure 2: Pattern of invasion -Grade 1: Tumour cells are arranged in solid sheets sheets, have well delineated infiltrating margins and extends beyond the lamina propria. There is minimal keratin production and a scant inflammatory cell infiltrate (H&E stain, original magnification, X20)

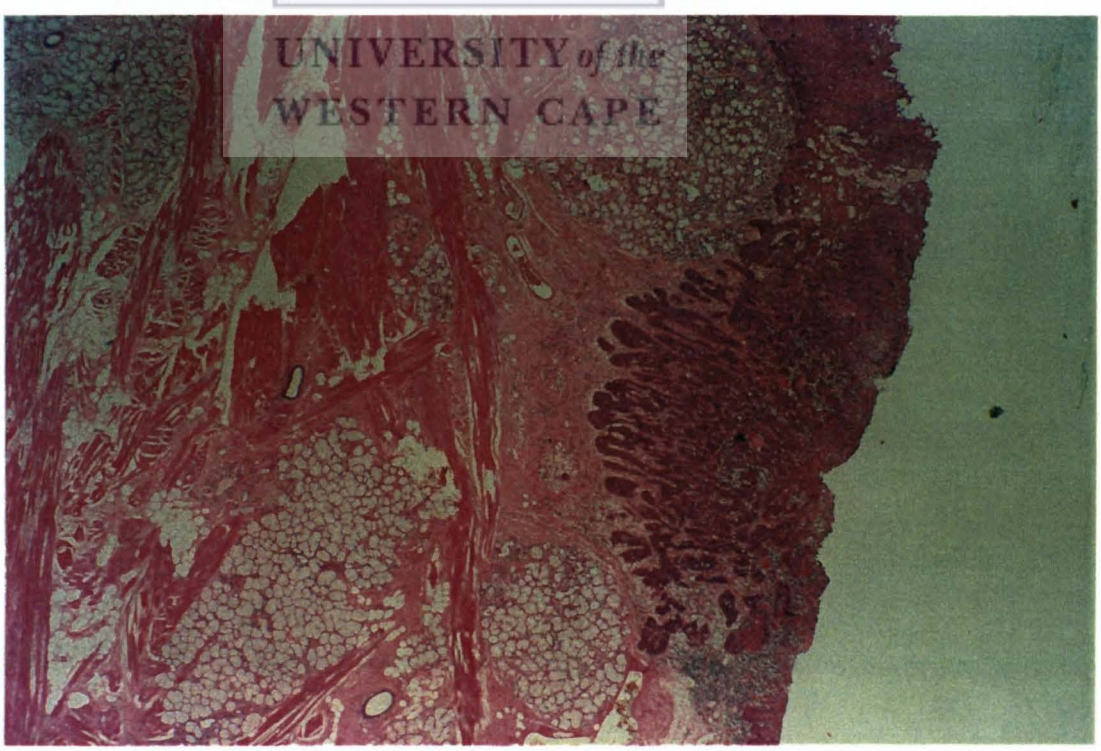


Figure 3: Pattern of invasion. Grade 2: Tumour cells form bands and strands and extends beyond the lamina propria (H&E stain, original magnification, X20)

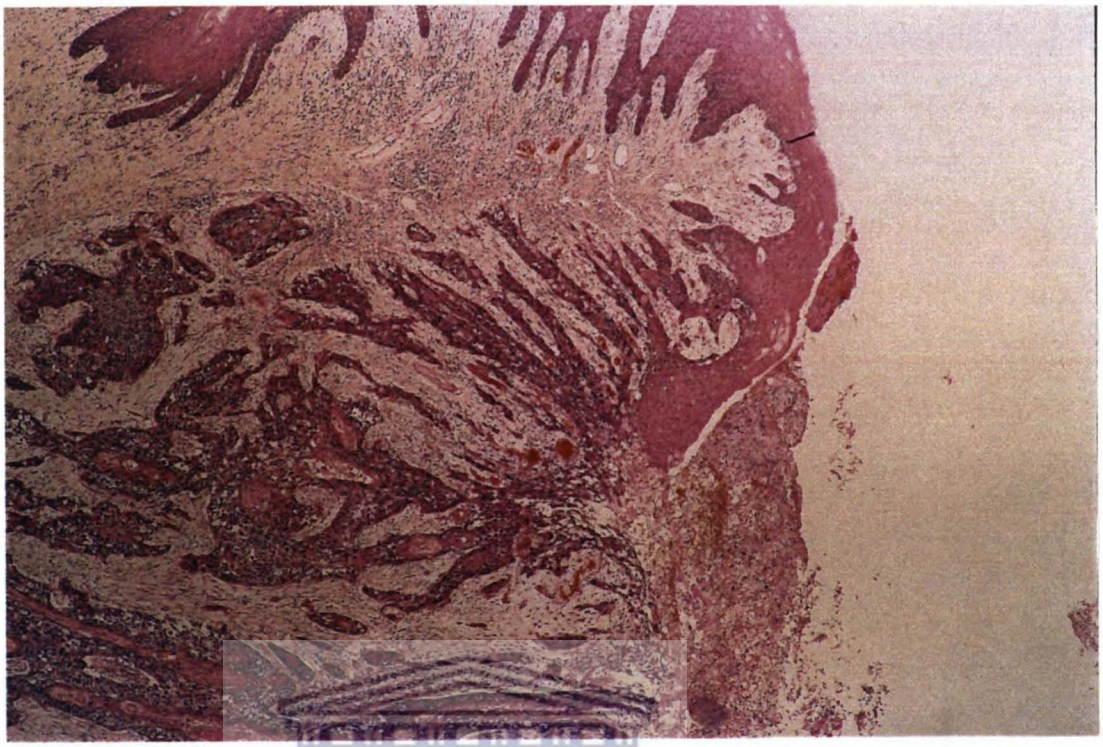


Figure 4: Patterm of invasion. Grade 3: Small groups of cells have been detached from papillary projections. There is a moderate amount of keratin production (H&E stain, origninal magnification, X100)

Figure 5: Pattern of invasion. Grade 4: Marked cellular dissociation (H&E stain, original magnification, X 20)

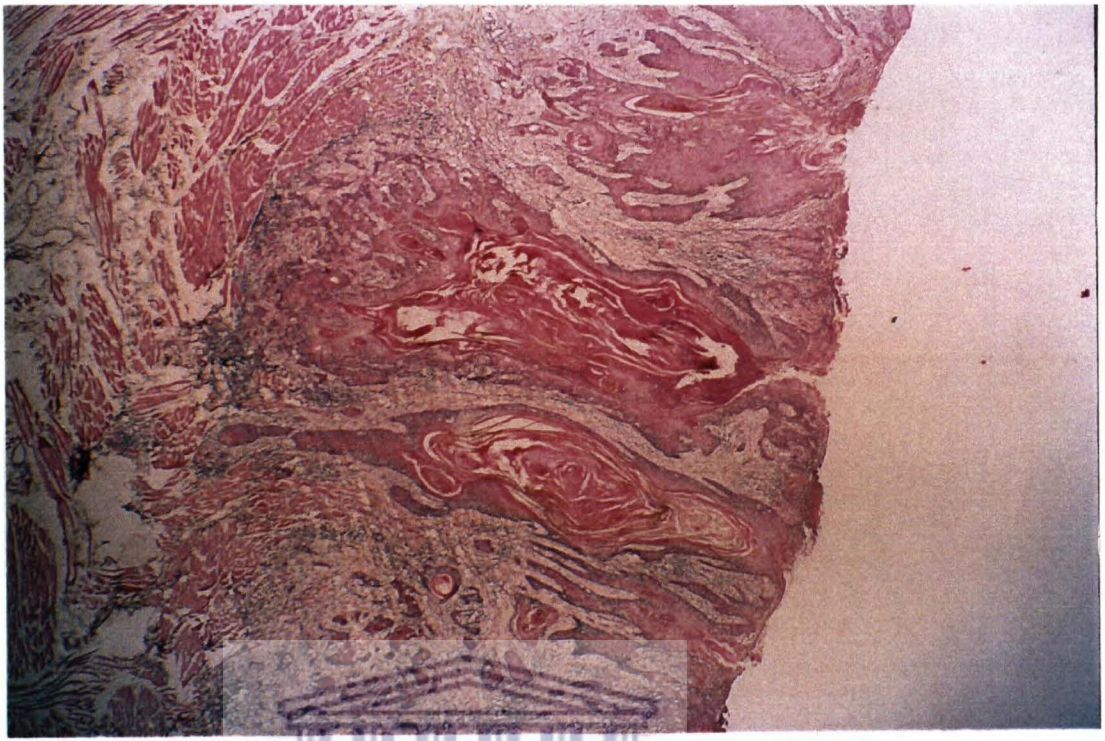


Figure 6: Marked keratin production associated with a mild inflammatory cell infiltrate in the lamina propria (H&E stain, original magnification, X20).

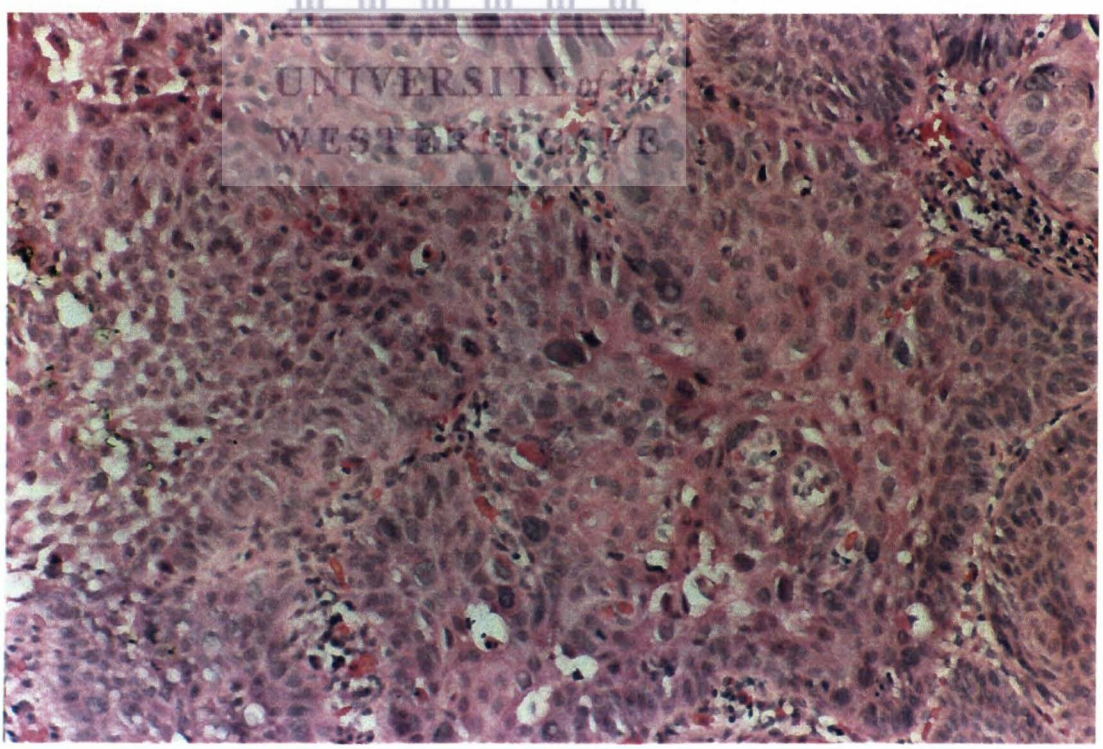


Figure 7: Cellular pleomorphism (H&E stain, original magnification, X40).

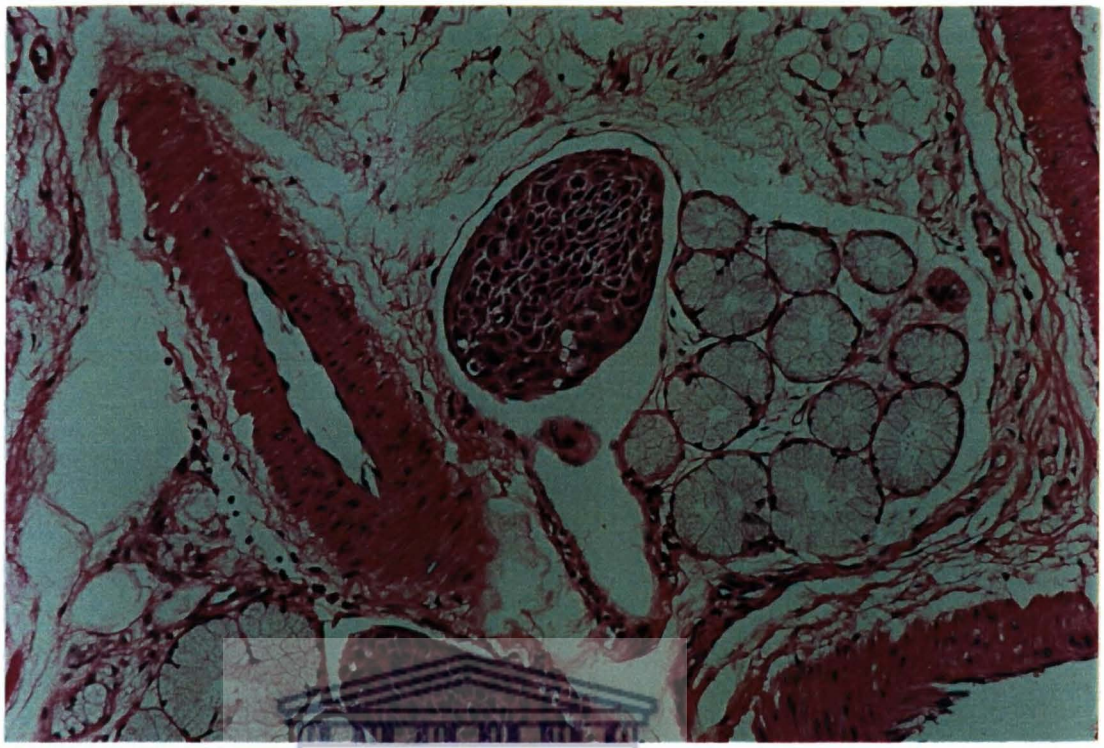


Figure 8: Tumour embolis within a thin-walled vascular channel (H&E stain, original magnification, X100)

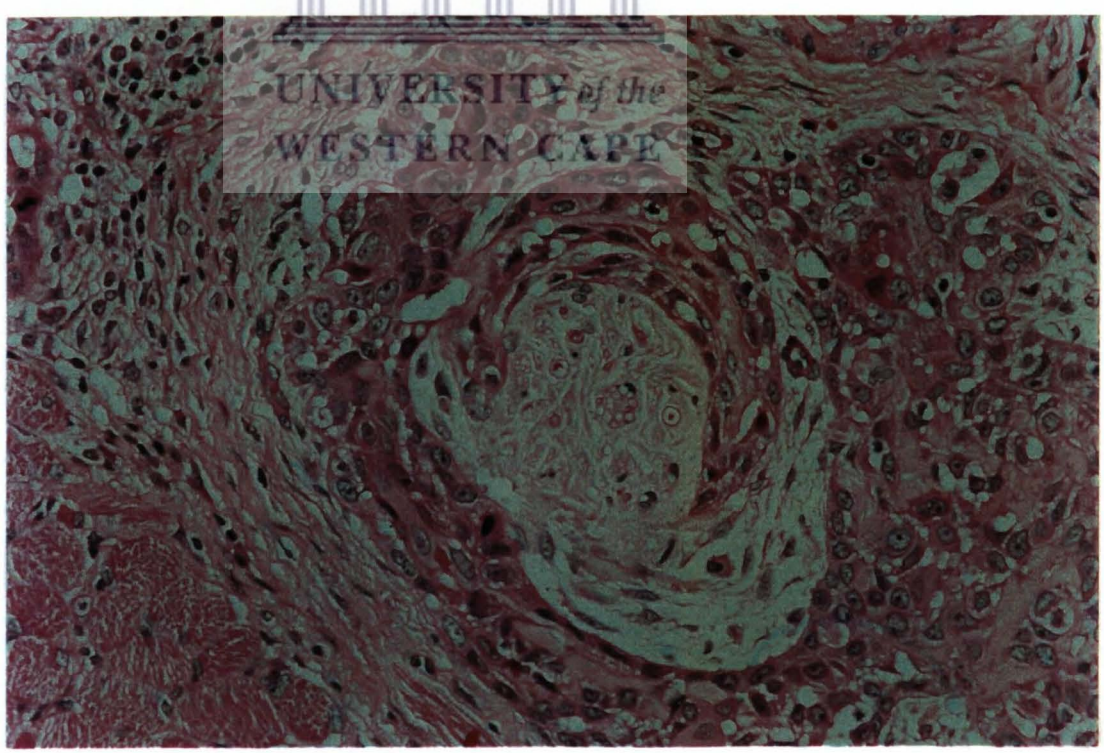


Figure 9: Tumour deposits surrounding a nerve fibre (H&E stain original magnification, X200)

4.AIMS AND OBJECTIVES

The study will assess various histological parameters in grading squamous cell carcinomas of the tongue and floor of the mouth to evaluate their reproducibility and to establish their value in predicting prognosis.

Objectives:

1. To determine:
   - the age and gender of the patients,
   - the follow-up period of the sample,
   -the primary site of the neoplasm and
   -the clinical stage of the tumor at the time of diagnosis
2. To compare Broders' grade with time of survival, time to nodal recurrence and time to primary recurrence.
3. To grade individual neoplasms according to a point score using defined criteria.
4. To determine the level of agreement between two observers and calibrate the results of individual observers.
5. To repeat the grading after a month and determine the level of agreement between observers for a second time.
6. To determine the whether there was a significant improvement after calibrating the results.
7. To determine the intra observer variablility.
8. Establish the clinical outcome of the study sample at the end of the study.

9. Establish the disease-free period at the end of the study.

10. Establish the survival rates at the end of the end of the study.

11. Establish the probability of survival and remaining free of nodal metastasis and primary recurrences after 2 and 5 years.

12. Determine the frequency distribution of carcinomas with each grading parameter.

13. Determine the frequency distribution of carcinomas developing nodal metastasis with each grading parameter.

14. Determine the frequency distribution of carcinomas developing primary recurrences with each grading parameter.

15. Determine the frequency distribution of carcinomas causing death of the patient with each grading parameter.

16. Determine the frequency distribution of disease-free patients with each grading parameter.

17. Compare disease-free patients with non-surviving patients in relation to each histological parameter.

18. Compare disease-free patients with patients developing nodal metastasis in relation to each histological parameter

19. Compare disease-free patients with patients developing priamary recurrence in relation to each histological parameter

20. To attempt to identify individual parameters that may correlate significantly with survival, nodal recurrence and local or primary recurrence.

21. To recommend a grading system for all oral squamous cell carcinomas.

5. MATERIALS AND METHODS

5.1 Definition of Terms

"The clinical stage" of the tumour (TNM classification) was based on the clinical size of the lesion, the presence of lymph node or distant metastases (see Appendix 1 for TNM classification).

"Regional metastases" referred to the presence of metastatic tumour deposits in the submandibular and deep cervical lymph nodes.

"Distant metastases" referred to the presence of neoplastic at (distant) sites other than in regional lymph nodes.

"Recurrence" occurred after initial treatment and consisted of local recurrence at the site of the first oral primary lesion, regional lymph node and distant sites.

" Keratinisation " referred to the quantity of keratin produced by the neoplastic cells and often reflects cellular differentiation

"Pattern of invasion" was based on the capacity of tumour cells' cohesiveness to keep the tumour population together

"Tumour thickness" or depth of invasion referred to the extension of tumour growth into the tissue below the epithelial surface

"Nuclear aberrations" included nuclear pleomorphism (variations in size and shape of tumour cell nuclei); increased nuclear-cytoplasm ratio; hyperchromatic and multiple nuclei and atypical mitoses.

"Absolute survival" was defined as the status of the patient at the time of evaluation, regardless

of the cause of death.

"Censored survival" excluded patients who were clinically free of disease at evaluation and those who died of causes other than disease.

5.2 Study Design

The study was based on a retrospective review of records and histological examination of surgical and biopsies specimens of T1 and T2 squamous cell carcinomas of the floor of the mouth and tongue.

5.2.1 Study Population and Sampling

Histological material and clinical details of patients with squamous cell carcinomas of the tongue and floor of the mouth were retrieved from the Department of Radiotherapy at Groote Schuur Hospital, the Department of Anatomical Pathology, University of Cape Town medical school and the Department of Oral and Maxillofacial Pathology, University of the Western Cape. All tumours clinically measured less than 4cm in diameter (TNM stage 1 and 2). Only specimens meeting the following criteria were included in the study.

Criteria for inclusion

- formalin fixed and paraffin embedded sections,
- material available in the archives of the respective institution,

- T1 and T2 primary squamous cell carcinomas from the tongue and floor of the mouth,
- representative sections of deep margins centrally, together with clinical and follow- up data,
- lesions within the confines of the lateral excision margins,
- primary neoplasms and
- lesions without preoperative chemotherapy, radiotherapy or surgery other than routine dental surgery and recent biopsy.

Exclusion criteria

- T3 and T4 squamous cell carcinomas from the tongue and floor of the mouth
- squamous cell carcinomas from other intraoral sites,
- malignancies other than squamous cell carcinomas,
- metastatic and recurrent neoplasms,
- second primary neoplasms,
- dysplastic lesions or carcinoma in situ,
- lesions of uncertain invasiveness,
- superficial biopsies,
- punch biopsies, and
- biopsy specimens that could not be located because of referral from centres other than UCT and UWC and slides not present in the archives.

The original slides of 48 patients were used for this evaluation. Patients had been evaluated and followed-up regularly at the Head and Neck Clinic of Groote Schuur Hospital.

5.2.2 Measurements

The following data was recorded:

- age

- gender

- TNM classification

-time to presentation

- site of lesion

- follow-up period in days

- Broders' classification

- individual pathologist's scores

The clinical statuses at the last visit are recorded as:

1. Alive with no local or regional recurrences and no and metastases
2. Alive with residual primary squamous cell carcinoma
3. Alive with recurrent, local or regional squamous cell carcinoma or systemic metastases
4. Dead of local or regional squamous cell carcinoma or systemic metastases
5. Dead of other disease

5.3 Data Collection

Specimen reference numbers were retrieved from a Microsoft Access database set up by visiting students from the United Kingdom in 1996 in collaboration with the Department of Radiation Oncology at Groote Schuur Hospital. Only T1 and T2 squamous cell carcinomas meeting the abovementioned criteria were included. Omitted specimen numbers were located by cross-referencing radiotherapy reference numbers and patient folder numbers. Hematoxylin and Eosin (H & E) sections were retrieved from the archives of the Department of Anatomical Pathology, University of Cape Town and the Department of Oral and Maxillofacial Pathology, University of the Western Cape Dental Faculty.

5.3.1 Histological Grading

The slides were allocated random numbers by the Chief Medical Technologist at the Department of Oral and Maxillofacial Pathology, University of Western Cape. These were labelled to own references and were not revealed to the examiners until after evaluation of the specimens. Malignancy grading of the deep invasive margins was done individually by two pathologists who had no prior knowledge of the clinical outcome. For each tumour, the tendency to keratinisation, cohesiveness / pattern of invasion, tumour thickness, depth from surgical excision margin, stage of invasion, mitoses, nuclear aberration, perineural invasion, vascular invasion, tumour presentation and inflammatory response were assessed. A guide to the score allocation of each parameter and grading criteria was available throughout the study (see Appendix 3 for guide). These parameters represent both the characteristics of the tumour cell population and host factors.

It was assumed that the parameters used are of equal importance for the evaluation of the degree of malignancy and its correlation to prognosis and survival. One of the two examiners had more than 10 years experience as a qualified oral pathologist, while the other was a postgraduate student. The two examiners evaluated each case and designated a score between 1 and 4 to each parameter. Results were recorded on a specifically designed Proforma (see Appendix 2). A maximum score of 11 x 4 = 44 could be recorded for each case.

Individual results were analysed and the proportion of paired diagnosis that agreed was determined. A Kappa value was obtained on all paired diagnoses. Kappa score was used to determine the reliability of paired agreements against pure chance and score range between 0 and 1. A score of zero showed random agreement and a score of 1 represented perfect agreement. Values greater than 0.75 indicated excellent agreement; 0.40 and 0.75 indicated fair agreement and less than 0.40 was poor agreement.

The results were calibrated and individual criteria discussed in depth. The grading procedure was repeated a month later. The intra examiner agreement was established using the individual sets of data.

Kaplan-Meier estimates were used to analyse the time until death, nodal recurrence and primary recurrence.

Cox's Regression model analysed the relationship between explanatory variables i.e. degree of keratinisation, cohesiveness, tumour thickness, depth from surgical excision margin, stage of invasion, mitoses, nuclear aberration, perineural invasion, tumour presentation, vascular

involvement and inflammatory response and survival, primary recurrence and nodal recurrence.

5.3.2 Data Capture and Statistical Analysis

The data was captured into the QUATTRO - PRO 6.0 database package. The EPI - INFO Statistical package was used to analyse the data, to determine the frequency distributions of the different variables and to test the levels of agreement. Survival analysis was performed using the SAS statistical package and the graphical illustrations were done by the STATISTICA package. Significance for all statistical evaluations was reported when a value less than 0.05 was recorded. The Wilcoxon test was used for comparison and the Chi-square test was used to test the trend.

5.4 Staff facilities and equipment

The respective archives of the Department of Oral and Maxillofacial Pathology of the Faculty of Dentistry of the University of the Western Cape at the Oral Health Centre, the Department of Radiotherapy at Groote Schuur Hospital and the Department of Anatomical Pathology of the Faculty of Medicine of the University of Cape Town at Groote Schuur Hospital were used with clerical staff for accessing the records. The Department of Statistical Science of the University of Cape Town was consulted for the statistical analysis.

5.5 Ethical and legal considerations

Strict patient-pathologist confidentiality was maintained. Pathology reference numbers of the patients were used to access the clinical data and histology sections. Where pathology reference numbers were not available, folder numbers and/or radiotherapy numbers were used for the purposes of retrieval. Permission for the use of records was obtained from the heads of the departments at the respective institutions (see Appendix 4).

6. RESULTS

6.1 Sample size

A computerised search of the records from both institutions revealed that 549 patients received treatment for squamous cell carcinomas of the oral cavity from January 1985 to December 1997. After applying the aforementioned inclusion and exclusion criteria, **48** patients were included in this study.

6.2 Age and Gender distribution of the sample

The age of the patients ranged from 27 years to 93 years. The mean age was 57.8 years. The majority of patients presented with primary lesions between the ages of 40 and 59 years (77% females and 51% males). There was a substantial decrease in the number of females that presented after the age of 60 years. The decrease in the number of males presenting after the age of 60 years was more gradual (Table 1). The gender distribution showed that the sample comprised of 27% female patients and 73% male patients, a female to male ratio of approximately 1: 2.7.

Table 1: Percentage frequency distribution of age by gender

Age (years)	Female	Male
20-39	0	6
40-59	77	51
>60	23	43

6.3 Duration of follow-up in days

The time of follow-up from January 1985 to December 1997 ranged from 1 day to 10 years. The mean follow-up period was 3.5 years with 54% attending the clinic for regular follow-up for longer than 2 years.

Table 2: Frequency distribution of follow-up

Follow-up period	Number of patients	Percentage
<1 year	12	25
1-2 years	10	21
2-3 years	8	17
3-4 years	5	10
4-5 years	2	4
>5 years	11	23

6.4 Site of lesions

There were equal numbers of lesions from the tongue and floor of the mouth. The even distribution of the sample with regard to site removed a proportion of the bias from the study.

6.5 Clinical stage

The cohort included 22 clinically staged T1 and 26 clinically staged T2 squamous cell carcinomas. We did not expect the distribution to significantly influence the outcome. Initially, we intended

to include only T1 lesions. Unfortunately the sample size was too small for significant statistical analyses.

6.6 Broders' classification

The majority (50%) of the carcinomas were moderately differentiated, with 25% well differentiated. Very few were poorly differentiated. However, 25% were not classified.

6.7 Interobserver agreement levels

The interobserver agreement levels illustrated the reproducibility of the grading system.

6.7.1 Initial interobserver agreement levels

The agreement level was excellent (>0.75) for the following: depth from surgical excision margin, perineural invasion and vascular involvement. The agreement level was fair (0.4 - 0.74) for the following: degree of keratinisation, pattern of invasion / cohesion, tumour thickness, stage of invasion, mitoses, nuclear aberration, tumour presentation and inflammatory response. None of the parameters were poor.

Table 3 illustrates the initial level of agreement among the two reviewing pathologists. The agreement levels recorded ranged from 0.52 (mitoses) to 0.89 (perineural involvement).

Table 3: Initial interobserver agreement levels

PROGNOSTIC INDICATOR	KAPPA SCORE
Degree of keratinisation	0.62
Pattern of invasion / cohesion	0.56
Tumour thickness	0.70
Depth from surgical excision margin	0.85
Stage of invasion	0.58
Mitoses	0.52
Nuclear aberration	0.58
Perineural invasion	0.89
Tumour presentation	0.64
Vascular involvement	0.75
Inflammatory response	0.56

6.7.2 Final Interobserver agreement levels

After calibration of the initial results and discussion of the individual criteria, the levels of agreement of the two reviewing pathologists were recorded once more (Table 4). The Kappa scores for agreement ranged from 0.60 (mitoses) to 0.93 (perineural involvement). The agreement level was excellent (>0.75) for the following: degree of keratinisation, pattern of invasion, tumour thickness, depth from surgical excision margin, perineural invasion, tumour presentation and vascular involvement. The agreement level was fair (0.4 - 0.74) for the following: stage of invasion, mitoses, nuclear aberration and inflammatory response. None of the parameters was poor.

Table 4: Interobserver agreement after calibration

PROGNOSTIC INDICATOR	KAPPA SCORE
Degree of keratinisation	0.79
Pattern of invasion / cohesion	0.75
Tumour thickness	0.81
Depth from surgical excision margin	0.89
Stage of invasion	0.66
Mitoses	0.60
Nuclear aberration	0.66
Perineural invasion	0.93
Tumour presentation	0.91
Vascular involvement	0.85
Inflammatory response	0.66

6.7.3 Comparison of interobserver agreements

To determine whether there were significant improvements in the agreement levels, the initial and the final agreement levels were compared. The agreement levels improved significantly for the following: degree of keratinisation, pattern of invasion / cohesion and tumour presentation. There were no significant improvements recorded for the remaining parameters (Table 5).

Table 5: Initial vs final interobserver levels

PROGNOSTIC INDICATOR	P-VALUE
Degree of keratinisation	**0.072**
Pattern of invasion / cohesion	**0.053**
Tumour thickness	0.200
Depth from surgical excision margin	0.542
Stage of invasion	0.391
Mitoses	0.410
Nuclear aberration	0.390
Perineural invasion	0.680
Tumour presentation	**0.001**
Vascular involvement	0.200
Inflammatory response	0.294

6.8 Intraobserver agreement

The intra-observer agreement levels represented the consistence of scoring by each examiner (Tables 6 and 7). The second examiner showed more consistent scoring rates when compared to the first.

6.8.1 Intra examiner agreement levels - examiner 1

The kappa scores for intraobserver agreement recorded by the first examiner ranged from 0.37 (mitoses) to 0.91 (perineural involvement). The agreement level was excellent (>0.75) for depth from surgical excision margin and perineural invasion. The agreement levels were fair (0.4 - 0.74) for the remaining parameters except mitoses that was poor (0.37).

Table 6: Intra examiner agreement: examiner 1

PROGNOSTIC INDICATOR	KAPPA SCORE
Degree of keratinisation	0.54
Pattern of invasion / cohesion	0.49
Tumour thickness	0.67
Depth from surgical excision margin	0.79
Stage of invasion	0.58
Mitoses	0.37
Nuclear aberration	0.52
Perineural invasion	0.91
Tumour presentation	0.50
Vascular involvement	0.71
Inflammatory response	0.60

6.8.2 Intra examiner levels - examiner 2

The kappa scores for intra examiner agreement recorded by the second examiner ranged from 0.29 (mitoses) to 0.87 (perineural involvement). The agreement levels were excellent (>0.75) for the majority of parameters except degree of keratinisation, nuclear aberration and inflammatory response that attained fair levels of agreement (0.4 - 0.74). Mitoses achieved a poor level of agreement (0.29).

Table 7: Intra examiner agreement: examiner 2

PROGNOSTIC INDICATOR	KAPPA SCORE
Degree of keratinisation	0.58
Pattern of invasion / cohesion	0.71
Tumour thickness	0.85
Depth from surgical excision margin	0.92
Stage of invasion	0.75
Mitoses	0.29
Nuclear aberration	0.63
Perineural invasion	0.87
Tumour presentation	0.79
Vascular involvement	0.85
Inflammatory response	0.62

6.9 Clinical outcome

6.9.1 Patient status

Of the 48 patients included in the study, 10.4% were completely lost because of an inadequate follow-up period. The majority of the sample was alive with nodal metastasis (42%). 8.3% of the cohort developed primary recurrence. Nodal metastasis accounted for all the disease-related deaths (12,5%). None of the deaths had resulted from primary recurrence. A relative small percentage of the cohort (39.6%) had remained alive and free of carcinoma at the end of the study.

Table 8: Clinical outcome

STATUS	FREQUENCY
Dead of squamous cell carcinoma (SCC) - lymph node metastasis	12.5%
Dead of other cause	4%
Alive with recurrent SCC	8.3%
Alive with lymph node metastasis	42%
Alive and well	39.6%

6.9.2 Disease free period

This period represents the interval from time of operation to the time of diagnosis of recurrent or metastatic disease. The majority of nodal metastasis and primary recurrences developed within

2 years (80% and 75% respectively). The mean time to nodal metastasis was 3 years and the median was 2.5 years. The mean time to primary recurrence was 1.5 years and the median was less than 1 year (approximately 9 months). In contrast, more deaths occurred after a 2 - year period. The mean time to death was 2.3 years and the median was 2.1 years.

Table 9: Time till the events death, nodal metastasis and local recurrence.

TIME PERIOD	DEATH (%)	NODAL METASTASIS (%)	PRIMARY RECURRENCE (%)
< 1 year	17	70	75
1 - 2 years	17	10	0
2 - 3 years	50	5	0
3 - 4 years	0	5	25
4 - 5 years	17	5	0
>5 years	17	5	0

6.10 Survival probabilities of death, nodal metastasis and primary recurrence

6.11.1 Death

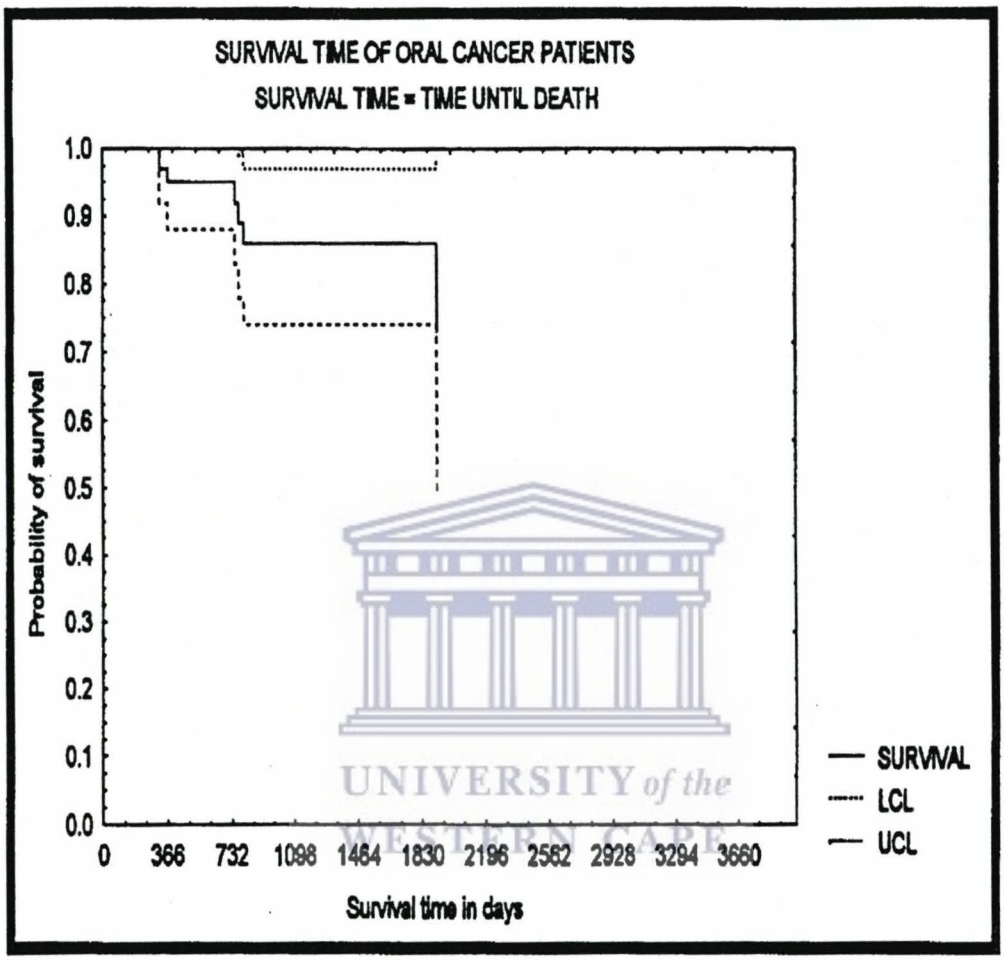
The 2-year survival probability is 95% and decreased to 86% after 2 years. A further notable decrease occurred after 5 years (73%).

The following table contains the product-limit (Kaplan-Meier) survival estimates for the case when the observed event is death.

Table 10: Kaplan-Meier estimates for the event death

Time period	Probability of Survival	Lower 95% confidence limit	Upper 95% confidence limit	Number of deaths
0	1	1	1	0
<1 year	0.97	0.92	1	1
1 - 2 years	0.95	0.88	1	1
2 - 3 years	0.86	0.74	0.97	3
3 - 4 years	0.86	0.74	0.97	0
4 - 5 years	0.86	0.74	0.97	0
>5 years	0.73	0.49	0.95	1

Figure 2: The following graph represents the tabulated values given in Table 12 and illustrates the survival curve for times till death.



LCL= Lower critical limit

UCL=Upper critical limit

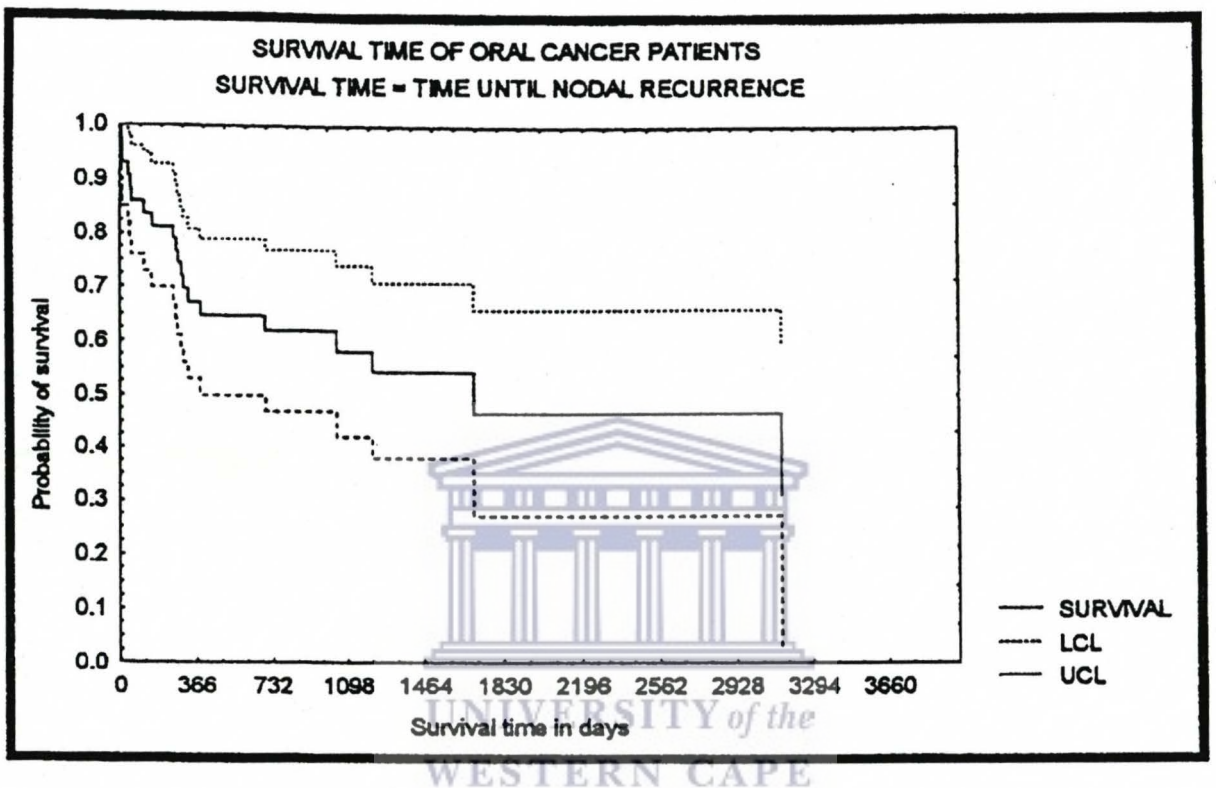
6.10.2 Nodal metastases

Nodal metastasis was a common complication of squamous cell carcinoma (41,5%) and accounted for 30% of deaths. The probability of remaining free of nodal disease within 2 years was 62% and decreased to 46% before 5 years. A further noticeable decrease occurred after 5 years (31%). Table 11 details the results for the Kaplan-Meier survival estimates.

Table 11: Kaplan-Meier estimates for the event nodal metastasis

Time period	Probability of Survival	Lower 95% confidence limit	Upper 95% confidence limit	Number of nodal metastasis
0	1	1	1	0
<1 year	0.67	0.53	0.81	14
1- 2 years	0.62	0.47	0.77	2
2 - 3 years	0.58	0.42	0.74	1
3 - 4 years	0.54	0.38	0.71	1
4 - 5 years	0.46	0.27	0.66	1
>5 years	0.31	0.03	0.96	1

Figure 3: The following graph represents the tabulated values given in Table 13 and illustrates the survival curve for times till nodal metastases.



LCL=Upper Critical Limit

UCL=Lower Critical Limit

6.10.3 Primary recurrence

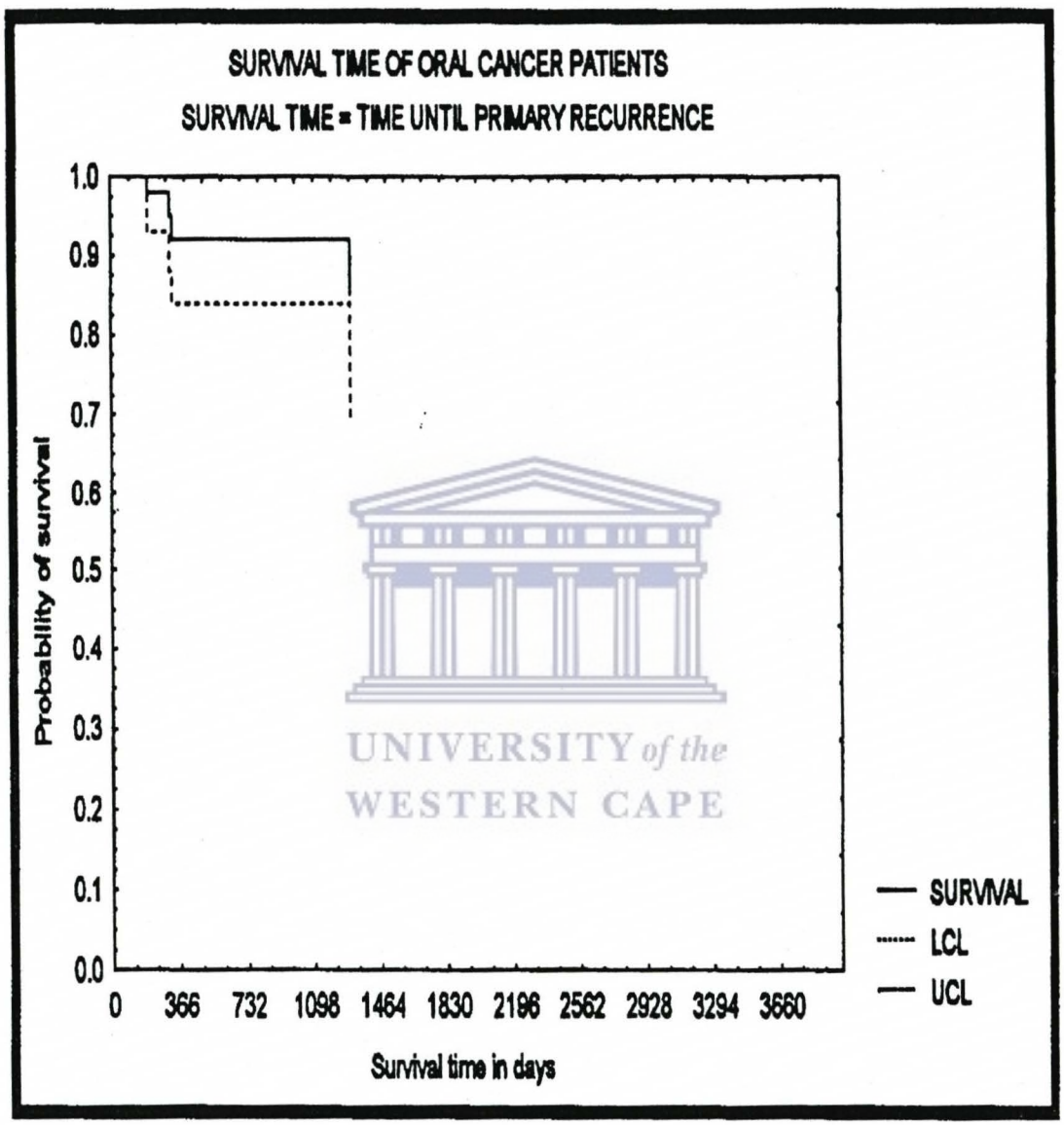
Approximately 8% (4 patients) of the cohort developed primary recurrence of which 50% also developed nodal metastases. The probability of remaining free of primary recurrence within 1 year was 92% and decreased to 85% after 3 years.

Table 12 illustrates the results for the Kaplan-Meier survival estimates. There are 4 patients who developed primary recurrence.

Table 12: Kaplan-Meier estimates for the event primary recurrence

Time Period	Probability of Survival	Lower 95% confidence limit	Upper 95% confidence limit	Number of primary recurrences
0	1	1	1	0
<1year	0.92	0.84	1	3
1-2 years	0.92	0.84	1	0
2-3 years	0.85	0.70	1	1
3-4 years	0.85	0.70	1	0
4-5 years	0.85	0.70	1	0
3-4 years	0.85	0.7	1	0

Figure 4: The following graph represents the tabulated values given in Table 14 and illustrates the survival curve for time till primary recurrence.



LCL=Upper Critical Limit

UCL=Lower Critical Limit

6.11 Parameter scores

The first examiner's score distribution illustrated the scoring pattern of carcinomas with each grading parameter for the overall sample, nodal metastasis, primary recurrence, death and disease-free cases. A score of 3 and /or 4 was unfavourable whereas 1 and/or 2 was less unfavourable.

6.11.1 Frequency distribution of carcinomas with each grading parameter

The score rates were unfavourable for the following parameters: degree of keratinisation, pattern of invasion, tumour thickness, stage of invasion, tumour presentation and vascular involvement. The score rates were less unfavourable for depth from surgical excision margin, mitoses, nuclear aberration, perineural invasion and tumour presentation.

The overall frequency distribution of carcinomas with each grading parameter score is illustrated by Table 13.

Table 13: Frequency distribution of carcinomas with each grading parameter

INVASIVE FRONT PARAMETERS	GRADE			
	1 (%)	2 (%)	3 (%)	4 (%)
Degree of keratinisation	8	35	**37**	**19**
Pattern of invasion / Cohesiveness	10	4	**77**	**8**
Tumour thickness	10	12	**50**	**27**
Depth from surgical excision margin	**86**	**10**	4	0
Stage of invasion	8	8	**37**	**50**
Mitoses	**35**	**40**	14	12
Nuclear aberration	**18**	**37**	40	2
Perineural invasion	**77**	0	0	23
Tumour presentation	**87**	0	0	13
Vascular invasion	21	0	0	**79**
Inflammatory response	8	29	**63**	0

6.11.2 Frequency distribution of carcinomas developing nodal metastases with each grading parameter score

The score rates were unfavourable for the following grading parameters: degree of keratinisation, pattern of invasion, tumour thickness, stage of invasion, vascular invasion and inflammatory response. The score rates were less unfavourable for depth from surgical excision margin, mitoses, nuclear aberration, perineural invasion and tumour presentation.

Table 14: Frequency distribution of carcinomas developing nodal metastasis

INVASIVE FRONT PARAMETERS	GRADE			
	1 (%)	2 (%)	3 (%)	4 (%)
Degree of keratinisation	5	35	**40**	**20**
Pattern of invasion / Cohesiveness	15	5	**75**	**5**
Tumour thickness	15	15	**40**	**30**
Depth from surgical excision margin	**95**	0	5	0
Stage of invasion	10	10	**40**	**40**
Mitoses	**25**	**55**	55	10
Nuclear aberration	**15**	**45**	40	0
Perineural invasion	**70**	0	0	30
Tumour presentation	**90**	0	0	10
Vascular invasion	15	0	0	**85**
Inflammatory response	10	25	**65**	0

6.12.3 Frequency distribution of carcinomas developing primary recurrence with each grading parameter

The score rates were unfavourable for the following grading parameters: degree of keratinisation, nuclear aberration and inflammatory response. The score rates were less unfavourable for depth from surgical excision margin, mitoses, perineural invasion and tumour presentation. There was an even distribution of scores for pattern of invasion, tumour thickness, stage of invasion and vascular invasion.

Table 15: Frequency distribution of carcinomas developing primary recurrences with each grading parameter score.

INVASIVE FRONT PARAMETERS	GRADE			
	1 (%)	2 (%)	3 (%)	4 (%)
Degree of keratinisation	0	0	**50**	**50**
Pattern of invasion / Cohesiveness	50	0	50	0
Tumour thickness	25	25	25	25
Depth from surgical excision margin	**100**	0	0	0
Stage of invasion	25	25	0	50
Mitoses	25	50	0	25
Nuclear aberration	25	0	**75**	0
Perineural invasion	**100**	0	0	0
Tumour presentation	**100**	0	0	0
Vascular invasion	50	0	0	50
Inflammatory response	0	25	**75**	0

6.12.4 Frequency distribution of carcinomas causing death of the patient with each grading parameter

The scoring rates were unfavourable for the following parameters: degree of keratinisation, pattern of invasion, tumour thickness, stage of invasion and vascular invasion. The scoring rates were less unfavourable for depth from surgical excision margin, mitoses, perineural invasion and tumour presentation. There was an even score distribution of scores for nuclear aberration and inflammatory response.

Table 16: Frequency distribution of carcinomas causing death of the patient with each grading parameter score.

INVASIVE FRONT PARAMETERS	GRADE			
	1 (%)	2 (%)	3 (%)	4 (%)
Degree of keratinisation	17	17	**50**	**17**
Pattern of invasion / Cohesiveness	17	0	**66**	**17**
Tumour thickness	17	0	**17**	**66**
Depth from surgical excision margin	**100**	0	0	0
Stage of invasion	17	0	**17**	**66**
Mitoses	**50**	**33**	0	17
Nuclear aberration	33	33	33	0
Perineural invasion	**66**	0	0	33
Tumour presentation	**100**	0	0	0
Vascular invasion	0	0	0	**100**
Inflammatory response	0	**50**	**50**	0

6.12.5 Frequency distribution of carcinomas of disease-free patients with each grading parameter

The scoring rates were unfavourable for the following grading parameters: pattern of invasion, tumour thickness, stage of invasion and vascular invasion. The scoring rates were less unfavourable for depth from surgical excision margin, mitoses, nuclear aberration, perineural invasion, tumour presentation and inflammatory response. There was an equal score distribution for degree of keratinisation throughout the sample.

Table 17: Frequency distribution of carcinomas of disease-free patients with each grading parameter score

INVASIVE FRONT PARAMETERS	GRADE			
	1 (%)	2 (%)	3 (%)	4 (%)
Degree of keratinisation	12	37	33	17
Pattern of invasion / Cohesiveness	0	4	**83**	**12**
Tumour thickness	0	12	**63**	**25**
Depth from surgical excision margin	**79**	**17**	5	0
Stage of invasion	5	5	**40**	**50**
Mitoses	**37**	**37**	3	10
Nuclear aberration	**17**	**46**	6	4
Perineural invasion	**79**	0	0	21
Tumour presentation	**83**	0	12	17
Vascular invasion	21	0	0	**79**
Inflammatory response	8	25	**67**	0

6.12 Comparison of disease-free patients and patients that died, developed nodal metastasis and local recurrence.

To establish whether a significant difference in the score rates existed, the grade scores of the patients who remained disease-free at the time of analysis and the cases that resulted in death, developed nodal metastasis and primary recurrence were compared (Tables 18 to 21)

6.12.1 Disease-free vs death

The Wilcoxon test showed no significant difference in the scoring rates of the disease-free cases and those that had died in relation to any of the histological parameters (Table 18).

Table 18: Disease-free vs death

Parameter	Chi-square probabilities	Wilcoxon probabilities
Degree of keratinisation	0.888	0.837
Pattern of invasion / Cohesiveness	0.149	0.456
Tumour thickness	0.014	0.130
Depth from surgical excision margin	0.353	0.170
Stage of invasion	0.52	0.684
Mitoses	0.697	0.721
Nuclear aberration	0.835	0.457
Perineural involvement	0.423	0.454
Tumour presentation	0.259	0.288
Vascular involvement	0.198	0.221
Inflammatory response	0.365	0.573

6.12.2 Disease-free vs nodal metastasis

There was a significant difference between the scoring rates of the disease-free group and those that developed nodal metastasis in relation to pattern of invasion (p= 0.0234). There was a strong association between the two groups regarding depth from the surgical excision margin (p=0.0738).

Table 19: Disease-free vs nodal metastasis

Parameter	Chi-square probabilities	Wilcoxon probabilities
Degree of keratinisation	0.824	0.623
Pattern of invasion / Cohesiveness	0.112	**0.023**
Tumour thickness	0.142	0.759
Depth from surgical excision margin	0.075	**0.074**
Stage of invasion	0.776	0.387
Mitoses	0.456	0.639
Nuclear aberration	0.761	0.797
Perineural involvement	0.369	0.384
Tumour presentation	0.449	0.467
Vascular involvement	0.524	0.541
Inflammatory response	0.976	0.844

6.12.3 Disease-free vs primary recurrence

Both the Chi-square and Wilcoxon tests showed a significant difference between the scoring rates of the disease-free group and patients who developed primary recurrences in relation to pattern of invasion (p=0.014). There was a strong association between the scoring rates of these groups in relation to the degree of keratinisation (p=0.802) as illustrated by the Wilcoxon test. Although the Chi-square test showed a significant difference in relation to tumour thickness, it did not consider ordinate variables and only tested the score trends.

Table 20: Disease-free vs primary recurrence

Parameter	Chi-square probabilities	Wilcoxon probabilities
Degree of keratinisation	0.325	**0.080**
Pattern of invasion / Cohesiveness	0.002	**0.014**
Tumour thickness	0.075	0.348
Depth from surgical excision margin	0.909	0.927
Stage of invasion	0.15	0.5083
Mitoses	0.569	0.653
Nuclear aberration	0.463	0.593
Perineural involvement	0.354	0.394
Tumour presentation	0.354	0.394
Vascular involvement	0.258	0.288
Inflammatory response	0.821	0.759

7. DISCUSSION

7.1 Age and Gender

The female to male ratio of 1:3 differs considerably from that in Hille and co-workers' study (1996) who recorded approximately equal ratios. Although this study was specific for a South African population, the data reviewed by these authors included a range of clinical stages and intraoral sites. These results also differ from those of earlier international studies which involved similar sites (Holm et al, 1981; Anneroth et al, 1986). This study showed that the lesion in males occurred over a wide age range from before 30 years to older than 60 years. A high incidence of males presented for treatment between the ages of 40 and 69 years (51%) which gradually decreased after the age of 70 years (43%). The majority of female patients (77%) included in the study presented between the ages of 40 and 69 years with a notable decrease (23%) after this age.

7.2 Duration of follow-up

The mean follow-up period for this study sample was 3.5 years. In view of this, the two-year survival analysis did not prove to be a problem but only 23% of the patients were eligible for inclusion in the 5-year analysis. Ideally, regular patient examination should continue for at least 2 years.

Reasons for the poor follow-up could include the following:

- lack of accessibility: patients are referred from as far as the Eastern Cape,
- lack of affordability: financial implications involved in travel, absenteeism from work etc.,
- the reconstruction of the health care systems: after initial treatment, patients are sent back to the health care facilities from which they are referred,

- patients referred from private practitioners are regularly examined by them and
- poor patient compliance

7.5 Broders' classification

The distribution of squamous cell carcinomas graded according to Broders' classification was as follows: 12% were well differentiated, 50% moderately differentiated, 4% poorly differentiated and 25% unclassified squamous cell carcinomas. We had intended to compare the lesions classified according to Broders' grade with survival, nodal metastasis and primary recurrence. The high percentage of unclassified lesions in the study (21%), may have greatly affected the results because of the uncertainty regarding its grade (according to Broders classification). Several investigators (Mendelson et al, 1976; Enroth et al, 1973; Frierson et al, 1986; Hambreus et al, 1988; Ragson et al, 1989; Odell et al, 1994) have documented the significance of Broders' grade as a prognostic parameter for survival, lymph node metastasis and primary recurrence. Arthur and Farr (1972) stated Broders' grade to be a reflection of tumour aggression.

7.6 Interobserver agreement level

Physical and psychological factors, different levels of visual alertness and perceptions affect the judgement of examiners from time to time and at different levels. Although criteria for the histological assessment of squamous cell carcinomas were described, reevaluated and discussed in detail, the ultimate interpretation of sections remained subjective. Assessment of histological

sections may not only vary between observers, but may also do so by the same observer given different circumstances (Shear et al, 1985). In view of this, it is logical to conclude that absolute agreement can never be obtained. However, this should not dissuade examiners to strive for the highest attainable level of agreement.

The importance of consistency and reproducibility in oral health surveys and clinical trials have been emphasised by the Federation Dentaire Internationale in 1982. The World Health Organisation (Eklund et al, 1993) outlined guidelines for establishing inter and intra examiner agreement.

There are two main reasons for establishing agreement in clinical trials. First, it is used in examiner training and calibration to identify areas of disagreement. In turn these are discussed and reduced to the lowest attainable levels. As a result, the examiners can develop a clear understanding of the criteria and a consistent method of applying them. The second reason is to detect whether the examiners in the study have achieved and are sustaining the preferred level of agreement (Eklund et al, 1993).

Our initial results show kappa values for interexaminer agreement ranging from 0.52 to 0.89 which represents a spectrum of fair to excellent levels of agreement. The parameter that scored the highest Kappa value was perineural invasion with a score of 0.89. The Kappa values of the remaining parameters ranged from between 0.52 and 0.75 which shows a fair level of agreement (Table 3). After the results were calibrated and the criteria discussed, a significant improvement in the agreement levels of the degree of keratinisation, pattern of invasion and tumour presentation was recorded (Table 5). The agreement levels of the remaining parameters also

improved, but were not statistically significant. Kappa values in the second phase ranged from 0.60 to 0.93. The degree of keratinisation, tumour thickness, depth from surgical excision margin, perineural invasion, tumour presentation and vascular invasion, scored Kappa values greater than 0.75, indicating excellent levels of agreement. These findings concur with those of Bichell and co-workers (1991) who recorded increased levels of agreement in a study of squamous cell carcinomas of the cervix after calibration of criteria. Reasons for the improved levels of agreement include an enhanced understanding of individual criteria, increased familiarity with the grading system and a more consistent manner of applying the grading system, especially by the inexperienced examiner. Conversely, familiarity with the sections could introduce bias to the results. Both first and second phase Kappa values were markedly higher than those obtained by authors like Anneroth and co-workers (1986) and Bryne and co-workers (1991).

8.7 Intra - examiner agreement

When intra-examiner reproducibility was evaluated, the second, more experienced examiner recorded considerably high Kappa values (Table 8) ranging from 0.29 (mitoses) to 0.92 (depth from surgical excision margin). Tumour thickness, depth from surgical excision margins, perineural invasion, vascular involvement and tumour presentation had Kappa values of greater than 0.75 indicating excellent levels of agreement. A fair level of agreement was established in the remaining parameters except number of mitoses (0.29 which is poor). These levels of agreement are also considerably higher than those recorded by the first examiner. The Kappa values for this examiner ranged from 0.37 to 0.91. Perineural invasion scored the highest and mitoses the lowest. The first examiner recorded fair levels of agreement in most of the remaining

parameters except mitoses in which a poor level of agreement was established. Reasons for the inconsistent score of the first examiner could be the result of one or more of the following: lack of understanding of criteria, inconsistent application of criteria, lack of familiarity with the grading system and inconsistent interpretation of criteria.

Judging from the marked increase in the level of agreement after calibration of the criteria, it was that most of these deficiencies occurred in the initial phase of the study, thereby adversely affecting the outcome of the overall results.

Throughout the study the parameter that the observers disagreed on most was the number of mitoses. This was in keeping with previous reports (Bundgaard et al, 1991; Odell et al, 1994). Reasons for the poor reproducibility includes the difficulty to standardise mitotic counts (Bryne et al, 1991; Odell et al, 1994). Bryne and co-workers (1991) reported that the omission of mitotic counts from their grading system, improved its reproducibility without compromising the prognostic value.

Authors like Crissman and co-workers (1984) on the other hand reported frequency of mitoses to be a most significant prognostic indicator of survival.

7.8 Disease-free interval

A minimum period of 2 years was allowed for inclusion and review of new cases into the study. Although this period is short and the possibility of nodal metastasis and local recurrence developing after completion exists, authors such as Vikram and co-workers (1984) and Foote and

co-workers (1993) reported these events to take place within 1 year of treatment. The results of the present study confirms these findings. The highest rate of treatment failure occurred within a year (70% of nodal metastasis and 75% of primary recurrences). In contrast, the majority of deaths (66%) were recorded after 2 years (Table 8).

7.9 Clinical outcome and survival probabilities

The outcome of patients in the current study is similar to (83%) those reported by Jones and co-workers, 1992 (overall survival of 82%) and Odell and co-workers, 1994 (overall survival rate of 93%) whose study samples were also similar. Ideally these figures show a good prognosis (Platz and co-workers, 1986; Moore and co-workers, 1986; Odell and co-workers, 1994). The positive results obtained in the study are overshadowed by the combined recurrence rate of primary recurrence and regional nodal metastasis (50%) which reflects the high morbidity associated with these lesions. Although Odell and co-workers (1994) argue that the metastasis rates (40,4%) in their series of squamous cell carcinomas of the tongue, reflect high cure rates and excellent prognosis, we fail to agree with them. These results are also not welcomed by the clinicians who regard them as treatment failures because recurrences are ultimately associated with poor prognosis for survival (Bundgaard and co-workers, 1991).

Of the 48 patients 12.5% died of the disease and 4% of unknown causes. The mean time to death was 2.3 years and the median 2 years. The use of survival analysis allowed for the appropriate censorship of data (Altman, 1991). Actuarial analysis showed the overall 2-year survival probability was 92% which decreased to 73% at 5 years (Table 10). These results are somewhat

higher than those recorded by Woolgar and co-workers (1995) in their study of squamous cell carcinomas of oral/oropharyngeal mucosa who recorded a 2-year survival probability of 69% which dropped to 65% at 5 years. Reasons for the discrepancies between the two studies include:

- the homogeneity of the present cohort with regard to site and clinical size,
- the complete excision of 95% of the lesions,
- early detection of local recurrences and nodal metastases,
- regular follow-up visits by patients and
- the inclusion only of primary lesions in the present cohort

The majority of deaths occurred after 2 years (Table 9).

Forty-two percent of the study sample developed nodal metastasis. Our results are in keeping with those of Cunningham and co-workers (1986) and Odell and co-workers (1994) who reported nodal metastasis in 42% and 40%of their respective study samples. Both these samples were similar to the present cohort with regard to site and clinical stage. In contrast, Spiro and co-workers (1984) reported a 24% rate of nodal recurrence in their sample of squamous cell carcinomas from the tongue and the floor of mouth. The mean time to nodal metastasis was 3.2 years and the median time was 2.5 years. Actuarial survival analysis showed that the probability of remaining free of nodal metastasis after a year was 67% and decreased to 46% after less than 5 years (Table 11). The results were recorded at a 95% confidence interval of (115; 1041) which is relatively wide, thereby increasing the uncertainty of nodal recurrence occurring at this time. Eighty percent of patients developing nodal metastasis, did so within two years of treatment (Table 9). Thirty percent of patients who developed nodal metastasis, subsequently died (30%).

Approximately 8.3% of the cohort developed recurrences at the site of the primary lesion. These

results are higher than those reported by Cunningham and co-workers (1986) who reported a local recurrence rate of 2%, but are marginally less than those reported by Spiro and co-workers (1986) and Odell and co-workers (1994) who reported a primary recurrence rate of 12% and 12.8% in their respective studies. In contrast, Jones and co-workers (1994) reported a local recurrence rate of 60% in their series of stage I and II squamous cell carcinomas of the oral cavity. The mean time to primary recurrence of squamous cell carcinomas of the tongue and floor of mouth was 1.5 years and the median was 325 days. Both these measures of central tendancy occurred before a period of 2 years. Actuarial survival analysis showed that the probability of remaining free of local recurrence after 1 year was 92%. Two-year analysis could not be estimated. Seventy-five percent of the primary recurrences occurred within 1 year of treatment (Table 9).

It is important to take cognisance of the fact that the measures of central tendency of the sample are influenced by the presence of outliers, which is data with extreme values, resulting in an uneven distribution (skewing) of data. Consequently, the values reflected by our study sample cannot be regarded as representative and are best ignored.

Our results show a significant relationship between lymph node metastasis and survival. All patients dying of oral squamous cell carcinoma had lymph node metastasis. Of the 20 cases that demonstrated lymph node metastasis, 2 also developed recurrent carcinoma at the site of the primary tumour. Failure to control clinically small tumours my result from both systemic factors e.g., impaired immune response (Scully et al, 1984) and environmental factors such as poor diet and continuous use of tobacco after initial treatment.

7.10 Parameter scores

The scoring rates of each parameter showed similar trends irrespective of whether the patient remained disease free, developed primary recurrence, nodal metastasis or died. Apart from depth from surgical excision margin and perineural invasion , the prognostic significance of the remaining parameters that attained less unfavourable scoring rates, is either controversial or not mentioned in the literature (Frierson et al, 1986; Bryne et al, 1991). In contrast, the prognostic significance of all the parameters obtaining unfavourable score rates have well documented and generally correlates to poor prognoses (Hambreus et al, 1988; Odell et al, 1994; Woolgar et al, 1995; Sarbia et al, 1995; Martinez-Gimeno et al, 1995; Fukano et al, 1997). The score rate trends of the study reflect unfavourable histological patterns (Tables 15 to 19). One can therefore conclude that although our cohort of tumours are clinically small, they are histologically aggressive lesions. One can also make the assumption that the biological behaviour of these lesions is sight specific, that is, that squamous cell carcinomas of the tongue and floor of the mouth are more aggressive than those at other sites and that indeed the tongue and floor of mouth are high risk areas for oral squamous cell carcinomas. To challenge these statements, comparative studies of similar lesions at other intraoral sites should be undertaken.

7.11 Comparison of disease-free patients and death, nodal metastasis and local recurrences

There was no significant difference between the score rates for the disease-free patients and those

dying from carcinoma in relation to any of the parameters investigated (Table 18).

There was a significant correlation between the score rates of the pattern of invasion and both nodal metastasis and local recurrence (p=0.0234 and p=0.0138 respectively) when the groups were compared to disease-free patients (Tables 19 and 20). Of the 36 cases with unfavourable pattern of invasion, 39 % developed nodal metastasis and 4.6% developed primary recurrences compared to 4 of 7 and 2 of 7 with less unfavourable patterns. This agrees with the findings of Odell and co-workers (1994) who studied 47 small squamous cell carcinomas of the tongue and found pattern of invasion statistically significant to both local (p=0.001) and regional recurrence (p=0.0195). Woolgar and co-workers (1995) reported cervical metastasis to occur more frequently in tumours with a histologically unfavourable pattern of invasion.

McGraven and co-workers (1961) initially identified two types of growth patterns that is "infiltrative" and "pushing". They reported significant correlations between the type of invasive pattern and nodal metastases and tumour differentiation in a study of laryngeal carcinomas. This parameter was first incorporated into an acceptable histological grading system by Jakobsson and co-workers (1973) which formed the platform of many multifactorial grading systems. The significance of the pattern of invasion within a multifactorial grading system was reported in studies from the larynx (Jakobsson et al, 1973), palate (Enroth et al, 1973), tongue (Odell,1994) and various oral sites (Yamamoto et al,1984; Woolgar et al, 1995). Crissman and co-workers (1984) reported the pattern of invasion to be the only prognostic indicator of survival in squamous cell carcinomas of the oropharynx.

Carcinoma approached the surgical resection margin within 1mm in 4% of the cases. Of the two cases, one developed nodal metastasis (50%) compared with none of the 45 patients cases with

favourable margins (i.e. those greater than 2mm as well as those between 1 and 2mm from the surgical excision margin). The presence of the single case that approached within 1mm of the resection margin case led to the development of significant association in relation to nodal metastasis (p=0.0738). Although this represents a small percent of the total number of cases, it resulted in a value that approached significance and may therefore have a significant influence on the outcome of a larger sample.

Scholl and co-workers (1986) described a significant relationship between local recurrence and positive surgical margins in a series of T1 and T2 squamous cell carcinomas of the tongue. They also noted that recurrence rates increased by up to 11% in the presence of positive margins. Recent studies have indicated similar findings with regard to both local recurrence and regional lymph node metastasis (Jones et al, 1992; Woolgar et al, 1995).

There was a significant association between disease-free patients and those developing local recurrences in relation to degree of keratinisation. Four of the 24 cases showing a lower degree of keratinisation developed local recurrences compared to none of the 24 (16%) cases with a higher degree of keratinisation. These findings are similar to those reported by Odell and co-workers (1994) and Woolgar and co-workers (1995), but contradicts those reported by earlier workers such as Crissman and co-workers (1980,1984) and Okamoto and co-workers (1988). There was a strong association between local recurrence and the degree of keratinisation (p=0.0802). Of the 24 cases with a less favourable keratin pattern, 4 developed local recurrence compared to 0 of 19 cases with a more favourable pattern.

7.12 Shortcomings of the study:

Some of the difficulties encountered in this study were:

- large amount of time spent retrieving material from different institutions,
- lack of communication between various institutions,
- the lack of communication between staff members at the various institutions,
- relative small sample size,
- the number and level of involved lymph nodes were not evaluated,
- the involvement of lymph node capsules were not evaluated,
- the time from diagnosis to treatment was not recorded and
- lack of locally available expertise in the area of statistical analysis of survival rates

8. CONCLUSION

An analysis of the data shows that the mean age of the sample was 57.8 years and the female: male ratio was 1:2.7. Forty-six percent of the patients were followed up for less than two years.

Equal numbers of squamous cell carcinomas from the floor of the mouth and the tongue were evaluated.

Forty-six percent of the study sample comprised of T1 squamous cell carcinomas with remaining lesions were clinically graded as T2 squamous cell carcinomas.

In view of the high percentage of unclassified squamous cell carcinomas, the relationship between Broders' grade and time to survival, time to nodal recurrence and time to local recurrence could not be established.

A significant level of agreement between two examiners can be attained if the criteria of a proposed grading system are sufficiently discussed and understood by the examiners.

The reproducibility of the grading system improved with increased use.

The first examiner's results were markedly altered after discussion with a senior, more experienced examiner. This resulted in low intra-examiner agreement levels for the inexperienced examiner. The second, more experienced examiner, recorded very similar scores in both trials. The results of our study therefore show the importance of applying criteria consistently within a

grading system. The experience of the examiner may also play a key role in the in the evaluation of a grading system..

The parameter least agreed on was the frequency of mitoses and perineural invasion was the histological parameter consistently agreed on.

Results that could be quantified by measurement (for example "tumour thickness") or were recorded as being "present" or "absent" (for example tumour presentation) were more agreed on when compared to those that required subjected measurement (for example inflammatory response).

This study confirms the validity of invasive front grading for T1 and T2 squamous cell carcinomas of the tongue and floor of the mouth. Although the recorded overall survival rate was high, the accompanying high recurrence rate could not be justified. The only cause of disease-related death was metastatic disease. Fifty-percent of deaths occurred within 2 years. The 2-year survival probability was 92% which decreased to 73% within 5 years.

Eighty-percent of the patients that developed lymph node metastasis did so within two years of initial diagnosis. The probability of remaining free of nodal disease after 2 years was 65% and decreased to 46% within 5 years.

Four patients developed local recurrences. Two of these also developed nodal metastasis. Seventy-five percent of primary recurrences occurred within 1 year. The probability of remaining free of local recurrence after 1 year was 92%.

A follow-up period of two years is adequate for data analysis of T1and T2 oral squamous cell

carcinomas as a high percentage of treatment failures occur within this period. Outliers or extreme values affected the central measures of tendancy and resulted in an uneven distribution of data.

Because the overall histological scores were high, one can deduce that all squamous cell carcinomas of the tongue and the floor of the mouth are aggressive. Surgeons should be aware of the aggressive nature of these lesions when planning treatment. Neck dissections should be considered as part of the initial surgical procedure of all squamous cell carcinomas of the tongue and floor of the mouth.

This study suggests that certain histological parameters have a significant prognostic value which will determine the possible outcome and aid treatment planning. The pattern of invasion, in particular, is a significant prognostic indicator of both lymph node metastasis and local recurrence of small squamous cell carcinomas of the tongue and the floor of the mouth. Depth from surgical excision margin is associated with lymph node metastasis and the degree of keratinisation with local recurrence. Careful assessment of these parameters are important when grading T1 and T2 squamous cell carcinomas.

An alternative grading system evaluating the following parameters may be useful: degree of keratinisation, pattern of invasion, depth from the surgical excision margin, tumour thickness, stage of invasion, perineural invasion, vascular invasion and inflammatory response. Tumour presentation was not a significant parameter.

10. RECOMMENDATIONS

It is recommended that:

- clinicians should be encouraged to take biopsy specimens that include the deep part of the tumour to facilitate grading at the invasive front,
- standardised, computerised data base of all oral squamous cell carcinomas should be compiled and the data entered by an individual who is familiar with the terminology and procedures,
- histology reference numbers should routinely be recorded,
- a special area should be designated to head and neck pathology in the archives to facilitate retrieval of sections,
- databases of individual institutions should be linked for easy access and queries.
- a Proforma designed for recording of histological score to be used for aiding further research,
- additional studies are indicated to investigate the various aspects of tumour behaviour,
- an alternative grading system which only includes degree of keratinisation, pattern of invasion, depth from surgical excision margin, stage of invasion, tumour thickness, perineural invasion, vascular invasion and inflammatory response, be used to grade squamous cell carcinomas,
- all lesions be classified according to Broders' grade,
- elective neck dissections are performed as part of the initial treatment regime of T1 and T2 squamous cell carcinomas from the tongue and floor of the mouth,
- patients are carefully followed-up for at least 2 years and

- intensive postgraduate training in statistical methods especially for individuals who are serious in pursuing specific research areas.

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APPENDICES

Appendix 1

UICC staging of squamous cell carcinomas

TNM staging system for oral squamous cell carcinoma	
T-Tumour	
T1	Tumour less than 2 cm in diameter
T2	Tumour 2-4 cm in diameter
T3	Tumour greater than 4cm in diameter
T4	Tumour invading adjacent structures
N-Node	
N0	No clinically palpable nodes
N1	Ipsilateral palpable nodes
N2	Contralateral or bilateral nodes
N3	Fixed palpable nodes
M-Metastasis	
M0	No distant metastasis
M1	Clinical or radiographical evidence of distant metastasis

Appendix 2

PROFORMA

PARAMETER	1	2	3	4
Tendancy to keratinisation				
Pattern of invasion/Cohesion				
Tumour Thickness				
Depth from surgical excision margin				
Stage of invasion				
Mitoses				
Nuclear aberration				
Perineural invasion				
Tumour presentation				
Vascular invasion				
Inflammatory Response				

Appendix 3

Guidelines to histological grading

Parameter 1

Morphological parameter	Points			
Tendency to keratinisation	1	2	3	4
	Highly	Moderate	Minimal	None

Keratinisation was graded irrespective of whether it occurred as individually keratinized cells or in the form of pearls within the tumour cell population.

Grade 1 - large amounts of keratin

Grade 2 - moderate amounts of keratin

Grade 3 - minimal keratin production

Grade 4 - no keratinisation of tumour cells

Parameter 2

Morphological parameter	Points			
Pattern of invasion \Cohesiveness	1	2	3	4
	Solid sheets or papillary configuration (or both)with well delineated infiltrating margins	Bands and strands	Small groups of cells	Marked cellular dissociation

The basis of this point grading was the capacity of tumour cells' cohesiveness to keep the tumour population together.

Grade 1 - tumour cells arranged solid sheets or in a papillary pattern or both

Grade 2 - tumour cells form bands and strands

Grade 3 - small groups of cells resulting from loss of cellular adhesion

Grade 4 - marked dissociation of tumour cells resulting in single cells

Parameter 3

Morphological parameter	Points			
Tumour thickness	1	2	3	4
	<1.5mm	1.5>-2mm	2>-6mm	>6mm

Grade 1 - less than 1.5mm(Mohit-Tabatabai)

Grade 2 -between 1.5mm and 2.0mm

Grade 3 - greater than 2mm but less than 6mm (Spiro)

Grade 4 - greater than 6mm (Frierson and co-workers)

Parameter 4

Morphological parameter	Points			
Depth from surgical excision margins	1	2	3	4
	>2mm	1-2mm	<1mm	onto \ beyond

Grade 1 - neoplasm extends to a distance more than 2mm away from the deep surgical excision margin

Grade 2 - neoplasm is between 1 and 2mm away from the deep surgical excision margins

Grade 3 - neoplasm is less than 1mm away from the deep surgical excision margin

Grade 4 - the lesion infiltrates onto or beyond the deep surgical excision margin

Parameter 5

Morphological parameter	Points			
Stage of invasion	1	2	3	4
	Microinvasion	Distinct invasion confined to the lamina propria	Invasion below the lamina propria	Massive deep and wide invasion

Grade 1 - Microinvasion

Grade 2 - distinct invasion confined to the lamina propria

Grade 3 - invasion below the lamina propria

Grade 4 - massive deep and wide invasion

Parameter 6

Morphological parameter	Points			
Mitoses	1	2	3	4
	Few 0-2	Moderate 3-4	Numerous 5-6	Abundant >6

The number of mitoses were estimated in one field using a 40 X /0.25 lens

Grade 1 - included not more than 2 mitoses per 40 x high power field

Grade 2 - moderate numbers of mitoses (3-4)

Grade 3 - numerous mitoses (5-6)

Grade 4 - abundant mitoses (more than 6)

Parameter 7

Morphological parameter	Points			
Nuclear aberration	1	2	3	4
	Few	Abundant	Abundant with few anaplastic nuceli	Abundant with many anaplastic nuclei

Nuclear aberrations included nuclear pleomorphism (variations in size and shape of tumour cell nuclei); increased nuclear-cytoplasm ratio; hyperchromatic and multiple nuclei and atypical mitoses.

Grade 1 - few nuclear abberations in a uniform cell population

Grade 2 - abundant nuclear abberations

Grade 3 -abundant nuclear abberations with few anaplastic nuclei

Grade 4 - abundant nuclear abberations, numerous, large anaplastic nuclei.

Parameter 8

Morphological parameter	Points	
Perineural invasion	1	4
	No	Yes

Grade 1 - no perineural invasion

Grade 4 - presence of perineural involvement

Parameter 9

Morphological parameter	Points	
Tumour presentation	1	4
	Single	Multifocal

Tumour presentation was evaluated on whether the lesion appeared as a single focus or whether it was multifocal

Grade 1 - Single tumour

Grade 4 - Multifocal tumour

Parameter 10

Morphological parameter	Points	
Vascular invasion	1	4
	No	Yes

Evaluation of invasion included perivascular as well intravascular involvement

Grade 1 - no perivascular involvement

Grade 4 -perivascular or intravascular (or both) involvement

Parameter 11

Morphological parameter	Points			
Inflammatory response	1	2	3	4
	Marked	Moderate	Mild	None

The occurrence of any infiltrate of plasma cells and lymphocytes in the juxtaepithelial area was evaluated:

Grade 1 - marked

Grade 2 - moderate

Grade 3 -mild

Grade 4 - none

The intensity of the inflammatory cell reaction was judged in the lamina propria in close proximity to the tumour tissue and, if possible, not adjacent to areas of ulceration.

Appendix 4

Dear Sir/Madam

RE: PERMISSION FOR THE USE OF CLINICAL RECORDS

As a registrar in the Department of Maxillofacial and Oral Pathology, I am required to submit a dissertation in partial fulfilment of the degree MCHD at the Faculty of Dentistry, University of the Western Cape.

The topic of my thesis is "The role of histological parameters in the prediction of prognosis for oral cancer."

Your permission is hereby requested for the use of the relevant clinical records and histological sections. Acknowledgements of your department will be made should any academic papers arise from this study.

Thanking you
Yours faithfully

.....
Dr.T.S.Roberts

.....
Prof.J.J.Hille/Dr.R.Lalloo/Dr.C.Stannard
(Supervisors)