ANTICANCER RESEARCH *41*: 269-277 (2021)
doi:10.21873/anticanres.14773

Brush Samples of Oral Lesions to FTA Elute Card for High-risk Human Papilloma Virus Diagnosis

CHRISTINA RUNOW STARK[1,2], INGER GUSTAVSSON[3], PETER HORAL[4], MARIA KOTOPOULI[5], ULF GYLLENSTEN[3] and JAN-MICHAÉL HIRSCH[1,2]

[1]*Department of Research & Development, Folktandvården Stockholms län AB, Stockholm, Sweden;*
[2]*Department of Surgical Sciences, Oral & Maxillofacial Surgery, Medical Faculty, Uppsala University, Uppsala, Sweden;*
[3]*Department of Immunology, Genetics and Pathology, Medical Genetics and Genomics, Uppsala University, Uppsala, Sweden;*
[4]*Department of Clinical Virology, Sahlgrenska University Hospital, Gothenburg, Sweden;*
[5]*Division of Biostatistics, Institute of Environmental Medicine, Karolinska Institute, Solna, Sweden*

Abstract. *Aim: To investigate the level of agreement between three non-invasive methods for hrHPV diagnosis in oral and oropharyngeal squamous cell carcinoma (OSCC, OPSCC) and in oral mucosal lesions. Materials and Methods: For hrHPV DNA FTA Elute card™ and Anyplex II HPV28™ were used and for hrHPV mRNA PreTect SEE™ in tumour patients (n=60), non-tumour lesions (n=51), immunosuppression or previous hrHPV-infection (n=32). Results: The level of agreement between the DNA-methods was 82.2% (k=0.54, p=0.001). Pair-wise comparison for the FTA Elute card were close to the reference (AUC=0.83, 95% CI=0.73-0.90). hrHPV mRNA was diagnosed in 50% of the tumours, with an agreement level of 58.3%, compared to Anyplex II (k=0.17, p=0.04). The hrHPV positivity in oral lesions was 3.9% for immunosuppression and for previous HPV infection 9.4%. Conclusion: The FTA card is reliable for hrHPV DNA diagnosis while mRNA gives an insight into viral activity and correlates with severity of the lesion.*

The intention with this study was to evaluate the efficacy of non-invasive brush methods for diagnosis of hrHPV in oral- and oropharyngeal lesions. In 2018 the global burden of oral cancer was 280,000 and that of oropharyngeal cancer 140,000, whereof 5,900 and 42,000 were attributable to hrHPV infection respectively (1). This compared to the incidence of cervical cancer in 2018 which accounted for 570,000 cases, with more than 90% caused by high-risk human papillomavirus (hrHPV) and existing screening programs (2). In Sweden, the number of oral and oropharyngeal cancer, ~1,000 cases yearly, exceeds the incidence of cancer in cervix uteri ~528 cases, in lack of screening program (3-5). Between 2008 to 2017 the incidence of oropharyngeal cancer in Sweden increased by 65% and that of oral cancer by 26% predominantly caused by hrHPV related oropharyngeal carcinomas (6), particularly in the tonsils and at the base of the tongue (7-13). In addition, hrHPV infections have also been argued to be associated with the malignant transformation of the oral mucosa as HPV is recognized as the cause of precancerous and cancerous cervical lesions (14-16). The IARC concluded that in the oral cavity there is enough evidence of the carcinogenicity of HPV16, but only limited evidence for HPV18 (17). One of the key questions is whether hrHPV is the driver of the malignant transformation of potentially malignant oral disease (PMOD). The prevalence of reported hrHPV PMOD varies extensively from 0% to 53% (18-21). Precursor lesions such as the PMOD oral leukoplakia developed to OSCC in 12% during a 7.5 years follow-up period according to Holmstrup *et al.* (22). The role of hrHPV in malignant transformation of PMOD is still debated. HPV infection of the oral mucosa is reported to be a frequent finding *e.g.* in youth, similar among males and females (9.3% *vs.* 9.8%), but higher for females with (17.1%) than without (4.4%) cervical HPV infection (23). Subclinical chronical oral human HPV infection is suspected to be the main factor for development of squamous cell carcinoma of the head and neck. This is a remaining research question to

Correspondence to: Christina Runow Stark, DDS, Ph.D., Department of Maxillofacial Surgery and Orofacial Medicine, Blekingesjukhuset Karlskrona, SE371 41 Karlskrona, Sweden. Tel: +46 455731088, e-mail: christina.runowstark@regionblekinge.se

Key Words: FTA Elute card, oral- and oropharyngeal cancer, potentially malignant oral disorders, human papillomavirus, hrHPV, real-time PCR, DNA, mRNA.

Table I. *Descriptive patient data*.

Category	Group I % (n)	Group II % (n)	Group III % (n)	All groups % (n)
Number included	60	51	32	143
Age, years (m±sd)	62.42±9.43	61.12 ±12.88	44.34±14.07	57.90±13.87
Gender				
Men	73.33 (44)	41.18 (21)	68.75 (22)	60.84 (87)
Women	26.67 (16)	58.82 (30)	31.25 (10)	39.16 (56)
Tobacco users, n(%)	20.00 (12)	13.70 (7)	15.60 (5)	17.00 (24)
Diagnosis				
Tonsillar scc	66.67 (40)	.	.	27.97 (40)
Base of tongue scc	26.67 (16)	.	.	11.19 (16)
Scc, other surface	6.67 (4)	.	.	2.80 (4)
Dysplasia	.	19.61 (10)	.	6.99 (10)
Leukoplakia	.	31.37 (16)	9.38 (3)	13.29 (19)
Follicular epithelial hyperplasia	.	1.96 (1)	9.38 (3)	2.80 (4)
Healthy mucosa	.	1.96 (1)	59.37 (19)	13.99 (20)
Oral lichen planus	.	43.13 (22)	6.25 (2)	16.78 (24)
Papilloma	.		9.38 (3)	2.10 (3)
Syphilis	.	.	3.13 (1)	0.70 (1)
Verruca	.	1.96 (1)	3.13 (1)	1.40 (2)

scc; Squamous cell carcinoma.

clarify the steps between initial infection and a later possible development of cancer (24). An extensive number of the HPV infections heal as shown by a meta-regression analysis that estimated the 12-month cumulative incidence of oral HPV to be 4.8% and clearance for HPV16 was 43-83% from 7 to 22 months but with extensive variability (25). Since hrHPV infections have been implicated to be a contributing factor for malignant transformation in the oral cavity it is of interest to elucidate methods for oral HPV diagnosis and possible viral genome integration in the host cell. This work reports on the level of agreement between different methods for hrHPV DNA and mRNA diagnosis in hrHPV-positive OSCC and OPSCC cancers in oral mucosal lesions and clinically healthy oral mucosa.

Patients and Methods

Study population. A total of 152 patients treated at the Department of Orofacial Medicine at Södersjukhuset, Stockholm, Sweden, between October 2015 and October 2019, were recruited for this study. However, 9 patients with tumours without hrHPV were excluded later. The final 143 consisted of 39.2 % women (n=56) and 60.8 % men (n=87), with a mean age of 57.9 years (SD±13.87) (Table I). Informed consent was obtained from all subjects. The patients were categorized into groups (Figure 1).

Group I. Control group (n=60): hrHPV positive OSCC or OPSCC consecutively referred by the Department of Oncology, Karolinska University Hospital with a mean age of 62.4 (SD±9.43), 73% (n=44) were men and 27% (n=16) women. Tumour diagnosis was tonsillar 66,7% (n=40); base of tongue 26,7% (n=16); and lateral border of tongue or floor of mouth 6,7% (n=4); (Table I).

Group II. Patients with PMOD, ulceration, and follicular epithelial hyperplasia (n=51) referred by general dental practitioners (GDP) with a mean age of 61.1 years (SD±12.88), 41.2% were men (n=21) and 58.8% women (n=30). The clinical diagnoses were oral lichen planus 43.1% (n=22), leukoplakia 31.4% (n=16); dysplasia 19.6% (n=10); follicular epithelial hyperplasia 1,9% (n=1); ulceration in healthy mucosa 2% (n=1); and verruca 2% (n=1), all histologically examined; (Table I).

Group III. Patients previously diagnosed with either HPV positive ano-genital condyloma or immunosuppression due to HIV, or cervix dysplasia, referred by the Department of Infectious Diseases and the Department of Obstetrics and Gynecology, at Södersjukhuset (n=32). The mean age was 44.3 years, (SD±14.07), 69% whereof men (n=22) and 31% women (n=10). Oral lesions were observed in 40.6 % (n=13) of the patients with the following clinical diagnoses: oral leukoplakia 9.4% (n=3); follicular epithelial hyperplasia 9.4% (n=3); papilloma 9.4% (n=3); oral lichen planus 6.2% (n=2); syphilis 3% (n=1); and verruca 3% (n=1), all histologically examined; (Table I).

Prior HPV analysis of Group I. HPV analyses (n=60) conducted prior to inclusion were performed at the Department of Pathology and Cytology, Karolinska University Hospital, with 2 different methods randomly. Positivity for hrHPV was confirmed using $p16^{INK4a}$ immunohistochemistry and with a real-time PCR assay with the Cobas® 4800 (Roche molecular systems, 4300 Hacienda Rd., Pleasanton, CA 94588, USA) that tested for 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), the most prevalent HPV16 and 18 were detected as single genotypes while the other types were reported as a group denoted by 'Other HPV types'.

Sample collection. Brush samples were obtained from Groups I, II and III plus healthy contralateral mucosa of all patients and a punch

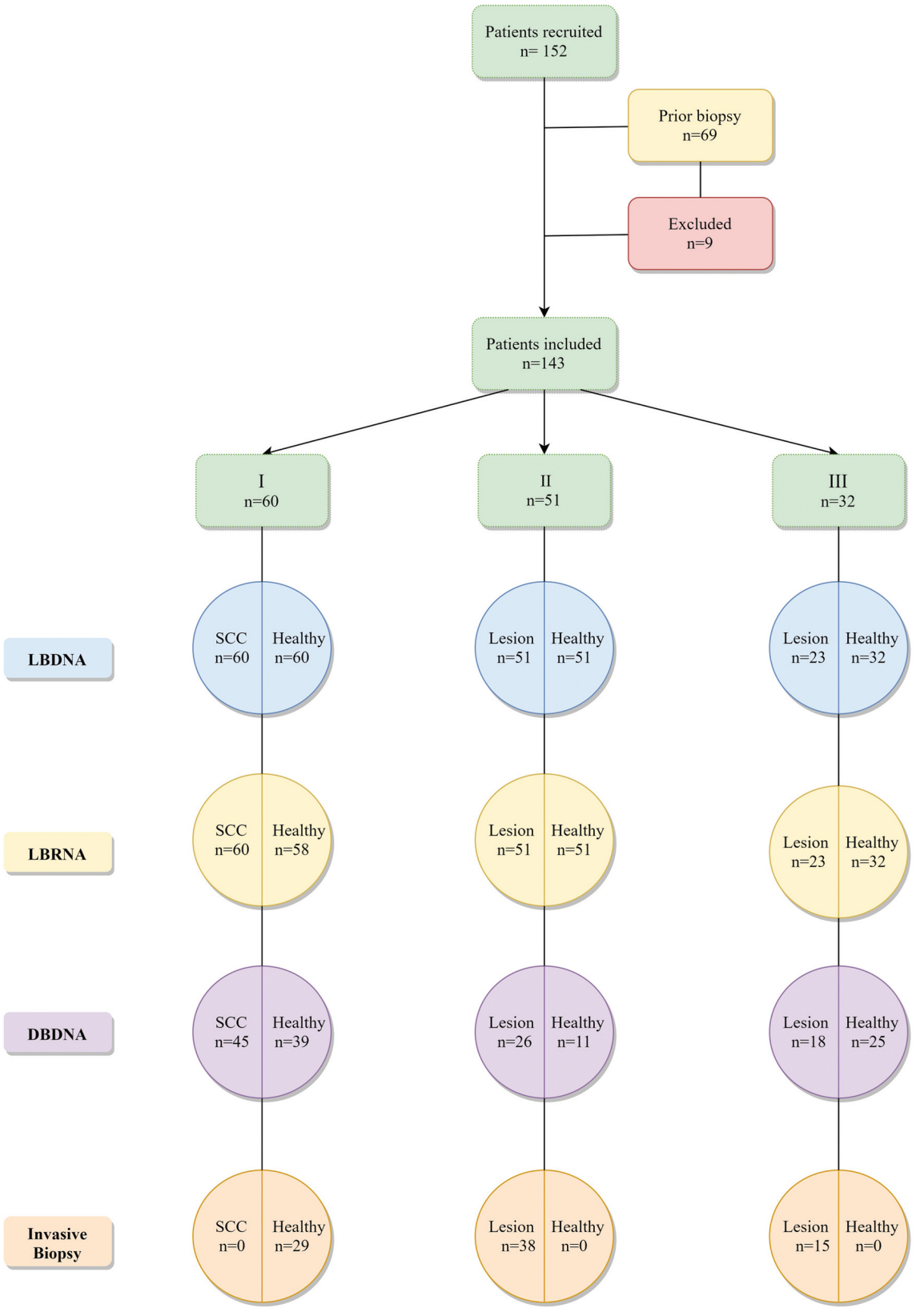

Figure 1. *Flow chart*.

Table II. *Prevalence of different hrHPV-types among tumour samples.*

Method:	Number, n	HPV16, single % (n)	HPV16+other % (n)	Other hrHPV, except HPV16 % (n)
All methods	60	80.0 (48)	10.0 (6)	10.0 (6)
Liquid-base DNA	60	83.3 (50)	10.0 (6)	5.0 (3)
Dry-base DNA	45	60.0 (27)	1.6 (1)	6.7 (3)
Liquid-base RNA	60	50.0 (30)	0 (0)	0 (0)
Standard:				
Prior tumour biopsy: p16	27	Indicative	-	-
Prior tumour biopsy: PCR	33	100 (33)	0	0

hrHPV: High-risk HPV.

Table III. *hrHPV detection per group and method.*

Group	Invasive biopsy % (n)	Brush LBDNA % (n)	Brush DBDNA % (n)	Brush LBRNA % (n)
I: Tumour site	100 (60)	85.0% (51)	66.7% (30)	50.0% (30)
Number tested	60	60	45	60
I: Healthy site	0%	41.7% (25)	25.6% (10)	5.2% (3)
Number tested	29	60	39	58 (2 invalid)
II: Lesion	0%	0%	7.7% (2)	0%
Number tested	46	51	26	51
II: Healthy	0%	2% (1)	0%	0%
Number tested	46	51	11	51
III: Lesion	0%	4.3% (1)	0%	0%
Number tested	15	23	18	23
III: Healthy	0%	9.4% (3)	0%	0%
Number tested	15	32	25	32

hrHPV: High-risk HPV; LBDNA: liquid-base DNA; DBDNA: dry-base DNA; LBRNA: liquid-base RNA.

biopsy (5 mm Ø) from each site, with the exception of already diagnosed OSCC or OPSCC, and sites in contralateral non-tumour tonsils or base of tongue.

Liquid base DNA (LBDNA). Cells collected were transferred to a sterile test tube containing 1 ml of sterile physiological saline (9mg/ml) and subsequently analyzed for HPV using a DNA-array based HPV assay (Anyplex 28™ II HPV28 Detection, Seegene) at the Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden. Anyplex 28™ assay is based on Tagging Oligonucleotide Cleavage and Extension (TOCE TM) technology (26).

Liquid base RNA (LBRNA). Samples were collected using an Isohelix SK-3S brush (Cell Projects Ltd, Harrietsham, Kent, ME17 1AB, UK) and preserved in a standard commercially available methanol-buffer solution (PreTect™), and sent to PreTect AS, Klokkarstua, Norway. PreTect® SEE is a real-time nucleic acid amplification and detection assay used for qualitative determination of E6/E7 viral messenger RNA (mRNA) from the 3 most prevalent carcinogenic HPV types; 16, 18 and 45 in a single analysis (27).

Dry base DNA (DBDNA). Indicating FTA Elute micro cards (art. No WB129308, GE Healthcare, Cardiff CF14 7YT, UK) were used for dry collection, transportation and storage of brush samples. Indicating FTA elute cards contain chemicals that lyse cells, denature proteins and protect, immobilizes and stabilizes nucleic acids, thereby making the samples non-infectious. FTA cards were processed using a dedicated automated laboratory system (easyPunch STARlet; Hamilton Robotics, Bonaduz, Switzerland). The system collects each card, acquires a photographic image of the sample collection area and uses machine learning software to identify parts of the sampling deposition area containing the highest amount of cellular material. Using a 3-mm Ø knife, punches were taken from the area containing most material and deposits and placed in a single well of a 96-well microtiter plate. DNA extraction from the punches was performed as described earlier (28). HPV testing was performed using a real-time PCR-based hpVIR assay (28,29). Briefly, this test detects and quantifies the following HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. It also measures a human single copy gene (*HMBS*), which serves as a control. For this purpose, the samples must contain sufficient amounts of cellular material so that the test would be

Table IV. *Kappa agreement test results for control, tumour and contralateral mucosa groups.*

Group/Method	Agreement %	Kappa coefficient	*p*-value
I: Control tumour			
LBDNA *vs*. DBDNA	82.22	0.54	<0.001
DBDNA *vs*. LBRNA	66.67	0.38	0.001
LBDNA *vs*. LBRNA	58.33	0.17	0.04
I: Control contralateral			
LBDNA *vs*. DBDNA	69.23	0.42	0.001
DBDNA *vs*. LBRNA	78.38	0.27	0.01
LBDNA *vs*. LBRNA	63.79	0.14	0.02

LBDNA: Liquid-base DNA; DBDNA: dry-base DNA; LBRNA: liquid-base RNA.

Table V. *ROC analysis of tumour sample sites. Comparison of combined curves in the diagnosis of hrHPV.*

Method	Number tested	AUC	95% CI	Sensitivity	Specificity
I: Control					
Lesion					
LBDNA	60	0.93	0.83-0.98	86.44%	100%
DBDNA	45	0.84	0.71-0.94	68.18%	100%
LBRNA	60	0.75	0.62-0.85	50.85%	100%
Gold standard ROC			*p*-Value		
LBDNA *vs*. DBDNA	39	0.83	0.03		

LBDNA: Liquid-base DNA; DBDNA: dry-base DNA; LBRNA: liquid-base RNA.

informative. The limit of detection for both *HMBS* and HPV was set to 10 copies per PCR.

Fresh frozen tissue sample for PCR analysis. Punch biopsies (5 mm Ø) of Group II and contralateral healthy oral mucosa were immediately submerged in RNAlater™ stabilization solution (Invitrogen, Thermo Fischer Scientific, Stockholm, Sweden). Thereafter, the samples were frozen at –20˚C and analyzed at the Department of Clinical Virology, Sahlgrenska University Hospital, Gothenburg, Sweden. A real-time TaqMan PCR assay targeting 12 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and 2 low-risk HPV types (6 and 11) was used for the identification of HPV DNA. Real-time PCR for the human betaglobin gene was performed in parallel for sample and amplification quality control; only samples with Ct-values below 35 were included (30).

Statistical analysis. Categorical data were expressed as numbers and percentages, while continuous data were expressed as mean±standard deviation. Descriptive values and differences were calculated using the Kruskal-Wallis rank test. Cohen's κ (kappa) was used to evaluate agreement between sample sites for pairwise comparisons. A k <0.40 signifies poor agreement, a k from 0.40-0.75 signifies fair to good agreement and a k>0.75 signifies excellent agreement. Receiver Operator Characteristic (ROC) curve and area under the curve (AUC) analyses were performed to assess the diagnostic value of DBDNA. The statistical package StataCorp. 2017, Stata Statistical. Software, Release 15 (College Station, TX, USA) and StataCorp LLC® for Windows was used. Statistical significance was set at <0.05.

Ethical approval. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Review Board Stockholm Sweden (2015-1213-31).

Results

Prevalence of hrHPV in group I. The hrHPV16 type was found in 90% (n=54) of tumour sample sites, whereof 10% (n=6) of cases in combination with other hrHPV, followed by single representations of hrHPV33 (n=3), hrHPV 35 (n=1), hrHPV53 (n=1) and hrHPV54 (n=1); (Table II). Positives detected for hrHPV among tumour sites was highest for LBDNA at 85% (n=51), followed by 66.7% (n=30) for DBDNA and 50% (n=30) for LBRNA. The rate of hrHPV positives detected for contralateral healthy mucosa was highest for LBDNA with 41.7% (n=25) followed by 25.6% for DBDNA (n=10) and 5.2% (lowest) for LBRNA (n=3); (Table III).

Prevalence of hrHPV for group II. Overall hrHPV findings were low in group II with only 7.7% (n=2) positive cases for lesion sites detected via DBDNA, of which one patient with dysplasia presented a positive value for the healthy sample site tested with LBDNA); (Table III).

Prevalence of hrHPV for group III. LBDNA detected hrHPV positives in the healthy contralateral mucosa of 9.4% (n=3) of Group III patients of which one patient with oral leukoplakia was positive for both lesions and healthy sites (Table III).

Results from fresh frozen invasive biopsies for PCR analysis. PCR analysis did not detect any clinically relevant levels of hrHPV among the incisional biopsies of healthy oral mucosa, lesions or PMOD (Table III).

HPV detection agreement for the different sampling methods. All pairwise agreements between the sampling methods for all participants were calculated, using the kappa coefficient. For tumour sites, the kappa based level of agreement between DBDNA and LBDNA was 82.22% (k=0.54, p=0.001) followed by that between LBRNA and LBDNA, which was 58.33% (k=0.17, p=0.04). The agreement between DBDNA and LBDNA was characterized as "fair" whereas the agreement between LBRNA and LBDNA was characterized as "poor". Both agreements were statistically

significant. The level of agreement between DBDNA and LBRNA was 66.67% (k=0.38, p=0.001) and characterized as "poor" (Table IV).

Similar results were obtained for contralateral healthy sites. Kappa for the level of agreement between DBDNA and LBDNA was 69.23% (k=0.42, p=0.001) followed by that between LBRNA and LBDNA which was 63.79% (k=0.14, p=0.02). The agreement between DBDNA and LBDNA was characterized as "fair" and the agreement between LBRNA and LBDNA was characterized as "poor". Both agreements were statistically significant. The agreement between DBDNA and LBRNA was the highest for these sites with 78.38% (k=0.27, p=0.001). However, it was characterized as "poor" (Table IV).

Diagnostic value of sampling methods using ROC analysis. ROC analysis was used to test the accuracy and predictability of tumours verified as hrHPV-positive. The first indicative HPV-test was performed on tissues excised via a biopsy prior to oral brush sampling. LBDNA showed the highest accuracy for detecting HPV (AUC=0.93, 95% CI=0.83-0.98), with a sensitivity of 86.44%. The DBDNA method showed good predictability (AUC=0.84, 95% CI=0.71-0.94), with a sensitivity of 68.18%. The LBRNA method resulted in a lower detection rate of HPV-mRNA with a sensitivity of 50.85%, indicating that the accuracy of LBRNA was lower compared to that of DNA-methods (AUC=0.75, 95% CI=0.62-0.85). All 3 methods showed 100% specificity. The difference between the 2 ROC curves was statistically tested and showed a p-value of 0.03, which proved that LBDNA was the most predictive for HPV (Table V).

Discussion

The FTA card has been reported to contain the amount of genomic DNA (>10 copies of a single copy gene) that is sufficient for HPV typing (27) and we found a sensitivity of the FTA card to be comparable to that of the LBDNA method. This result is substantiated by previous reports that used the FTA card for detecting high-grade cervical intraepithelial neoplasia (29, 31). The current prospective study had limited options to compare the different methods, because the prevalence of HPV in the non-tumour lesions was low. However, with optimization of workflow, FTA card-based sampling may be used for hrHPV diagnosis and continuous clinical control of oral cavity lesions (32, 33). We surmised that self-sampling of the oral cavity can be introduced using FTA technology, as shown by repeated sampling of patients with potentially malignant cervical lesions, which has been proven to be practical and well accepted (34-36). Furthermore, dry FTA cell sampling technology is an easy system to use in primary care, an effective, reliable and sensitive method for transporting and storing of specimens (37).

The results of our LBRNA assay showing 50% HPV in OPSCC were in accordance with those of a previous study by Koneva *et al.* (15). Previous *in situ* hybridization results for the detection of hrHPV E6/E7 mRNA in OPSCC were found to be highly specific, sensitive, and clinically relevant to the de-escalated treatment approach that was used for retesting p16-positive tumours (38). The prevailing consensus on cervical cancer is that upregulated expression of E6/E7 allows insight into viral activity and correlates well with severity of the lesion (39). Detection of mRNA transcripts may, therefore, be a better indicator of the increased risk for progression to neoplasia than HPV DNA (40-42). However, in HPV-positive OPSCC, the incidence of viral integration is reportedly lower, and many tumours contain either extrachromosomal or mixed extrachromosomal and integrated viral DNA (43-46). Whether HPV is a driver of malignant transformation of oral cavity lesions such as PMOD is debatable. The number of infected oral lesions and in clinical healthy oral mucosa in our study was 3.9%. Our records are substantiated by the earlier findings by Sundberg *et al.*, who reported that none of the 74 oral leukoplakia cases were found to be infected (21). This contradicts the results of several previous studies that reported the presence of a significant number of HPV infections in OSCC and in PMOD (14, 19). Pierangeli *et al.* reported that 53.2% of patients with PMOD were HPV-infected, with HPV16 and 18 being the most frequently detected subtypes (20). The significant levels of variance in prevalence are most probably due to the quality of samples and the geographical region from which the samples were obtained (32, 47). In clinical practice $p16^{INK4a}$ is frequently used as a biomarker of HPV. However, a major issue arises since overexpression of p16 does not correlate with HPV infection in PMOD, OSCC or cervical carcinoma, the latter being one of the main causes for variation in HPV prevalence (14, 21). The large variation in age of patients between studies, as well as the methodology used for sampling and analysis, may also account for these differences (48). In the case of large tumours, cells may contaminate healthy sample sites that account for HPV positivity in healthy contralateral mucosa of the control group. The fact that brush samples from the oral cavity mucosa came from lesions obtained from various areas with different surface layers, some of which were keratinized, may explain the difficulties encountered in acquiring representative samples. Della Vella *et al.* (49) found the HPV prevalence in incisional biopsies of oral leukoplakia to be 17% compared to 5% in brush biopsies analyzed with the same method as in our study. Their result differs significantly from ours as we found a level of agreement between the two DNA-methods to be 82.2% (k=0.54, p=0.001). This indicates that the brush sampling technique may need to be adjusted to fit both the region of interest and the tentative tumour diagnosis.

Conclusion

The FTA card is a robust and reliable method for detecting hrHPV DNA in potentially malignant oral disorders, as well as post treatment follow-up of OSCC and OPSCC. However, since the prevailing consensus is that upregulated expression of E6/E7 allows insight into viral activity and correlates with severity of the lesion, detection of mRNA transcripts is suggested to be a better indicator of the increased risk for progression to neoplasia.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization: CRS, IG, PH, UG, JMH. Data curation: CRS, IG, PH, MK, UG, JMH. Formal analysis: CRS, IG, PH, MK, UG, JMH. Funding acquisition: CRS, IG, PH, UG, JMH. Investigation: CRS, IG, PH, UG, JMH. Methodology: CRS, IG, PH, UG, JMH. Project administration: CRS, JMH. Resources: CRS, IG, PH, UG, JMH. Supervision: CRS, JMH. Validation: CRS, IG, PH, MK, UG, JMH. Writing of draft: CRS, IG, PH, MK, UG, JMH. Writing and editing: CRS, IG, PH, MK, UG, JMH.

Acknowledgements

This study was supported by Region Uppsala; the Thuréus Foundation (Grant number 463349110), Region Stockholm (SOF 4-640/2016) and Folktandvården Stockholms län AB (Grant number 7072) for the conduct of the research.

References

1 Martel C, Georges D, Bray F, Ferlay J and Clifford GM: Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health *8(2)*: e180-90, 2020. PMID: 31862245. DOI: 10.1016/S2214-109X(19)30488-7

2 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *68(6)*: 394-424, 2018. PMID: 30207593. DOI: 10.3322/caac.21492

3 Globocan 2018, Sweden. Available at: https://gco.iarc.fr/today/data/factsheets/populations/752-sweden-fact-sheets.pdf [Last accessed on October, 2020]

4 The Swedish cancer register, Socialstyrelsen Sweden. Available at: https://sdb.socialstyrelsen.se/if_can/val_eng.aspx [Last accessed on May 5, 2019]

5 Cancer statistics for the Nordic countries. Available at: https://www-dep.iarc.fr/nordcan/sw/frame.asp [Last accessed on March 26, 2019]

6 The Swedish head and neck cancer register. Swe HNCR. Available at: https://www.cancercentrum.se/globalassets/cancerdiagnoser/huvud-och-hals/kvalitetsregister/presentation-in-english-swehncr.pdf [Last accessed on September 4, 2020]

7 Attner P, Du J, Nasman A, Hammarstedt L, Ramqvist T, Lindholm J, Marklund L, Dalianis T and Munck-Wikland E: The role of human papillomavirus in the increased incidence of base of tongue cancer. Int J Cancer *126(12)*: 2879-2884, 2010. PMID: 19856308. DOI: 10.1002/ijc.24994

8 Kreimer AR, Clifford GM, Boyle P and Franceschi S: Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev *14(2)*: 467-475, 2005. PMID: 15734974. DOI: 10.1158/1055-9965.EPI-04-0551

9 Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Dahlgren LO, Joneberg J, Creson N, Lindholm J, Ye W, Dalianis T and Munck-Wikland E: Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. Int J Cancer *119(11)*: 2620-2623, 2006. PMID: 16991119. DOI: 10.1002/ijc.22177

10 Hammarstedt L, Dahlstrand H, Lindquist D, Onelov L, Ryott M, Luo J, Dalianis T, Ye W and Munck-Wikland E: The incidence of tonsillar cancer in Sweden is increasing. Acta Otolaryngol *127(9)*: 988-992, 2007. PMID: 17712680. DOI: 10-1080/00016480601110170

11 Gillison ML, Chaturvedi AK, Anderson WF and Fakhry C. Epidemiology of human papillomavirus–positive head and neck squamous cell carcinoma. J Clin Oncol *33(29)*: 3235-3242, 2015. PMID: 26351338. DOI: 10.1200/JCO.2015.61.6995

12 Chaturvedi AK: Epidemiology and clinical aspects of HPV in head and neck cancers. Head Neck Pathol *6(Suppl 1)*: 16-24, 2012. PMID: 22782220. DOI: 10-1007/s12105-012-0377-0

13 Jia WH and Qin HD: Non-viral environmental risk factors for nasopharyngeal carcinoma: a systematic review. Semin Cancer Biol *22(2)*: 117-126, 2012. PMID: 22311401. DOI: 10.1016/j.semcancer.2012.01.009

14 Syrjanen S, Lodi G, von Bültzingslöwen I, Aliko A, P Arduino P, Campisi G, Challacombe S, Ficarra G, Flaitz C, Zhou HM, Maeda H, Miller C and Jontell M: Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis *17(Suppl 1)*: 58-72, 2011. PMID: 21382139. DOI: 10.1111/j.1601-0825.2011.01792.x

15 Koneva LA, Zhang Y, Virani S, Hall PH, McHugh JB, Chepeha DB, Wolf GT, Carey TE, Rozek LS and Sartor MA: HPV integration in HNSCC correlates with survival outcomes, immune response signatures, and candidate drivers. Mol Cancer Res *16(1)*: 90-102, 2018. PMID: 28928286. DOI: 10.1158/1541-7786.MCR-17-0153

16 Nkili-Meyong AA, Moussavou-Boundzanga P, Labouba I, Koumakpayi IH, Jeannot E, Descorps-Declère S, Xavier Sastre-Garau X, Leroy EM, Belembaogo E and Berthet N: Genome-wide profiling of human papillomavirus DNA integration in liquid-based cytology specimens from a Gabonese female population using HPV capture technology. Sci Rep *9(1)*: 1504, 2019. PMID: 30728408. DOI: 10.1038/s41598-018-37871-2

17 Human papillomaviruses. IARC monographs on the evaluation of carcinogenic Risks to humans, Vol 90, pp. 222-230, 2007.Available at: https://publications.iarc.fr/108 [Last accessed Dec 2007]

18 Chen X and Zhao Y: Human papillomavirus infection in oral potentially malignant disorders and cancer. Arch Oral Biol *83*: 334-339, 2017. PMID: 28886585. DOI: 10.1016/j.archoralbio.2017.08.011

19 Syrjanen SM, Syrjanen KJ and Happonen RP: Human papillomavirus (HPV) DNA sequences in oral precancerous

lesions and squamous cell carcinoma demonstrated by *in situ* hybridization. J Oral Pathol *17(6)*: 273-278, 1988. PMID: 2849647. DOI: 10.1111/j.1600-0714.1988.tb01536.x

20 Pierangeli A, Cannella F, Scagnolari C, Gentile M, Sciandra I, Antonelli G, CiolfiC, Russo C, Palaia G, Romeo U and Polimeni A: Frequent detection of high human papillomavirus DNA loads in oral potentially malignant disorders. Clin Microbiol Infect *22(1)*: 95.e9-95e15, 2016. PMID: 26408278. DOI: 10.1016/j.cmi.2015.09.011

21 Sundberg J, Korytowska M, Burgos MP, Blomgren J, Blomstrand L, De Laras S, Sand L, Hirsch JM, Holmberg E, Giglio D, Öhman J, Kovács A, Horal P, Lindh M, Kjeller G and Hasséus B: Combined testing of p16 tumour-suppressor protein and human papillomavirus in patients with oral leukoplakia and oral squamous cell carcinoma. Anticancer Res *39(3)*: 1293-1300, 2019. PMID: 30842161. DOI: 10.21873/anticanres.13241

22 Holmstrup P, Vedtofte P, Reibel J and Stoltze K: Long-term treatment outcome of oral premalignant lesions. Oral Oncol 42(5): 461-474, 2006. PMID: 16316774. DOI: 10.1016/j.oraloncology.2005.08.011

23 Du J, Nordfors C, Ahrlund-Richter A, Sobkowiak M, Romanitan M, Näsman A, Andersson S, Ramqvist T and Dalianis T: Prevalence of oral human papillomavirus infection among youth, Sweden. Emerg Infect Dis *18(9)*: 1468-1471, 2012. PMID: 22932445. DOI: 10.3201/eid1809.111731

24 Kreimer AR, Chaturvedi AK, Alemany L, Devasena L, Anantharaman D, Bray F, Carrington M, Doorbar J, D'Souza G, Fakhry C, Ferris RL, Gillison M, Hayes ND, Hildesheim A, Huang SH, Kowalski LP, Lang Kuhs KA, Lewis Jr J, Lowy DR, Mehanna H, Ness A, Pawlita M, Pinheiro M, John Schiller J, Shiels MS, Tota J, Mirabello L, Warnakulasuriya S, Waterboer T, Westra W, Chanock S and Brennan P: Summary from an international cancer seminar focused on human papillomavirus (HPV)-positive oropharynx cancer, convened by scientists at IARC and NCI. Oral Oncol *108*: 104736, 2020. PMID: 32502860. DOI: 10.1016/j.oraloncology.2020.104736

25 Wood ZC, Bain CJ, Smith DD, Whiteman DC and Antonsson A: Oral human papillomavirus infection incidence and clearance: a systematic review of the literature. J Gen Virol *98(4)*: 519-526, 2017. PMID: 28150575. DOI: 10.1099/jgv.0.000727

26 Rollo F, Donà MG, Pichi B, Pellini R, Covello R and Benevolo M: Evaluation of the Anyplex II HPV28 Assay in the detection of human papillomavirus in archival samples of oropharyngeal carcinomas. Arch Pathol Lab Med *144(5)*: 620-625, 2020. PMID: 31509455. DOI: 10.5858/arpa.2019-0199-OA

27 Cuschieri K, Hardie A, Hovland S, Hoaas B, Karlsen F and Cubie H: Comparison of the sensitivities of three commercial assays for detection of the high risk HPV types 16,18 and 45. J Virol Methods *193*: 147-150, 2013. PMID: 23727117. DOI: 10.1016/j.jviromet.2013.05.013

28 Gustavsson I, Lindell M, Wilander E, Strand A and Gyllensten U: Use of FTA card for dry collection, transportation and storage of cervical cell specimen to detect high-risk HPV. J Clin Virol *46(2)*: 112-1166, 2009. PMID: 19628427. DOI: 10.1016/j.jcv.2009.06.021

29 Wolfgramm EV, de Carvalho FM, Aguiar VR, Sartori MP, Hirschfeld-Campolongo GC, Tsutsumida WM and Louro ID: Simplified buccal DNA extraction with FTA elute cards. Forensic Sci Int Genet *3(2)*: 125-127, 2009. PMID: 19215882. DOI: 10.1016/j.fsigen.2008.11.008

30 Lindh M, Gorander S, Andersson E, Horal P, Mattsby-Balzer I and Ryd W: Real-time taqman pcr targeting 14 human papilloma virus types. J Clin Virol *40(4)*: 321-324, 2007. PMID: 17981499. DOI: 10.1016/j.jcv.2007.09.009

31 Wang SM, Hu SY, Chen F, Chen W, Zhao FH, Zhang YQ, Ma XM and Qiao YL: Clinical evaluation of human papillomavirus detection by careHPV™ test on physician-samples and self-samples using the indicating FTA Elute® card. Asian Pac J Cancer Prev *15(17)*: 7085-7089, 2014. PMID: 25227795. DOI: 10.7314/apjcp.2014.15.17.7085

32 Kreimer AR, Shiels MS, Fakhry C, Johansson M, Pawlita M, Brennan P, Hildesheim A and Waterboer T: Screening for human papillomavirus-driven oropharyngeal cancer: considerations for feasibility and strategies for research. Cancer *124(9)*: 1859-1866, 2018. PMID: 29499070. DOI: 10.1002/cncr.31256

33 Alsarraf AH, Kujan O and Farah CS: The utility of oral brush cytology in the early detection of oral cancer and oral potentially malignant disorders: A systematic review. J Oral Pathol Med *47*: 104-116, 2018. PMID: 29130527. DOI: 10-1111/jop.12660

34 Gyllensten U, Sanner K, Gustavsson I, Lindell M, Wikstrom I and Wilander E: Short-time repeat high-risk HPV testing by self-sampling for screening of cervical cancer. Br J Cancer *105(5)*: 694-697, 2011. PMID: 21811250. DOI: 10.1038/bjc.2011.277

35 Lindström AK, Hermansson RS, Gustavsson I, Hedlund Lindberg J, Gyllensten U and Olovsson M: Cervical dysplasia in elderly women performing repeated self-sampling for HPV testing. PLoS One *13(12)*: e0207714, 2018. PMID: 30517176. DOI: 10.1371/journal.pone.0207714

36 Gustavsson I, Aarnio R, Berggrund M, Hedlund-Lindberg J, Strand AS, Sanner K, Wikström I, Enroth S, Olovsson M and Gyllensten U: Randomised study shows that repeated self-sampling and HPV test has more than two-fold higher detection rate of women with CIN2+ histology than Pap smear cytology. Br J Cancer *118(6)*: 896-904, 2018. PMID: 29438367. DOI: 10.1038/bjc.2017.485

37 Dong L, Lin C, Li L, Wang M, Cui J, Feng R, Liu B, Wu Z, Lian J, Liao G, Chen W and Qiao Y: An evaluation of clinical performance of FTA cards for HPV 16/18 detection using cobas 4800 HPV test compared to dry swab and liquid medium. J Clin Vir *94*: 67-71, 2017. PMID: 28763729. DOI: 10.1016/j.jcv.2017.06.008

38 Randén-Brady R, Carpén T, Jouhi L, Syrjänen S, Haglund C, Tarkkanen J, Remes S, Mäkitie A, Mattila PS, Silén S and Hagstrom J: In situ hybridization for high-risk HPV E6/E7 mRNA is a superior method for detecting transcriptionally active HPV in oropharyngeal cancer. Hum Pathol *90*: 97-105, 2019. PMID: 31121131. DOI: 10.1016/j.humpath.2019.05.006

39 Derbie A, Mekonnen D, Woldemanuel Y, Van Ostade X and Abebe T: HPV E6/E7 mRNA test for the detection of high grade cervical intraepithelial neoplasia (CIN2+): a systematic review. Infect Agent Cancer *15*: 9, 2020. PMID: 32047531. DOI: 10.1186/s13027-020-0278-x

40 Tropé A, Sjøborg K, Eskild A, Cuschieri K, Eriksen T, Thoresen S, Steinbakk M, Laurak V, Jonassen CM, Westerhagen U, Jacobsen MB and Lie AK: Performance of human papillomavirus DNA and mRNA testing strategies for women with and without cervical neoplasia. J Clin Microbiol *47(8)*: 2458-24564, 2009. PMID: 19535524. DOI: 10.1128/JCM.01863-08

41 Molden T, Kraus I, Karlsen F, Skomedal H, Nygard JF and Hagmar B: Comparison of human papillomavirus messenger RNA

and DNA detection: a cross-sectional study of 4,136 women >30 years of age with a 2-year follow-up of high-grade squamous intraepithelial lesion. Cancer Epidemiol Biomarkers Prev *14(2)*: 367-372, 2005. PMID: 15734959. DOI: 10.1158/1055-9965.EPI-04-0410

42 Molden T, J. Nygard JF, Kraus I, Karlsen F, Nygard M, Baadstrand Skare G, Skomedal H, Thoresen SO and Hagmar B: Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-Proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear. Int J Cancer *114(6)*: 973-976, 2005. PMID: 15645423. DOI: 10.1002/ijc.20839

43 Parfenov M, Pedamallu CS, Gehlenborg N, Freeman SS, Danilova L, Bristow CA *et al*: Characterization of HPV and host genome interactions in primary head and neck cancers. Proc Natl Acad Sci USA *111(43)*: 15544-15549, 2014. PMID: 25313082. DOI: 10.1073/pnas.1416074111

44 Vojtechova Z, Sabol I, Salakova M, Turek L, Grega M, Smahelova J, Vencalek O, Lukesova E, Klozar J and Tachezy R: Analysis of the integration of human papillomaviruses in head and neck tumours in relation to patients' prognosis. Int J Cancer *138(2)*: 386-395, 2016. PMID: 26239888. DOI: 10.1002/ijc.29712

45 Gao G, Johnson SH, Vasmatzis G, Pauley CE, Tombers NM, Kasperbauer JL and Smith DI: Common fragile sites (CFS) and extremely large CFS are targets for human papillomavirus integrations and chromosome rearrangements in oropharyngeal squamous cell carcinoma. Genes Chromosomes Cancer *56(1)*: 59-74, 2017. PMID: 27636103. DOI: 10.1002/gcc.22415

46 Olthof NC, Speel EJ, Kolligs J, Haesevoets A, Henfling M, Ramaekers FC, Preuss SF, Drebber U, Wieland U, Silling S, Lam WL, Vucic EA, Kremer B, Klussmann JP and Huebbers CU: Comprehensive analysis of HPV16 integration in OSCC reveals no significant impact of physical status on viral oncogene and virally disrupted human gene expression. PLoS One *9(2)*: e88718, 2014. PMID: 24586376. DOI: 10.1371/journal.pone.0088718

47 Isayeva T, Li Y, Maswahu D and Brandwein-Gensler M: Human papillomavirus in non-oropharyngeal head and neck cancers: A systematic literature review. Head Neck Pathol *6 Suppl 1(1)*: 104-120, 2012. PMID: 22782230. DOI: 10.1007/s12105-012-0368-1

48 Tota JE, Best AF, Zumsteg ZS, Gillison ML, Rosenberg PS and Chaturvedi AK: Evolution of the oropharynx cancer epidemic in the United States: moderation of increasing incidence in younger individuals and shift in the burden to older individuals. J Clin Oncol *37(18)*: 1538-1546, 2019. PMID: 31026209. DOI: 10.1200/JCO.19.00370

49 Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D and Petruzzi M: Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig *24*: 1845-1851, 2020. PMID: 31402398. DOI: 10.1007/s00784-019-03048-y

Received October 12, 2020
Revised November 16, 2020
Accepted November 23, 2020