HPV Status Improves Classification of Head and Neck Gray Zone Cancers

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Appendix

Appendix Table 1. Definitions for HPV seropositivity. *[1]The cut-off for positivity for HPV 16 E6 is different between Standards 1 and 2; the Standard 1 cut-off is > 1000 MFI and thus the panel label is s1-16E6, while for standard 2 the cut-off is >484 MFI, and is labelled s2-16E6.*

Definition	HPV Seromarkers, definition of positive	Panel Label
Standard 1	HPV 16 E6 alone (> 1000 MFI)	s1-16E6
Standard 2: either of the two definitions on the right	3 of 4 high levels of the following markers: HPV 16 E1 (s2-16E1), HPV 16 E2 (s2 16E2), HPV 16 E6 (s2 16E6), and HPV 16 E7 (s2 16E7)	s2 16
	3 of 4 high levels of the following markers: HPV 18 E1 (s2 18E1), HPV 18 E2 (s2 18E2), HPV 18 E6 (s2 18E6), and HPV 18 E7 (s2 18E7)	s2 18
Standard 3: type-specific combinations of E6 AND E7	High levels of both HPV 18 E6 (s3 18E6) AND E7 (s3 18E7)	s3 18
	High levels of both HPV 31 E6 (s3 31E6) AND E7 (s3 31E7)	s3 31
	High levels of both HPV 33 E6 (s3 33E6) AND E7 (s3 33E7)	s3 33
	High levels of both HPV 35 E6 (s3 35E6) AND E7 (s3 35E7)	s3 35
	High levels of both HPV 45 E6 (s3 45E6) AND E7 (s3 45E7)	s3 45
	High levels of both HPV 52 E6 (s3 52E6) AND E7 (s3 52E7)	s3 52
	High levels of both HPV 58 E6 (s3 58E6) AND E7 (s3 58E7)	s3 58
Combined Standards	Either one of Standard 1 or Standard 2 or Standard 3	HPV$_{sero}$

Appendix Table 2. Comparison between HPV-positive grey zone and HPV-positive OPC

Covariate	Categories	HPV-positive OPSCC (n=737)	HPV-positive Discrepancy (n=28)	HPV-positive SCCUPHN (n=67)	p-value
Age	Mean (standard deviation)	60 (9.1)	58.7 (10.2)	59.2 (9.4)	0.82
	Median (Range)	59.4 (33.2,85.3)	59.4 (22.7,77.4)	59.6 (34.9,88.2)	
Sex	Female/Male	15%/85%	4%/96%	13%/87%	0.25
Marital status	Married/Never	92%/8%	96%/4%	96%/4%	0.54
BMI	Mean (sd)	27.4 (5.1)	26.3 (3.8)	28 (4.5)	0.16
	Median (Range)	26.7 (13.7,49)	25.9 (21.2,36.2)	27.8 (17,39.8)	
CCI	0/1/2/3+	73%/19%/6%/2%	71%/21%/4%/4%	78%/13%/4%/4%	0.54
Smoker	Current/Ex-smoker	20%/46%	29%/39%	28%/34%	0.31
	Never	34%	32%	37%	
Smoking pack-year	Mean (standard deviation)	25.5 (22.6)	16 (17.1)	22.5 (17.5)	0.10
	Median (Range)	20.9 (0.008,220)	10 (0.5,51)	20 (1,80)	
Alcohol Status	Current/Former	52%/27%	72%/11%	67%/17%	0.43
	Never	21%	17%	17%	
Alcohol Use	Heavy/Moderate	11%/16%	11%/29%	14%/9%	0.22
	Non or light drinker	72%	61%	77%	
Urban rural	R/U	16%/84%	18%/82%	21%/79%	0.53
Subsite	OPC:Tongue Base/Tonsil/Other	40%/58%/2%	77%/14%/5%	0%/0%/0%	<0.001
	Tongue	0%	5%	0%	
	Unknown primary	0%	18%	100%	
T-stage	T0/T1/T2	0%/19%/40%	21%/21%/25%	100%/0%/0%	<0.001
	T3/T4	26%/15%	14%/18%	0%/0%	
N-stage	N0/N1	6%/7%	7%/7%	0%/13%	0.25
	N2/N3	80%/6%	79%/7%	78%/9%	
M-stage	M0/M1	99%/1%	100%/0%	100%/0%	1.00
Clinical Stage	0/I/II	0%/0%/2%	0%/0%/7%	0%/0%/0%	0.47
	III/IV	9%/88%	7%/86%	12%/88%	
Follow-up time	Median (Range)	3.4 (0,9.8)	1.8 (0.1,8)	4.2 (0.2,8.4)	0.09
Treatment	CRT/RT/surgery only	68%/29%/4%	45%/52%/3%	59%/39%/1%	0.08
Chemo	No/Yes	41%/59%	32%/68%	55%/45%	0.06

Legend: for significance testing, Kruskal-Wallis test was used for continuous variables, while the chi-squared test was used for categorical variables.

Appendix Table 3. Comparisons among HPV-negative grey zone groups, oral cavity squamous-cell carcinoma and HPV-negative oropharyngeal squamous-cell carcinoma.

Covariate	Categories	HPV-negative SCCUPHN vs. OSCC			HPV-negative discrepancy vs. HPV-negative		
		HPV negative SCCUPHN (n=25)	OSCC (n=721)	p-value	HPV negative discrepancy (n=38)	HPV negative OPSCC (n=201)	p-value
Age	Mean (sd)	62.4 (9.9)	63.4 (12.5)	0.50	62.1 (10.4)	63.3 (10)	0.61
	Median (Range)	59.8 (46.8,87.1)	63.5 (21.8,96.4)		64.5 (38.5,81.7)	63.8 (33,88.5)	
Sex	Female/Male	16%/84%	34%/66%	0.09	34%/66%	25%/75%	0.23
Marital	Married/Never	84%/16%	92%/8%	0.13	92%/8%	85%/15%	0.31
BMI	Mean (sd)	27.1 (4.4)	25.8 (5.3)	0.09	24.8 (5.4)	24.1 (5.6)	0.29
	Median (Range)	26.6 (17.3,36.5)	25.1 (14.3,53.3)		24.1 (15.4,36)	23.2 (13.2,46.3)	
CCI	0/1/2/3+	68%/20%/8%/4%	61%/28%/8%/4%	0.82	74%/13%/8%/5%	58%/30%/8%/3%	0.11
Smoker	Current/ex-smoker	44%/32%	35%/32%	0.56	45%/42%	54%/36%	0.09
	Never	24%	33%		12%	9%	
Smoking pack-year	Mean (sd)	30.9 (14)	32.1 (22.9)	0.84	36.4 (31.9)	41 (25.2)	0.10
	Median (Range)	30 (4,55)	30 (0,125)		30 (1.9,150)	40 (0.6,145)	
Alcohol Status	Current/Former	56%/16%	53%/14%	0.85	66%/18%	52%/37%	0.07
	Never	28%	33%		16%	11%	
Alcohol Use	Heavy/Moderate	16%/24%	15%/12%	0.15	23%/22%	40%/16%	0.08
	Non- or light drinker	60%	73%		55%	44%	
Urban rural	R/U	12%/88%	11%/89%	0.75	11%/89%	13%/87%	0.80
Subsite	OPC: Tongue	-	-	Not applicable	17%/14%/22%	28%/41%/31%	**<0.001**
	OSCC: Tongue/other	0%/0%	48%/52%		31%/17%	0%/0%	
	Unknown primary	25	0		2	0	
T-stage	T0/T1/T2	100%/0%/0%	4%/32%/30%	**<0.001**	6%/25%/22%	0%/10%/30%	**0.002**
	T3/T4	0%/0%	13%/21%		33%/14%	30%/30%	
N-stage	N0/N1	0%/4%	57%/13%	**<0.001**	50%/6%	25%/12%	**0.03**
	N2/N3	68%/28%	29%/1%		42%/3%	56%/7%	
M-stage	M0/M1	92%/8%	98%/2%	0.07	95%/5%	97%/3%	0.63
Clinical Stage	0/I/II	0%/0%/0%	2%/26%/17%	**<0.001**	0%/17%/11%	0%/4%/11%	**0.01**
	III/IV	5%/95%	14%/41%		25%/47%	15%/69%	
Follow-up time	Median (Range)	2.0 (0.2,5.4)	3.2 (0,10.6)	0.09	1.7 (0.2,8)	3.3 (0.1,9.7)	0.35
Received Chemo	No/Yes	48%/52%	84%/16%	**<0.001**	76%/24%	66%/34%	0.26

Appendix Table 4. Multivariable analysis for overall survival among each comparison groups.

OPSCC HPV-positive vs. HPV-negative				HPV-positive OPSCC vs. HPV-positive Grey zone			
Covariate		**HR (95%CI)**	**p-value**	**Covariate**		**HR (95%CI)**	**p-value**
Group	HPV+ OPC	reference	**<0.001**	**Group**	HPV+ OPC	reference	0.35
	HPV- OPC	3.26 (2.38,4.46)			HPV+ Discrepancy	1.22 (0.52,4.42)	
					HPV+ SCCUPHN	0.81 (0.29,1.42)	
Age	per year increase	1.00 (0.99,1.02)	0.61	**Age**	per year increase	1.02 (1.00,1.05)	0.03
Stage	0/I/II	reference	<0.001	**Stage**	0/I/II	reference	0.16
	III/IV	3.21 (1.75,5.89)			III/IV	2.73 (0.66,11.25)	
Pack years	per pack-year increase	1.01 (1,1.01)	0.006	**Pack years**	per pack-year increase	1.01 (1.01,1.02)	<0.001
Chemo	NO	reference	<0.001	**Chemo**	NO	reference	0.0089
	YES	0.45 (0.33,0.62)			YES	0.59 (0.40,0.88)	
SCCUPHN: HPV-positive vs. HPV-negative				HPV-negative OPSCC vs. HPV-negative clinician-ICD coding discrepancy			
Group	HPV+ SCCUPHN	reference	**0.015**	**Group**	HPV- OPC	reference	0.19
	HPV- SCCUPHN	4.61 (1.34,15.9)			HPV- discrepancy	0.83 (0.43,1.5)	
Age at Dx	per year increase	1.02 (0.96,1.08)	0.45	**Age**	per year increase	1.00 (0.97,1.02)	0.67
Stage	0/I/II	not applicable as by definition, almost all SCCUPHN would be Stage III/IV		**Stage**	0/I/II	reference	<0.001
	III/IV				III/IV	2.84 (1.56,5.18)	
Pack years	per pack-year increase	0.99 (0.95,1.02)	0.44	**Pack years**	per pack-year increase	1.00 (1,1.01)	0.59
Chemo	NO	reference	0.69	**Chemo**	NO	reference	<0.001
	YES	0.79 (0.25,2.51)			YES	0.35 (0.21,0.59)	
Clinician-ICD coding discrepancy: HPV-positive vs. HPV-negative				OSCC vs. HPV-negative SCCUPHN			
Group	HPV+ Discrepancy	reference	0.18	**Group**	OSCC	reference	0.37
	HPV- Discrepancy	2.54 (0.78,4.32)			HPV- SCCUPHN	0.88 (0.30,1.76)	
Age	per year increase	1.1 (1.02,1.18)	0.01	**Age**	per year increase	1.03 (1.02,1.05)	<0.001
Stage	0/I/II	reference	0.92	**Stage**	0/I/II	reference	<0.001
	III/IV	0.93 (0.23,3.68)			III/IV	3.19 (2.33,4.37)	
Pack years	per pack-year increase	1.01 (0.99,1.02)	0.34	**Pack years**	per pack-year increase	1.01 (1,1.01)	<0.001
Chemo	NO	reference	0.98	**Chemo**	NO	reference	0.81
	YES	0.98 (0.19,5.04)			YES	1.05 (0.72,1.53)	

Appendix Table 5. Multivariable analysis for disease-free survival among each comparison groups.

OPSCC HPV-positive vs. HPV-negative				HPV-positive OPSCC vs. HPV-positive Grey zone			
Covariate		**HR (95%CI)**	**p-value**	**Covariate**		**HR (95%CI)**	**p-value**
Group	HPV+ OPC	reference	<0.001	**Group**	HPV+ OPC	reference	0.21
	HPV- OPC	3.25 (2.45,4.31)			HPV+ Discrepancy	1.22 (0.78,2.53)	
					HPV+ SCCUPHN	0.86 (0.47,1.35)	
Age	per year increase	1.01 (1.00,1.03)	0.12	**Age**	per year increase	1.03 (1.01,1.05)	0.005
Stage	0/I/II	reference	<0.001	**Stage2**	0/I/II	reference	0.15
	III/IV	3.14 (1.8,5.5)			III/IV	2.36 (0.74,7.52)	
Pack years	per pack-year increase	1.01 (1.00,1.01)	0.01	**Pack years**	per pack-year increase	1.01 (1.00,1.02)	0.001
Chemo	NO	reference	<0.001	**Chemo**	NO	reference	0.01
	YES	0.56 (0.42,0.74)			YES	0.64 (0.45,0.9)	
SCCUPHN: HPV-positive vs. HPV-negative				HPV-negative OPSCC vs. HPV-negative clinician ICD coding discrepancy			
Group	HPV+ SCCUPHN	reference	0.006	**Group**	HPV- DISCREPANCY	reference	0.34
	HPV- SCCUPHN	5.35 (1.68,17.8)			HPV- OPC	1.26 (0.70,2.33)	
Age	per year increase	1.03 (0.97,1.09)	0.32	**Age**	per year increase	1.00 (0.98,1.02)	0.74
Stage	0/I/II	not applicable as by definition, almost all SCCUPHN would be Stage III/IV		**Stage**	0/I/II	reference	<0.001
	III/IV				III/IV	2.66 (1.51,4.66)	
Pack years	per pack-year increase	0.99 (0.96,1.02)	0.43	**Pack years**	per pack-year increase	1.00 (1.00,1.01)	0.31
Chemo	NO	reference	0.99	**Chemo**	NO	reference	0.008
	YES	0.99 (0.33,3.02)			YES	0.55 (0.36,0.85)	
Clinician-ICD coding discrepancy: HPV-positive vs. HPV-negative				OSCC vs. HPV-negative SCCUPHN			
Group	HPV+ Discrepancy	reference	0.16	**Group**	HPV- SCCUPHN	reference	0.45
	HPV- Discrepancy	1.94 (0.72,3.42)			OSCC	1.14 (0.62,2.32)	
Age	per year increase	1.06 (1.00,1.13)	0.05	**Age**	per year increase	1.03 (1.02,1.04)	<0.001
Stage	0/I/II	reference	0.96	**Stage**	0/I/II	reference	<0.001
	III/IV	0.97 (0.29,3.23)			III/IV	2.64 (1.99,3.50)	
Pack years	per pack-year increase	1.01 (1.00,1.02)	0.25	**Pack years**	per pack-year increase	1.01 (1.00,1.01)	0.01
Chemo	NO	reference	0.91	**Chemo**	NO	reference	0.77
	YES	1.08 (0.30,3.93)			YES	1.05 (0.74,1.5)	

Appendix Figure 1. Summary for the association between oropharyngeal squamous cell (OPSCC), oral cavity squamous cell carcinoma (OSCC), and grey zone cases (Clinician-ICD discrepant coding and squamous cell carcinoma of unknown primary of the head and neck region, SCCUPHN) by HPV status.