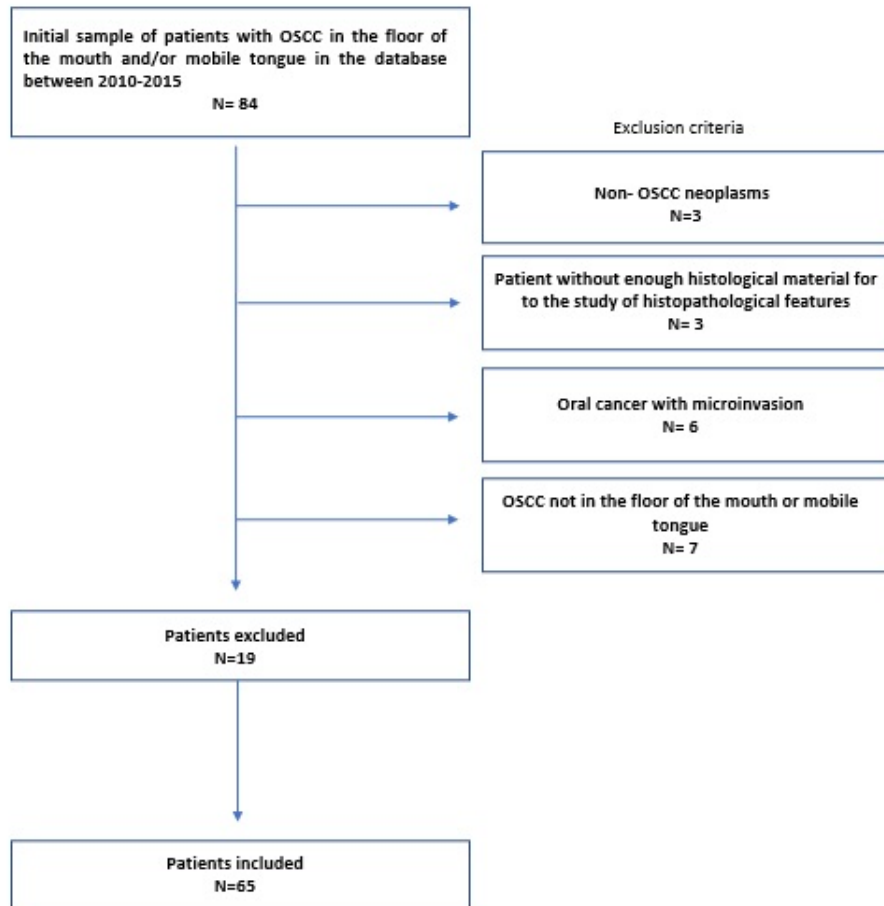


SUPPLEMENTARY INFORMATION

Supplementary Figure S1. Flow chart of the selected sample.



Supplementary Table S1. Descriptive statistics of biomarkers according to sex.

Variable	N	Overall, N = 65	Sex		p-value <sup>1</sup>
			Men, N = 40	Women, N = 25	
PD-1	65				0.838
Median (IQR)		0.01 (0.00, 0.05)	0.02 (0.00, 0.05)	0.01 (0.00, 0.03)	
PD-1	65				0.202
PD-1-Negative <0%		13 (20%)	10 (25%)	3 (12%)	
PD-1-Positive >0%		52 (80%)	30 (75%)	22 (88%)	
PD-L1 CPS	65				0.138
Median (IQR)		2 (0, 15)	1 (0, 6)	3 (1, 22)	
PD-L1 CPS	65				0.279
cps[0,1)		26 (40%)	15 (38%)	11 (44%)	
cps[1,20)		16 (25%)	8 (20%)	8 (32%)	
cps[20,100]		23 (35%)	17 (42%)	6 (24%)	
PD-L1 CPS cutoff >1	65				0.194
PD-L1 Negative CPS<=1		30 (46%)	21 (52%)	9 (36%)	
PD-L1 Positive CPS>1		35 (54%)	19 (48%)	16 (64%)	
PD-L1 intensity	65				0.250
Nil		25 (38%)	19 (48%)	6 (24%)	
Low		18 (28%)	10 (25%)	8 (32%)	
Moderate		18 (28%)	9 (22%)	9 (36%)	
Intense		4 (6.2%)	2 (5.0%)	2 (8.0%)	
PD-L1 TPS	65				0.037
Median (IQR)		0 (0, 19)	0 (0, 4)	1 (0, 40)	
PD-L1 TPS cutoffs 5%/10%	65				0.131
PD-L1 <5%		43 (66%)	30 (75%)	13 (52%)	
PD-L1 [5-10%)		4 (6.2%)	2 (5.0%)	2 (8.0%)	
PD-L1[10-100%]		18 (28%)	8 (20%)	10 (40%)	
PD-L1 TPS cutoff 5%	65				0.057
PD-L1 TPS<5%		43 (66%)	30 (75%)	13 (52%)	
PD-L1 TPS>=5%		22 (34%)	10 (25%)	12 (48%)	
PD-L1 TPS cutoff 10%	65				0.153
PD-L1 TPS <=10%		48 (74%)	32 (80%)	16 (64%)	
PD-L1 TPS >10%		17 (26%)	8 (20%)	9 (36%)	
FOXP3 (%)	65				0.073
Median (IQR)		15 (10, 20)	10 (7, 16)	15 (10, 20)	
FOXP3 cutoff 10%	65				0.033
[0-10)%		29 (45%)	22 (55%)	7 (28%)	
(10-100)%		36 (55%)	18 (45%)	18 (72%)	
CD4 (%)	65				0.223
Median (IQR)		25 (15, 35)	20 (15, 31)	30 (20, 35)	
CD4	65				0.126
[0.05,0.25)		31 (48%)	23 (57%)	8 (32%)	
[0.25,0.35)		13 (20%)	7 (18%)	6 (24%)	
[0.35,0.50]		21 (32%)	10 (25%)	11 (44%)	
P16	65				>0.999
Negative		63 (97%)	39 (98%)	24 (96%)	
Positive		2 (3.1%)	1 (2.5%)	1 (4.0%)	
CSF1R (%)	65				0.619
Median (IQR)		7 (3, 10)	7 (3, 10)	7 (3, 10)	
CSF1R	65				0.217
[0-8%)		38 (58%)	21 (52%)	17 (68%)	
[8-100]		27 (42%)	19 (48%)	8 (32%)	
CD8 (%)	65				0.644

Supplementary Table S1. Descriptive statistics of biomarkers according to sex.

Variable	N	Overall, N = 65	Sex		p-value <sup>1</sup>
			Men, N = 40	Women, N = 25	
Median (IQR)		30 (20, 40)	32 (20, 41)	30 (25, 35)	
CD8	65				0.099
CD8 [0-10%] Mild		5 (7.7%)	4 (10%)	1 (4.0%)	
CD8 [10-50%] Moderate		51 (78%)	28 (70%)	23 (92%)	
CD8 >=50% Severe		9 (14%)	8 (20%)	1 (4.0%)	

<sup>1</sup>Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Abbreviations: IQR: interquartile range.

Supplementary Table S2. Bivariate correlation between biomarkers Spearman's rank-order correlation

Parameter 1	Parameter 2	rho (CI95%)	<i>p</i> -value
PD-1	PD-L1	0.251 (0.001-0.472)	0.737
PD-1	FOXP3	0.065 (-0.189-0.311)	1.000
PD-1	CSF1R	0.079 (-0.175-0.324)	1.000
PD-1	CD4	0.092 (-0.163-0.335)	1.000
PD-1	CD8	-0.080 (-0.325-0.174)	1.000
PD-L1	FOXP3	0.038 (-0.215-0.286)	1.000
PD-L1	CSF1R	0.283 (0.034-0.498)	0.452
PD-L1	CD4	-0.160 (-0.395-0.094)	1.000
PD-L1	CD8	0.018 (-0.234-0.268)	1.000
FOXP3	CSF1R	0.196 (-0.058-0.426)	1.000
FOXP3	CD4	0.341 (0.099-0.545)	0.114
FOXP3	CD8	0.049 (-0.204-0.296)	1.000
CSF1R	CD4	0.014 (-0.238-0.264)	1.000
CSF1R	CD8	0.016 (-0.236-0.266)	1.000
CD4	CD8	0.243 (-0.008-0.465)	0.818

Abbreviations: CI: Confidence Interval.

Supplementary Table S3. Descriptive statistics of clinical and histopathological variables according to PD-1 expression and PD-L1 expression in TPS and CPS.

Variable	N	Overall, N = 65	PD-1 Expression		p-value <sup>1</sup>	PD-L1 Expression TPS			PD-L1 Expression CPS		p-value <sup>1</sup>
			Negative, <0% = 13	Positive, >0% N = 52		TPS <5%	TPS ≥5%	p-value <sup>1</sup>	CPS ≤ 1	CPS > 1	
Sex	65				0.202			0.057			0.194
Men		40 (62%)	10 (77%)	30 (58%)		30 (70%)	10 (45%)		21 (70%)	19 (54%)	
Women		25 (38%)	3 (23%)	22 (42%)		13 (30%)	12 (55%)		9 (30%)	16 (46%)	
Age at diagnosis	65				0.254			0.598			0.171
Mean (SD)		67 (57, 73)	65 (56, 68)	68 (59, 75)		66 (56, 73)	68 (59, 76)	0.598	66 (55, 71)	69 (60, 74)	
Tobacco use	63				0.539			0.405			0.249
Never smoker		30 (48%)	4 (33%)	26 (51%)		18 (42%)	12 (60%)		12 (40%)	18 (55%)	
Former smoker		16 (25%)	4 (33%)	12 (24%)		12 (28%)	4 (20%)		7 (23%)	9 (27%)	
Current smoker		17 (27%)	4 (33%)	13 (25%)		13 (30%)	4 (20%)		11 (37%)	6 (18%)	
Alcohol use	60				0.685			0.910			>0.999
Nondrinker		41 (68%)	7 (58%)	34 (71%)		27 (66%)	14 (74%)		20 (69%)	21 (68%)	
Former drinker		7 (12%)	2 (17%)	5 (10%)		5 (12%)	2 (11%)		3 (10%)	4 (13%)	
Current drinker		12 (20%)	3 (25%)	9 (19%)		9 (22%)	3 (16%)		6 (21%)	6 (19%)	
Mouth Primary Location	65	23 (35%)	5 (38%)	18 (35%)	>0.999	18 (42%)	5 (23%)	0.127	17 (57%)	6 (17%)	<0.001
Tongue Primary Location	65	48 (74%)	11 (85%)	37 (71%)	0.486	30 (70%)	18 (82%)	0.296	18 (60%)	30 (86%)	0.019
Tumor Size status	63				0.157			0.518			0.633
T1		13 (21%)	2 (17%)	11 (22%)		10 (24%)	3 (14%)		5 (17%)	8 (24%)	
T2		25 (40%)	2 (17%)	23 (45%)		14 (33%)	11 (52%)		10 (34%)	15 (44%)	
T3		13 (21%)	4 (33%)	9 (18%)		10 (24%)	3 (14%)		7 (24%)	6 (18%)	
T4		12 (19%)	4 (33%)	8 (16%)		8 (19%)	4 (19%)		7 (24%)	5 (15%)	
Nodal status	60				0.200			0.143			0.756
N0		36 (60%)	5 (45%)	31 (63%)		27 (68%)	9 (45%)		16 (59%)	20 (61%)	
N1		8 (13%)	3 (27%)	5 (10%)		5 (12%)	3 (15%)		4 (15%)	4 (12%)	
N2		14 (23%)	2 (18%)	12 (24%)		8 (20%)	6 (30%)		7 (26%)	7 (21%)	
N3		2 (3.3%)	1 (9.1%)	1 (2.0%)		0 (0%)	2 (10%)		0 (0%)	2 (6.1%)	
Metastasis status	60				0.330			0.404			0.222
M0		53 (88%)	8 (80%)	45 (90%)		33 (85%)	20 (95%)		21 (81%)	32 (94%)	
M1		7 (12%)	2 (20%)	5 (10%)		6 (15%)	1 (4.8%)		5 (19%)	2 (5.9%)	
Stage	63				0.574			0.612			0.680
Stage I		8 (13%)	1 (8.3%)	7 (14%)		7 (17%)	1 (4.8%)		4 (14%)	4 (12%)	
Stage II		13 (21%)	3 (25%)	10 (20%)		9 (21%)	4 (19%)		5 (17%)	8 (24%)	
Stage III		28 (44%)	7 (58%)	21 (41%)		17 (40%)	11 (52%)		15 (52%)	13 (38%)	
Stage IV		14 (22%)	1 (8.3%)	13 (25%)		9 (21%)	5 (24%)		5 (17%)	9 (26%)	
Histological Grade	65				0.478			0.239			0.456
Grade 1: WD		22 (34%)	4 (31%)	18 (35%)		17 (40%)	5 (23%)		11 (37%)	11 (31%)	
Grade 2: MD		37 (57%)	9 (69%)	28 (54%)		21 (49%)	16 (73%)		15 (50%)	22 (63%)	
Grade 3: PD		6 (9.2%)	0 (0%)	6 (12%)		5 (12%)	1 (4.5%)		4 (13%)	2 (5.7%)	
Oral potentially malignant disorders	65	10 (15%)	0 (0%)	10 (19%)	0.191	7 (16%)	3 (14%)	>0.999	6 (20%)	4 (11%)	0.493
Lymphoplasmacytic invasion	65				0.030			0.647			0.731
Nil		2 (3.1%)	0 (0%)	2 (3.8%)		2 (4.7%)	0 (0%)		1 (3.3%)	1 (2.9%)	
Low		33 (51%)	11 (85%)	22 (42%)		20 (47%)	13 (59%)		15 (50%)	18 (51%)	
Moderate		25 (38%)	1 (7.7%)	24 (46%)		18 (42%)	7 (32%)		13 (43%)	12 (34%)	
Intense		5 (7.7%)	1 (7.7%)	4 (7.7%)		3 (7.0%)	2 (9.1%)		1 (3.3%)	4 (11%)	
Vascular invasion	65	5 (7.7%)	1 (7.7%)	4 (7.7%)	>0.999	3 (7.0%)	2 (9.1%)	>0.999	3 (10%)	2 (5.7%)	0.655
Perineural invasion	65	26 (40%)	7 (54%)	19 (37%)	0.255	19 (44%)	7 (32%)	0.335	14 (47%)	12 (34%)	0.310
WPOI	65				0.313			0.102			0.016
WPOI 1		4 (6.2%)	1 (7.7%)	3 (5.8%)		4 (9.3%)	0 (0%)		4 (13%)	0 (0%)	
WPOI 2		30 (46%)	4 (31%)	26 (50%)		17 (40%)	13 (59%)		10 (33%)	20 (57%)	
WPOI 3		20 (31%)	7 (54%)	13 (25%)		13 (30%)	7 (32%)		8 (27%)	12 (34%)	
WPOI 4		4 (6.2%)	0 (0%)	4 (7.7%)		2 (4.7%)	2 (9.1%)		2 (6.7%)	2 (5.7%)	
WPOI 5		7 (11%)	1 (7.7%)	6 (12%)		7 (16%)	0 (0%)		6 (20%)	1 (2.9%)	
Risk Score	65				0.165			0.229			0.043
0-1		24 (37%)	2 (15%)	22 (42%)		17 (40%)	7 (32%)		12 (40%)	12 (34%)	
2-3		18 (28%)	4 (31%)	14 (27%)		9 (21%)	9 (41%)		4 (13%)	14 (40%)	
4-7		23 (35%)	7 (54%)	16 (31%)		17 (40%)	6 (27%)		14 (47%)	9 (26%)	
Depth of invasion	63				0.017			0.170			0.978

Median (IQR)		9 (3, 12)	12 (7, 16)	8 (3, 11)		7 (3, 11)	10 (8, 12)		7 (3, 12)	9 (4, 12)	
Local recurrence	65	65	19 (29%)	3 (23%)	0.740	10 (23%)	9 (41%)	0.139	5 (17%)	14 (40%)	0.039
Regional recurrence	65	65	6 (9.2%)	3 (23%)	0.089	5 (12%)	1 (4.5%)	0.655	3 (10%)	3 (8.6%)	>0.999
Distant recurrence	65	65	2 (3.1%)	1 (7.7%)	0.362	2 (4.7%)	0 (0%)	0.545	1 (3.3%)	1 (2.9%)	>0.999
DFS outcome	65				0.039						
Alive and without recurrence		26 (40%)	2 (15%)	24 (46%)		16 (37%)	10 (45%)		11 (37%)	15 (43%)	
Death		5 (7.7%)	1 (7.7%)	4 (7.7%)		4 (9.3%)	1 (4.5%)		4 (13%)	1 (2.9%)	
Death OSCC		11 (17%)	4 (31%)	7 (13%)		9 (21%)	2 (9.1%)		7 (23%)	4 (11%)	
Local recurrence		18 (28%)	3 (23%)	15 (29%)		10 (23%)	8 (36%)		5 (17%)	13 (37%)	
Regional or Nodal recurrence		5 (7.7%)	3 (23%)	2 (3.8%)		4 (9.3%)	1 (4.5%)		3 (10%)	2 (5.7%)	
DFS event	65	34 (52%)	10 (77%)	24 (46%)	0.047	23 (53%)	11 (50%)	0.790	15 (50%)	19 (54%)	0.730
DSS outcome	65				0.009			0.404			0.148
Alive with or without recurrence		33 (51%)	2 (15%)	31 (60%)		19 (44%)	14 (64%)		12 (40%)	21 (60%)	
Death		5 (7.7%)	1 (7.7%)	4 (7.7%)		4 (9.3%)	1 (4.5%)		4 (13%)	1 (2.9%)	
Death OSCC		27 (42%)	10 (77%)	17 (33%)		20 (47%)	7 (32%)		14 (47%)	13 (37%)	
DSS event	65	34 (52%)	10 (77%)	24 (46%)	0.004	20 (47%)	7 (32%)	0.255	14 (47%)	13 (37%)	
OS Outcome	65				0.004			0.138			0.108
Alive with or without recurrence		33 (51%)	2 (15%)	31 (60%)		19 (44%)	14 (64%)		12 (40%)	21 (60%)	
Death by any cause		32 (49%)	11 (85%)	21 (40%)		24 (56%)	8 (36%)		18 (60%)	14 (40%)	
Overall Survival event	65	32 (49%)	11 (85%)	21 (40%)	0.004	24 (56%)	8 (36%)	0.138	18 (60%)	14 (40%)	0.108

1 Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Abbreviations: DFS: Disease-free survival, DSS: Disease-specific survival, MD: Moderately differentiated, OSCC: Oral squamous cell carcinoma, OS: Overall survival PD: Poorly differentiated, SD: standard deviation, WD: Well differentiated, WPOI: worst pattern of invasion.

Supplementary Table S4. Univariate analysis of clinical and histopathological variables for the different survival rates (OS, DSS, DFS)

	Cox proportional Hazard Model					
	OS: HR(CI95%)	p-value	DSS: HR(CI95%)	p-value	DFS: HR(CI95%)	p-value
<b>Age at diagnosis</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
NA	1.02 (0.99-1.05)	0.107	1.01 (0.98-1.04)	0.373	1.02 (0.99-1.04)	0.177
<b>Gender</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Men						
Women	0.35 (0.15-0.82)	0.015	0.36 (0.14-0.89)	0.028	0.80 (0.40-1.63)	0.549
<b>Tobacco use</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Never smoker						
Former smoker	1.50 (0.59-3.84)	0.395	1.96 (0.73-5.31)	0.184	1.40 (0.62-3.19)	0.418
Current smoker	2.11 (0.90-4.90)	0.084	2.47 (0.97-6.27)	0.056	1.14 (0.50-2.59)	0.757
<b>Alcohol use</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Nondrinker						
Former drinker	1.73 (0.64-4.68)	0.280	2.07 (0.75-5.73)	0.162	1.78 (0.72-4.44)	0.211
Current drinker	1.28 (0.51-3.25)	0.596	1.25 (0.45-3.45)	0.667	1.13 (0.46-2.83)	0.787
<b>Tumor location</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Tongue						
Base of the Mouth	0.87 (0.36-2.05)	0.741	0.92 (0.36-2.35)	0.857	0.67 (0.29-1.57)	0.358
Tongue and Base of the Mouth	1.16 (0.34-3.93)	0.810	1.39 (0.40-4.78)	0.603	1.61 (0.55-4.68)	0.384
<b>Tumor status</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
T1						
T2	1.46 (0.47-4.54)	0.516	1.43 (0.38-5.31)	0.596	1.06 (0.40-2.83)	0.912
T3	2.16 (0.63-7.41)	0.222	2.43 (0.60-9.76)	0.211	1.04 (0.34-3.23)	0.944
T4	2.29 (0.69-7.65)	0.177	3.00 (0.79-11.38)	0.107	2.21 (0.80-6.10)	0.124
<b>Nodal Status</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
N0						
N1	1.21 (0.40-3.67)	0.741	1.07 (0.30-3.84)	0.912	1.57 (0.57-4.31)	0.384
N2/N3	1.04 (0.45-2.40)	0.920	1.12 (0.46-2.76)	0.795	0.88 (0.40-1.98)	0.764
<b>Metastasis status</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
M0						
M1	5.84 (2.18-15.64)	0.000	6.00 (2.02-17.81)	0.001	3.11 (1.15-8.44)	0.026
<b>Histological Grade</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Grade 1: WD						
Grade 2: MD	1.26 (0.54-2.95)	0.596	1.77 (0.64-4.92)	0.276	1.63 (0.72-3.71)	0.242
Grade 3: PD	5.07 (1.73-14.84)	0.003	8.09 (2.43-26.97)	0.001	5.15 (1.75-15.15)	0.003
<b>Stage</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Stage I						
Stage II	1.43 (0.28-7.39)	0.674	2.75 (0.32-23.63)	0.358	1.41 (0.36-5.48)	0.617
Stage III	2.24 (0.52-9.74)	0.280	3.88 (0.51-29.46)	0.190	1.56 (0.46-5.36)	0.478
Stage IV	1.71 (0.35-8.29)	0.503	2.39 (0.28-20.48)	0.430	1.32 (0.34-5.12)	0.689
<b>Oral potentially malignant disorders</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
No						
Yes	0.81 (0.28-2.32)	0.697	0.46 (0.11-1.94)	0.289	0.74 (0.26-2.10)	0.569
<b>Perineural invasion</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
No						
Yes	1.21 (0.58-2.51)	0.610	1.25 (0.56-2.79)	0.575	1.04 (0.52-2.08)	0.912
<b>Vascular invasion</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
No						
Yes	0.68 (0.16-2.95)	0.610	0.81 (0.18-3.59)	0.787	0.59 (0.14-2.53)	0.478
<b>Lymphoplasmacytic invasion</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Nil/Low						
Moderate	0.86 (0.40-1.89)	0.711	0.67 (0.28-1.64)	0.384	0.90 (0.43-1.88)	0.779
Intense	1.04 (0.24-4.61)	0.952	1.16 (0.26-5.19)	0.841	0.73 (0.17-3.15)	0.674
<b>Margins affected</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
No						
Yes	0.50 (0.07-3.71)	0.503	0.61 (0.08-4.53)	0.631	0.44 (0.06-3.22)	0.418
<b>Risk Score</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
0-1						
2-3	0.92 (0.36-2.37)	0.865	1.02 (0.37-2.86)	0.968	1.02 (0.43-2.41)	0.968
4-7	1.26 (0.54-2.94)	0.582	1.35 (0.53-3.41)	0.529	1.17 (0.52-2.63)	0.704
<b>WPOI 1+2+3 VS 4+5</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
WPOI 1+2+3						
WPOI 4+5	2.34 (1.00-5.47)	0.050	1.92 (0.72-5.12)	0.194	1.76 (0.72-4.28)	0.211
<b>Depth of Invasion</b>						
	Ref.Cat.	-	Ref.Cat.	-	Ref.Cat.	-
Less invasive						
Moderate invasive	0.93 (0.39-2.23)	0.873	0.89 (0.34-2.36)	0.818	1.07 (0.48-2.42)	0.865

Deeply invasive                    0.64 (0.26-1.57)    0.332    0.69 (0.26-1.81)    0.453    0.51 (0.21-1.20)    0.124