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Invasive pattern grading score designed as an independent prognostic indicator in oral squamous cell carcinoma

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Aims: To test the validity of an invasive pattern grading score (IPGS) developed for oral squamous cell carcinoma (OSCC) as a prognostic indicator and to elucidate the relationship between the IPGS and clinical parameters.
Methods and results: The IPGS was applied to a total of 153 cases of OSCC. There were significant correlations between IPGS and distant metastasis ($P = 0.01$) or recurrence ($P = 0.001$). However, there were no significant correlations between IPGS and gender, age, size or extent, location, status of lymph node metastasis, clinical staging, or histological grading. Cases of OSCC with higher IPGS were associated with poor patient survival ($P < 0.001$) and higher probability of tumour recurrence ($P = 0.001$). Intraobserver ($\kappa = 0.74$) and interobserver agreement ($\kappa = 0.67$) were very satisfactory.
Conclusions: Our study confirms the validity of the IPGS, an indicator that is simple and easy to use. IPGS not only provides histological assessment of biological behaviour, but also offers an independent prognostic factor that may influence the treatment of OSCC.

Keywords: clinical parameters, independent prognostic indicator, invasive pattern grading score, oral squamous cell carcinoma

Abbreviations: IPGS, invasive pattern grading score; OSCC, oral squamous cell carcinoma; POI, pattern of invasion; TMA, tissue microarray; TNM, tumour node metastasis

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common head and neck carcinomas, and its morbidity and mortality are increasing both in Taiwan and worldwide.[1] Despite substantial developments in both diagnosis and therapy in recent decades, the prognosis of male predominant OSCC remains poor.[2,3] Extensive local invasion and/or frequent regional lymph node metastases are usually present even at initial diagnosis, resulting in the unpredictable prognosis of OSCC.[4] Clinical assessment by the tumour node metastasis (TNM) system is widely and routinely used to define the extent of tumour load and thus determine treatment options for patients with OSCC.[5] One of the major criticisms of the TNM system is that it ignores individual histological characteristics of tumours.[6] Therefore, many workers have devised histological grading systems to predict the biological behaviour and recommended prognostic markers for OSCC, such as cell morphometry, proliferation-associ-

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ated markers, flow cytometry, and oncogene expression.[7–9] Although many of these systems have prognostic significance in patients with OSCC, no prognostic predictors are reliable enough for clinicopathological use. Multi-parameter prognostic models and scoring systems have been developed and refined over the last two decades based on histological variables that include nuclear pleomorphism, mitotic index, lymphocytic response, tumour growth pattern, tumour thickness, degree of keratinization, depth of invasion, and pattern of invasion (POI).[10–13] The histological features of OSCC may differ widely from area to area within the same tumour due to tumour heterogeneity and is subject to inter- and intraobserver disagreement.[14] There is general agreement that the most useful prognostic information can be deduced from the invasive front of the tumour, where the deepest and presumably most aggressive cells reside.[5,8,15,16]

In our previous publication,[17] we clearly demonstrated that intense staining of Fascin was identified at the invasive front of the tumour cell nests in patients with OSCC. This phenomenon has also been described by other investigators. We further noticed that increased Fascin expression is correlated with aggressive behaviour and poor patient outcome in addition to higher histological grades. Therefore, we designed a modified invasive pattern grading score (IPGS) inspired by the Gleason grading system, which gives a total IPGS consisting of the sum of the two most prevalent patterns present at on the invasive front of the tumour. The aim of the current study was to test the validity of the newly defined IPGS as a reliable and independent prognostic indicator by correlating IPGS with clinical parameters.

Materials and methods

PATIENT AND TISSUE SPECIMENS

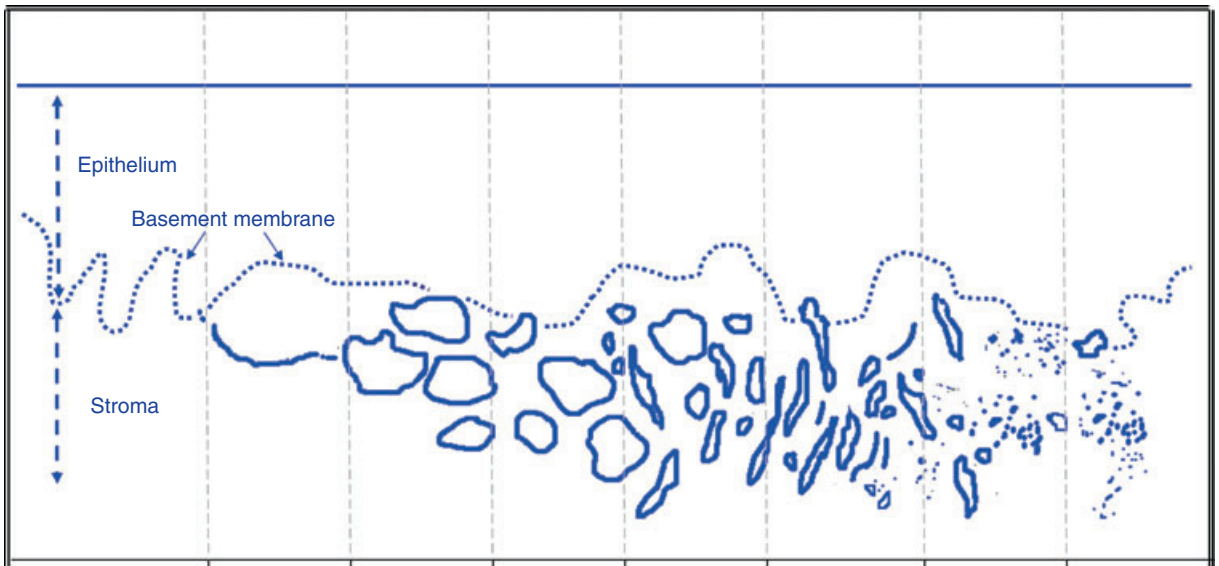
Tissue specimens of 176 patients with OSCC were retrieved from the archives of the Department of Pathology, Tri-Service General Hospital (Taipei, Taiwan) from January 2000 to December 2002. The srudy of all specimens received ethical approval from Tri-Service General Hospital Institutional Review Board (Board No. 096-05-0008). The majority of cases were selected from radical surgical specimens of OSCC. Twenty-three cases were lost to follow-up or had insufficient clinicopathological data for analysis and were therefore excluded. Initially, a tissue microarray (TMA) of OSCC was constructed and examined for analysis of IPGS but was found to be ineffective for selecting representative patterns of invasion. Cases of OSCC from TMA and small biopsy specimens other than widely excised tissues of OSCC were excluded owing to inadequate representation of the deepest invasive front. Thus, a total of 153 cases of OSCC were included in the study.

HISTOPATHOLOGICAL EVALUATION

All the histopathological slides were concurrently reviewed and evaluated independently by two qualified pathologists (the first observer and the second observer) using the same type of microscope without any prior knowledge of each patient's clinical details. When the opinions of the two evaluators differed, consensus was reached by discussion. To assess intraobserver agreement, the same slides were reassigned to the first observer, who was blinded to the results of the first assessment and to the outcome after an interval of 3 months.

INVASION PATTERN GRADING SCORE

The IPGS was based on the classification of POI originally introduced by Jakobsson *et al.*, and further defined by Bryne *et al.*[15,18] POI type 1 represents tumour invasion in a broad pushing manner with a smooth outline. POI type 2 represents tumour invasion with broad pushing 'fingers', or separate large tumour islands, with a stellate appearance. POI type 3 represents invasive islands of tumour greater than 15 cells per island. POI type 4 represents invasive tumour islands smaller than 15 cells per island including cord-like and single-cell invasion. We further modified the above-mentioned POI, leading to a newly designed IPGS. A search for the deep invasive front of OSCC interfacing with the stromal tissue to signify the invasive pattern or patterns was performed and scored correspondingly under both low- and high-power field microscopy. Because of the histological variations within each tumour, the two most prevalent patterns at the invasive front of OSCC, the predominant, or primary grade and the less extensive, or secondary grade, were given a total score, the IPGS. Tertiary patterns were not considered in the IPGS. Since each invasive pattern score was assigned a number between 1 and 4, the total summed scores ranged from 2 to 8. Furthermore, in the case of a consistent invasive pattern, if only one grade was present, IPGS was gained by doubling the invasive score. Twenty percent was used as the minimal cut-off for incorporating a tumour invasion pattern into the IPGS. Comparisons and illustrations of the four main previously described



Morphological characteristics	Solid sheet with a pushing border		Large islands		Small island and thin strands		Individual tumour cells
Invasion pattern	Pattern I		Pattern II		Pattern III		Pattern IV
Newly modified IPGS	I+I IPGS = 2	I+II / II+I IPGS = 3	II+II IPGS = 4	II+III / III+II IPGS = 5	III+III IPGS = 6	III+IV / IV+III IPGS = 7	IV+IV IPGS = 8

Figure 1. Diagrammatic illustrations and comparisons of four main patterns of invasion (POI) with corresponding IPGS of OSCC.

POIs of OSCC correlated with the new IPGS are shown in Figure 1.

STATISTICAL ANALYSIS

χ^2 test (with adequate Yates' correction when 2×2 table and d.f. = 1, or with Fisher's exact test when expected value <5 in >20% table cells) was used to measure the significance between clinical parameters and IPGS or recurrence. The log rank test was used to compare differences in survival between two groups. The prognostic significance of clinicopathological parameters and IPGS for overall survival was assessed by Cox's regression model. Survival curves were obtained by the Kaplan–Meier method. $P < 0.05$ was considered to be significant. κ statistics were used to investigate the reliability of the IPGS by measuring inter- and intraobserver agreement. A value of $\kappa < 0.20$ was considered as poor agreement, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as good and values >0.81 as very good agreement.[19] SPSS software v.13.0 (SPSS UK Ltd, Woking, UK) was used for analysis.

Results

MORPHOLOGICAL DESCRIPTION AND MEASUREMENT OF IPGS IN OSCC

The clinicopathological data of 153 patients with OSCC are summarized in Table 1. According to our newly modified IPGS as described in Figure 1, each case was assigned an IPGS between 2 and 8 (Figure 2). The two most frequent IPGS expressed in a total of 153 cases of OSCC were IPGS 4 ($n = 45$, III + I $N = 2$ and II + II $N = 43$) and IPGS 5 ($n = 39$, II + III $N = 38$ and III + II $N = 1$). Groups were classified as high IPGS (≥5) or low IPGS (≤4) with score 4 as the threshold point. There were 77 cases of OSCC with low IPGS and 76 cases of OSCC with high IPGS (Figure 3).

CORRELATION BETWEEN IPGS AND CLINICOPATHOLOGICAL PARAMETERS IN OSCC

When comparing IPGS with the clinicopathological parameters, there was no significant correlation between IPGS and gender, age, size or extent of the

Table 1. Clinicopathological data in 153 patients with oral squamous cell carcinoma

Clinicopathological parameters	Number of patients *n* (%)
Age	
Median (52.34)	
≤52	85 (55.6)
>52	68 (44.4)
Gender	
Male	129 (84.3)
Female	24 (15.7)
Location	
Tongue	115 (75.2)
Buccal mucosa	36 (23.5)
Gingiva	2 (1.3)
T stage	
1	31 (20.3)
2	50 (32.7)
3	33 (21.5)
4	39 (25.5)
N stage	
0	106 (69.3)
1	27 (17.6)
2	14 (9.1)
3	6 (4)
M stage	
0	132 (86.3)
1	21 (13.7)
TNM stage	
I	22 (14.4)
II	33 (21.6)
III	36 (23.5)
IV	62 (40.5)
Recurrence	
No	106 (69.3)
Yes	47 (30.7)
Differentiation	
Well	54 (35.3)
Moderate	82 (53.6)
Poor	17 (11.1)

tumour, location, status of lymph node metastasis, clinical staging or histological grading. However, there were statistically significant correlations between IPGS and distant metastasis ($P = 0.01$) and tumour recurrence ($P = 0.001$) of OSCC (Table 2).

IPGS USED AS AN INDEPENDENT PROGNOSTIC INDICATOR IN OSCC

When comparing the predictor of tumour recurrence with clinicopathological parameters, histological differentiation and IPGS, there were statistically significant correlations between tumour recurrence and age ($P = 0.036$), status of lymph node metastasis ($P = 0.039$), distant metastasis ($P < 0.001$), clinical staging ($P < 0.001$) and IPGS ($P = 0.001$). However, there were no significant correlations between tumour recurrence and gender, size or extent of the tumour, and histological grading (Table 3).

Follow-up of 153 cases with OSCC from initial diagnosis to 72 months was performed and the overall survival rate estimated at 42.5%. There was a statistically significant correlation between patient survival and the size or extent of the tumour ($P < 0.001$), lymph node metastasis ($P = 0.033$), distant metastasis ($P < 0.001$), clinical staging ($P < 0.001$), tumour recurrence ($P < 0.001$), histological grading ($P = 0.002$) and IPGS ($P = 0.001$) by the log rank test. Cox's multivariate analysis also showed that size or extent of the tumour ($P < 0.001$), positive lymph node metastasis ($P = 0.037$), distant metastasis ($P < 0.001$), clinical staging ($P < 0.001$), tumour recurrence ($P < 0.001$), histological grading ($P = 0.003$) and IPGS ($P = 0.001$) were significant prognosticators (Table 4). Kaplan–Meier plot analysis demonstrated statistical significance in terms of clinical staging, tumour recurrence, histological grading, and IPGS (Figure 4). Cases of OSCC with high IPGS were associated with strong invasive ability and the implication of poor prognosis.

IPGS ALSO REGARDED AS A RELIABLE INDICATOR IN OSCC

Interobserver agreement was calculated from the first assessment of the first observer and that of the second observer by double blind methods. The κ score of interobserver agreement in the original data was 0.69, which is good agreement. To assess intraobserver agreement, the same slides were reassigned to the first observer, who was blinded to the results of the first assessment after an interval of 3 months. The κ score for intraobserver agreement ($\kappa = 0.74$) was slightly higher than the interobserver scores ($\kappa = 0.69$), and

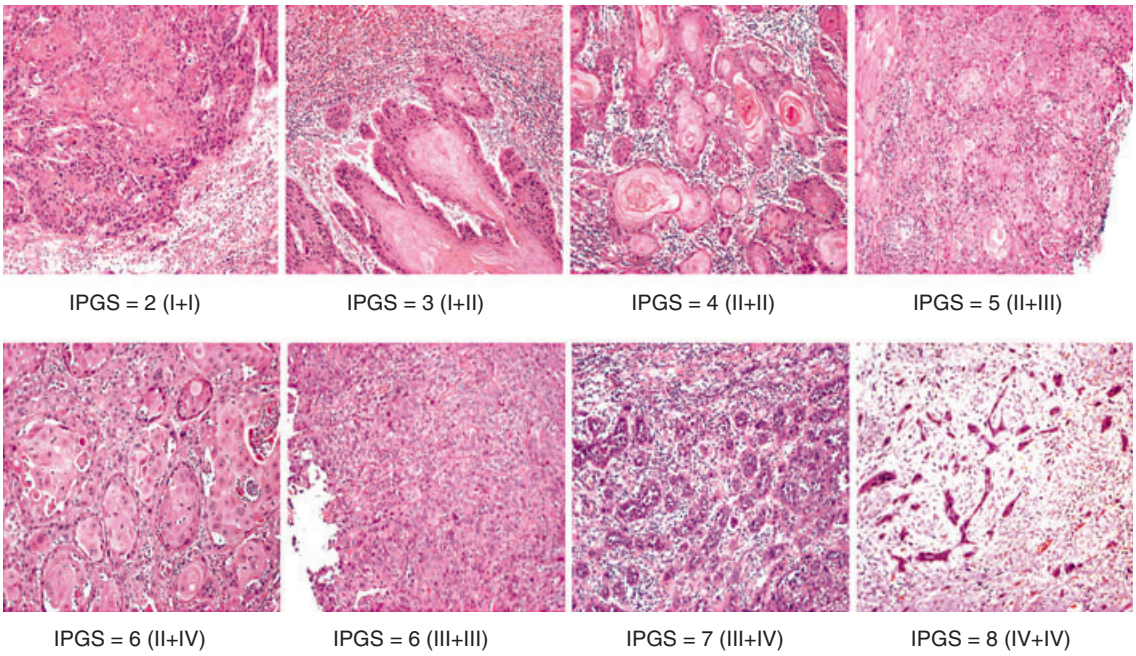


Figure 2. Representative microscopic photographs of OSCC with assigned IPGS from 2 to 8 (H&E stain, ×200).

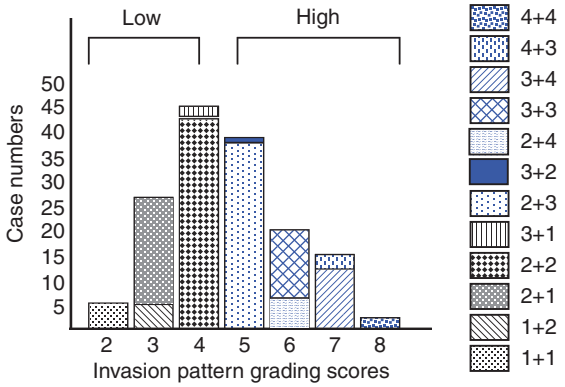


Figure 3. The distribution of each individual IPGS in a total of 153 patients with OSCC.

can also be categorized as good agreement. Similarly, based on the categories of high and low IPGS, the κ score of interobserver agreement was 0.72, which can also be considered good agreement, and intraobserver agreement was 0.81, which can be regarded as very good agreement (Table 5).

Discussion

The inevitable histological variations in OSCC can be closely related to prognosis. In addition to subjective histological interpretations of OSCC by pathologists, the major problem encountered when investigating OSCC is its heterogeneity both between tumours, within individual tumours, and between close but biologically different oral anatomical sites.[8] All of these factors complicate the prognosis of OSCC, resulting in unpredictability.

Many histological prognostic models and scoring systems have been developed in previous series for predicting the biological behaviour of OSCC. Broders' system was first established on the basis of the proportion of highly differentiated cells in 1920.[20,21] Although Broders' system was simple and widely used, it was a poor predictor for survival or metastasis.[6,22] In 1973, Jakobsson *et al.*[18] developed a multifactorial grading system which had the advantage of scoring tumour–host interactions and tumour characteristics, but eventually proved to only be useful when applied to tongue cancers.[23,24] Later, Anneroth *et al.*[25] proposed a modification of Jakobsson's system based on the assessments of six histomorphological parameters including degree of keratinization, nuclear pleomorphism, pattern of invasion, host response and mitotic activity. Bryne *et al.*[15] modified Anneroth's grading system and developed a malignancy grading system focusing only on the invasive front of the tumour. This method of grading appeared to be less time-consuming than those of Jakobsson *et al.* and Anneroth *et al.*[26,27] Nevertheless, this system was not sufficiently homogeneous to allow grading parameters to be assessed individually.

Table 2. Correlation of clinicopathological parameters with invasive pattern grading score (IPGS) in 153 patients with oral squamous cell carcinoma

Clinicopathological parameters	Low IPGS (*N* = 77)	High IPGS (*N* = 76)	*P*-value
Age			
≤52	41	44	NS
>52	36	32	
Gender			
Male	67	62	NS
Female	10	14	
Location			
Tongue	59	56	NS
Buccal mucosa	18	18	
Gingiva	0	2	
T stage			
T1/T2	41	40	NS
T3/T4	36	36	
N stage			
N0	54	52	NS
N1/N2/N3	23	24	
M stage			
0	72	60	0.01
1	5	12	
TNM stage			
I/II	33	22	NS
III/IV	44	54	
Recurrence			
No	63	43	0.001
Yes	14	33	
Differentiation			
Well	29	25	NS
Moderate	43	39	
Poor	5	12	

NS, not significant.

Table 3. Correlation of clinical parameters, differentiation and invasive pattern grading score (IPGS) with recurrence

		Recurrence		
Clinicopathological parameters	No. of cases	No (*N* = 106)	Yes (*N* = 47)	*P*-value
Age				
≤52	85	65	20	0.036
>52	68	41	27	
Gender				
Male	129	87	42	NS
Female	24	19	5	
T stage				
T1/T2	81	61	20	NS
T3/T4	72	45	27	
N stage				
N0	106	79	27	0.039
N1/N2/N3	37	27	20	
M stage				
0	132	102	30	<0.001
1	21	4	17	
TNM stage				
I/II	55	48	7	<0.001
III/IV	98	58	40	
Differentiation				
Well/moderate	136	96	40	NS
Poor	17	10	7	
IPGS				
Low (2∼4)	77	63	14	0.001
High (5∼8)	76	43	33	

NS, not significant.

A variety of clinical and histological parameters may indeed influence patient prognosis and outcome, but independence and reliability are important and essential for a chosen prognostic parameter, especially in regard to local recurrence and overall survival. The IPGS in our study was developed from original POI to satisfy the above-mentioned independence and reliability for prognostic evaluation of OSCC. As shown in Tables 2 and 3, we have demonstrated that IPGS is an independent parameter that does not interact with and is not influenced by the majority of the important clinicopathological parameters, including size or extent of the tumour, status of lymph node metastasis, clinical or histological grading. However, due to significant correlations between IPGS and distant metastasis and tumour recurrence, as well as poor survival, IPGS is considered to be a reliable prognostic parameter. From

Table 4. Statistical correlation of clinical parameters, differentiation and invasive pattern grading score (IPGS) with survival

Variables	Cut-off levels	No. of cases	Log-rank *P*-value	Cox regression *P*-value
Age	≤52 versus >52	85/68	0.513	0.518
Gender	Male versus female	129/24	0.714	0.717
T stage	T1/T2 versus T3/T4	81/72	<0.001	<0.001
N stage	N0 versus N1/N2/N3	106/47	0.033	0.037
M stage	M0 versus M1	132/21	<0.001	<0.001
TNM stage	I/II versus III/IV	55/98	<0.001	<0.001
Recurrence	No versus yes	106/47	<0.001	<0.001
Differentiation	Well/moderate versus poor	136/17	0.002	0.003
IPGS	Low versus high	77/76	<0.001	0.001

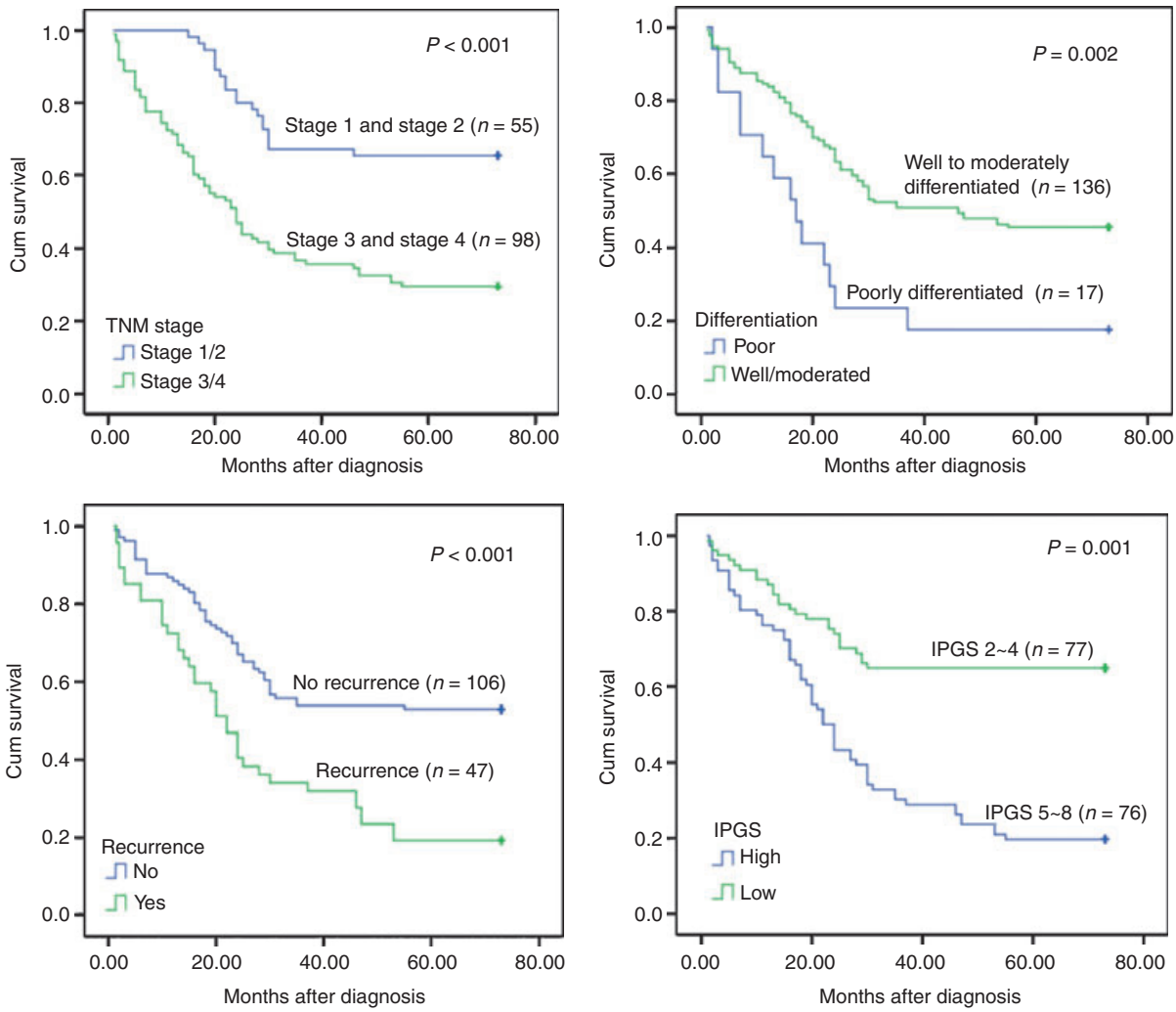


Figure 4. Statistical differences were found by comparisons of cumulative survival in terms of the clinical stage, histological grading, tumor recurrence, and IPGS in 153 patients with OSCC by Kaplan–Meier analysis.

Table 5. Interobserver and intraobserver agreements for the histological features of invasive pattern grading score (IPGS)

Kappa (κ) score			
Original score*		Score categorized†	
Interobserver	Intraobserver	Interobserver	Intraobserver
0.69	0.74	0.72	0.81

*In the original κ score, each case was scored from 2 to 8.
†While the categorized κ score was classified as low and high IPGS.

the practical standpoint, IPGS is simple and easy to use by calculating and scoring the most representative invasion pattern or patterns. Based on our data, cases of OSCC with low IPGS were associated with limited invasion and a favourable prognosis, including infrequent recurrence and relatively long survival, whereas cases of OSCC with high IPGS were associated with increased invasion and poor overall survival, a propensity to tumour recurrence and even distant metastasis. Thus, IPGS might thus influence the strategy for treatment.

As mentioned above, IPGS was derived from four types of POI. Each POI has its own morphological features and definition. Cases of verrucous carcinoma were not included in the study due to their predictable favourable outcomes and the monotonous histological presentation of pattern I without other invasion patterns.[28,29] Besides these four types of invasion pattern, a fifth pattern was introduced by Brandwein-Gensler *et al.*[30] by defining a separate and infiltrative invasion pattern as tumour satellites of any size with ≥1 mm distance of intervening normal tissue at the tumour–host interface. Although this type of POI can be seen in some cases or in some areas of the tumour, we believe that this widely dispersed pattern is rare, unpredictable and unmeasurable, as well as too rare to calculate. Furthermore, pattern 5 does not seem to be well established and accepted in the field of head and neck cancer and therefore we decided to exclude it from our study.

Based on our data, the κ scores indicated good inter- and intraobserver variability in assessment of IPGS. Compared with the study performed by Sawair *et al.*,[5] our data using IPGS derived by adding up the scores of the two most prevalent patterns at the invasive front of OSCC resulted in a more reproducible and reliable statistical κ score. To the best of our knowledge, the IPGS is reported for the first time and is a reliable and independent prognostic factor that may influence the treatment plan. This is an initial study and the assessment of more cases of OSCC is required to support and confirm its significance. We hope that the IPGS will also have application at other sites of the body for the prognosis of cancer.

Acknowledgements

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