Journal of Medical Virology 87:1397–1403 (2015)

Human Papillomavirus mRNA Testing for the Detection of Anal High-Grade Squamous Intraepithelial Lesions in Men who Have Sex With Men Infected With HIV

Elena Sendagorta,[1]* Maria P. Romero,[2] Jose I. Bernardino,[3] María J. Beato,[4] Mario Alvarez-Gallego,[5] and Pedro Herranz[6]

[1]*Department of Dermatology, Hospital Universitario La Paz-IdiPaz, Madrid, Spain*
[2]*Department of Microbiology, Hospital Universitario La Paz-IdiPaz, Madrid, Spain*
[3]*Department of Internal Medicine, Hospital Universitario La Paz-IdiPaz, Madrid, Spain*
[4]*Department of Pathology, Hospital Universitario La Paz, Madrid, Spain*
[5]*Department of Coloproctology, Hospital Universitario La Paz, Madrid, Spain*
[6]*Department of Dermatology, Hospital Universitario La Paz-IdiPaz, Madrid, Spain*

Currently, screening for anal high-grade squamous intraepithelial lesions (anal HSIL) relies on anal cytology and high-resolution anoscopy. Since this approach has limited sensitivity and specificity for detecting anal HSIL, there is increasing interest in the role of biomarkers for predicting anal HSIL. The aim of this study is to evaluate the diagnostic accuracy of HPV E6/E7-mRNA expression for the detection of anal HSIL in MSM infected with HIV, in comparison to DNA-HR-HPV and anal cytology. This cross-sectional screening study included 101 MSM followed at the HIV-unit of La Paz University Hospital. Intra-anal swabs from patients participating in a screening program including cytology, high-resolution anoscopy and histology were analyzed. HR-HPV-DNA detection was performed by means of the CLART® HPV2 assay (GENOMICA S.A.U., Madrid, Spain). E6/E7-mRNA detection of HR-HPV-types 16, 18, 31, 33, and 45 was performed using the NucliSENS-EasyQ assay (BioMérieux, Marcy ÍEtoile, France). HR-HPV DNA and HPVE6/E7 mRNA were detected in 82% and 57% of the anal smears respectively. Anal cytology screening was abnormal in 70.3%. For the detection of HSIL sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 71.7%, 55.6%, 57.9%, and 69.8% for E6/E7-mRNA testing, respectively, compared to 100%, 31.5%, 55.4%, and 100% for HR-HPV-DNA testing and to 83%, 40.7%, 54.9%, 73.3% of cytology testing. In comparison with the other tests, HPVE6/E7 mRNA testing yielded a lower clinical sensitivity but a higher clinical specificity and PPV for the detection of anal HSIL in MSM infected with HIV. ***J. Med. Virol. 87:1397–1403, 2015.*** © 2015 Wiley Periodicals, Inc.

KEY WORDS: Anal HSIL; Human Papillomavirus; E6/E7 RNA m; anal cytology; HIV

Grant sponsor: Instituto de Investigaciones Sanitarias del Hospital Universitario La Paz (IdiPAZ); Grant sponsor: Red de Investigacion en SIDA (AIDS Research Network) (RIS); Grant number: RD07/0006/2007.

Conflict of interest: Authors confirm there is no conflict of interest. This manuscript hasn't been previously published nor is under consideration for been published by any other journal.

Author Contributions: E.S.C., M.P.R.G.: Conception and design, acquisition of data, analysis and interpretation of data. J. I. B. D. L. S., P. H. P.: Manuscript critical revision for important intellectual content. M. J. B. M., M. A.-G.: acquisition of data.

Meetings at which parts of the data were presented—Oral comunication: "Human papillomavirus mRNA testing for the detection of anal high-grade squamous intraepithelial lesions in HIV-infected men who have sex with men." Presented at the first IANS (International Anal Neoplasia Society) innagural meeting. It was held 22-24 November 2013 in San Francisco. Poster: "Utilidad de la determinación del ARNm del virus del papiloma humano para el diagnóstico de lesiones intraepiteliales escamosas de alto grado en hombres que tienen sexo con hombres infectados por VIH". Presented at the V National Congress of GESIDA, Sitges 19-22 November 2013.

*Correspondence to: Elena Sendagorta, MD, Camino de la Huerta 128, 28050 Madrid, Spain.
E-mail: elenasendagorta@hotmail.com

Accepted 2 February 2015

DOI 10.1002/jmv.24188
Published online 1 May 2015 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

There has been a significant increase in the incidence of anal squamous cell carcinoma in the last 2 decades [Johnson et al., 2004; Kurdgelashvili et al., 2013]. This increase is particularly remarkable among patients infected with HIV and specially in men who have sex with men (MSM) [Bower et al., 2004; Kreuter et al., 2010; Stanley et al., 2011], with incidence rates of 65–109 per 100,000 person-years [Machalek et al., 2012]. Like cervical cancer, squamous cell carcinomas and their precursor lesions (high-grade squamous intraepithelial lesions or HSIL) are caused by persistent infections with high-risk oncogenic human papillomaviruses (HR-HPV [Machalek et al., 2012; Wentzensen et al., 2012]). Cervical and anal HPV infections usually clear spontaneously after a short period of time, but in some patients a transforming infection, characterized by increased expression of HPV oncogenes, may develop. HIV-infected patients are less likely to clear HPV and thus, the potential for subsequent development of anal neoplasia is increased [Critchlow et al., 1998].

Currently, up-regulated expression of E6/7 oncogenes is considered a crucial step for the initiation and progression of intraepithelial neoplasia. The E6 and E7 oncoproteins facilitate malignant transformation and are consistently expressed in HSIL. Their mechanism of action is based on inactivation of the p53 and pRb tumor suppressor proteins [Zur and ausen, 2002; Wentzensen et al., 2012]. Consequently, detection of E6/7 mRNA transcripts may be better than HPV DNA detection as a surrogated marker of increased risk of progression to neoplasia.

MSM infected with HIV are at an increased risk of anal HR-HPV persistent infection [Fox, 2009; Arbyn et al., 2012]. As a consequence, the prevalence of anal HSIL in these patients is extremely high [Berry et al., 2009]. In light of the increasing health burden of anal cancer and its similarities to cervical cancer, anal HSIL screening programs for this population are proposed and are currently based on anal cytology and high-resolution anoscopy [Palefsky et al., 2005; Kreuter et al., 2010]. Although universal screening is not widely accepted some HIV guidelines include screening some populations.[1]

The aim of this study is to evaluate the accuracy of the HPV E6/7-mRNA expression by means of the NucliSENS EasyQ HPV assay for the detection of anal histological HSIL in MSM infected with HIV, compared to HR-HPV-DNA testing and anal cytology.

[1]Management of STIs in HIV-Infected Patients. Human Papillomavirus (HPV). New York State Department of Health AIDS Institute. Available at: http://www.hivguidelines.org/clinical-guidelines/adults/management of-stis-in-hiv-infected-patients/human-papillomavirus-hpv/ (Accessed 10/03/2014).

PATIENTS AND METHODS

This study prospectively included 101 HIV-infected patients followed-up in the HIV Unit at the Hospital La Paz and consecutively referred to the Dermatology Department (anal intraepithelial neoplasm early detection unit) from March of 2012 to April of 2013.

Inclusion and exclusion criteria: Patients over 18 years of age, MSM infected with HIV who gave their written consent to participate in the study were included. Patients with a history of anal squamous cell carcinoma were excluded.

Study Protocol

All patients were evaluated at the first visit with a detailed medical history and a complete physical examination. Anal samples were taken by cytobrush, and collected in two containers with liquid transport medium (ThinPrep Pap Test. PreservCyt®, Marlborough, MA). One was sent to Pathology for study of the cytological characteristics and the other to the Microbiology Department for detection of HPV-DNA and E6/7 HPV-mRNA. All patients underwent high-resolution anoscopy and biopsy. This protocol was presented to and approved by our hospital's Clinical Research Ethics Committee.

Technical Specifications and Tests Performed

Anal cytology. The sample collection technique for the cytology study was always performed by the same dermatologist and collected blindly using the procedure initially described by Palefsky [Palefsky et al., 1997]. It consists of insertion of the cytobrush into the anal canal to a depth of 5–6 cm. The brush is then pressed and rotated in a spiral until removal. The sample is quickly fixed in a liquid medium (ThinPrep Pap Test, PreservCyt®) for cytological study.

The results were analyzed blindly by two pathologists specialized in the diagnosis of these lesions, who determined both the suitability criteria as well as their diagnosis according to the modified Bethesda classification criteria [Nayar and Solomon, 2004]. Squamous cell lesions were classified as follows: Normal, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL).

Extraction of Nucleic Acids.

Nucleic acids were extracted from anal cytology samples using the NucliSens easy MAG automatic extraction system (BioMérieux). The extraction protocol was performed as recommended by the manufacturer for these types of specimens.

HPV-DNA Testing

Testing for the different HPV genotypes was performed using genomic amplification CLART®

Papillomavirus 2 kit (GENOMICA S.A.U., Madrid, Spain). This microarray technique is capable of detecting the presence of 35 different HPV genotypes: (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89). Detection was carried out through amplification of a fragment of 450 bp within the L1 region of the virus, as this is a highly preserved sequence among the various HPV types. However, this region has sufficient variation that allows for each virus type to be differentiated with specific probes. In this way, the specificity of the test is ensured.

The HPV test results were only considered positive when the presence of one of the 20 HR-HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, and 85) as categorized by [Dunne et al., 2007] were detected.

It was also considered the analyzes using the detection of only 5 HR-HPV types (HPV 16, 18, 31, 33, and 45) from CLART for further comparison with NUcliSENS EasyQ HPV.

E6/7-m RNA Measurement

HPV-E6/7-mRNA testing was performed using the NucliSENSEasyQ HPV1.1 assay (BioMérieux) according to the manufacturer's instructions. NucliSENS-EasyQ is based on NASBA-technique and detects E6/7-mRNA of five HR-HPV-types (HPV16/18/31/33/45) and U1A-reference-mRNA.

The results of the HPV mRNA test were classified as absence of HPV mRNA, presence of HPV mRNA and presence of RNA from each of the genotypes included in the reagent.

High-resolution anoscopy and biopsy

High-resolution anoscopy was performed by visualizing the anal canal through a video-colposcope (Zeiss, OPMI pico). Disposable plastic anoscopes coated in 5% lidocaine lubricating gel were used; a stain with 5% acetic acid was also prepared and applied using a cotton swab for 1 min after removing the anoscope. Then, the video colposcope was used to examine the walls of the anal canal with the objective of identifying acetowhite lesions, followed by staining with Lugol's solution and a search for Lugol's-negative lesions. After the stains, biopsy samples were collected under local anesthesia with Baby-Tischler forceps. Visually atypical areas were chosen for biopsy using criteria that were predefined in the study: acetowhite plaques, areas with an anomalous vascular pattern and Lugol's-negative areas. When several areas suggestive of dysplasia were seen, multiple biopsies were taken and only the result with the greatest histological severity was recorded. In cases in which no area suggestive of dysplasia was found, a blind biopsy was taken at the squamocolumnar junction.

A total of 120 histological samples were obtained, though only one result was recorded per patient corresponding to the sample with the greatest level of histological severity.

The histological samples were evaluated blindly by two pathologists who did not know the previous cytological result and were classified as normal, LSIL and HSIL.

Statistical Analysis

The quantitative variables were described using the mean, (standard deviation) or median (interquartile range). The qualitative variables were described according to absolute frequency and percentages.

Fisher's exact test was applied to analyze the association between qualitative variables.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with 95% confidence intervals and Youden index were calculated for the endpoints histological HSIL (AIN2 and AIN3) Youden's index (YI) was calculated as sensitivity+specificity-1. Sensitivity and specificity with confidence intervals were plotted on a Receiver-Operator-Characteristics (ROC) curve graph. Percentage agreement between the two HPV-tests was calculated. Moreover, the chance-corrected agreement between HR-HPV-DNA and -mRNA testing was assessed by Cohen's kappa (<0.20, poor; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 substantial; >0.80 perfect agreement).

All statistical tests were bilateral and those with a *P*-value less than 0.05 were considered significant. The data was analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

One-hundred-one patients with a median age of 42 (IQR 33–50) years were included in the study. The mean time of HIV infection at the time of consultation was 10.5 ± 8.9 years. 90 patients (89%) were receiving HAART. Viral load was only detectable in 11 patients, all of them naïve to HAART treatment. The median CD4 counts and CD4 nadir were 569 (IQR: 396–747) cells/mm3 and 170 (IQR: 52–314) cells/mm3, respectively.

All the cytological samples obtained were considered satisfactory for diagnosis. Cytology was abnormal in 71 patients (70.3%) of patients, and results were classified as shown in Table I.

Samples for the DNA study were obtained from all patients, one of which was not evaluable for diagnosis due to an inhibited PCR result.

HR-HPV DNA was detected in 83 patients (82.2%) with a median number of genotypes of 2(IQR:1–4). HPV 16 was the most prevalent, being detected in 31.7% of patients. Of note, 55.4% ($n = 56$) patients presented DNA from at least one of the genotypes included in the E6/7 mRNA reagent.

TABLE I. Anal Cytology and Histological Results

Histology	Normal	LSIL	HSIL(AIN 2)	HSIL(AIN 3)	Total
Citology					
Normal	20	2	4	4	n:30 29.7%(20.8–38.6)
ASCUS	4	2	1	2	n = 9 8.9%(3.3–14.4)
LSIL	18	6	15	11	n = 50 49.5%(39.7–59.2)
HSIL	2	0	5	5	n = 12 11.9%(5.6–18.2)
Total	n = 44 43.6% (I33.9–53.2)	n = 10 9% (3.4–14.5)	n = 25 24.8 (16.3–33.2)	n = 22 2.8 (13.7–29.8)	n:101 100%

Samples for the E6/7 mRNA study were obtained from all patients, one of which was not evaluable for diagnosis due to an inhibited PCR result. E6/7 mRNA was detected in 57% (n = 57) of anal samples. E6/7 mRNA from HPV 16, 18, 31, 33, 45 was detected in 37%, 14%, 19%, 9%, and 11% of cases, respectively. The median mRNA types found were 1(IQR.0–1) per patient.

The results of the histological analysis are shown in Table I. In 75 patients, the biopsies were taken from lesions suggestive of dysplasia on high-resolution anoscopy. The anoscopy was normal in 26 patients, with only one sample taken for biopsy at the squamocolumnar junction. Only one of these biopsies revealed a low-grade lesion, the remainder being normal.

As seen in Table II, the sensitivity, specificity, positive, and negative predictive value for the detection of HSIL was 71.7%, 55.6%, 57.9%, and 69.8% for the E6/7 mRNA test compared to 100%, 31.5%, 55.4%, and 100% for the HR-HPV-DNA test and 83%, 40.7%, 54.9%, and 73.3% for anal cytology.

No statistically significant differences between the three ROC (receiver operating characteristics) curves were found, as shown in Figure 1.

Kappa-value for agreement between HPV-DNA and E6/7-mRNA testing was 0.16(*P*:0.05) When the analysis was performed including only the DNA results of the five HPV types detected by NucliSENS EasyQ HPV, kappa coefficient increased to 0.43(*P*:0.00)

Kappa-values for agreement between HPV-DNA and E6/7-mRNA testing for individual HPV-types were substantial for HPV16 (κ: 0.712) and HPV18 (κ: 0.680), moderate for HPV 33(κ: 0.550) and fair for HPV 31(κ: 0.297) and 45(κ: 0.329)

Discrepancies between the mRNA and HRHPV-DNA detection for each individual high risk genotype are shown in Table III.

DISCUSSION

This study evaluated the operational characteristics of the E6/7 mRNA HPV test versus anal cytology and HR-HPV-DNA detection in a population of HIV-infected MSM.

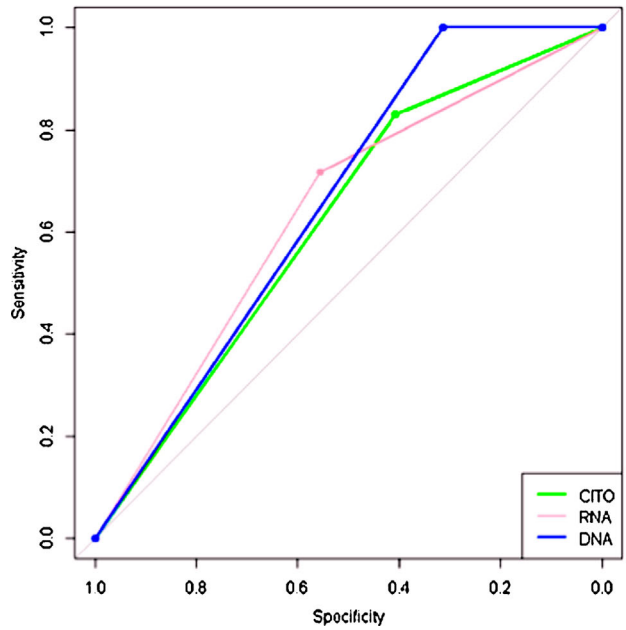
82.2% of men recruited for the study had HR-HPV DNA and 46.5% were diagnosed with HSIL, which confirms to the expected levels in a series with these characteristics according to the literature.

In this study, the sensitivity, specificity, positive, and negative predictive value for the detection of HSIL was 71.7%, 55.6%, 57.9%, and 69.8% for the E6/7 mRNA test compared to 100%, 31.5%, 55.4%, and 100% for the HR-HPV-DNA test and 83%, 40.7%, 54.9%, and 73.3% for anal cytology.

Anal HSIL screening currently relies on anal cytology and high resolution anoscopy in most clinical settings. Both approaches have limitations for detecting anal HSIL. HRA is a direct visual method that is not widely available, has limited reproducibility and depends on the expertise of the practitioner to identify HSIL [Swedish and Lee, 2011]. The sensitivity of anal cytology is moderate, similar to cervical cytology, and ranges between 69 and 93% [Chiao et al., 2006]. On the other hand, as only HSIL lesions are treated to prevent progression to invasive cancer, the specificity of anal cytology to diagnose this lesion is also important and this, unfortunately, is low with values ranging between 32 and 59% [Chiao et al., 2006]. This poor specificity limits the utility of anal cytology as a

TABLE II. Performance of the Three Tests for the Diagnosis of Histological HSIL

	Sensitivity	Specificity	PPV	NPV	Youden index
E6/7 mRNA	71% (95% CI 62.15–79.8)	55.6% (95% CI 45.9–65.2)	57.9% (95% CI 48.2–67.5)	69.8% (95% CI 60.8–78.7)	0.26
HR-HPV DNA	100% (95% CI 100–100)	31.5% (95% CI 22.4–40.5)	55.4% (95% CI 45.7–65)	100% (95% CI 100–100)	0.31
Anal cytology	83% (95% CI 75.6–90.3)	40.7% (95% CI 31.1–50.2)	54.9% (95% CI 45.2–64.6)	73.3% (95% CI 64.6–81.9)	0.23



	AUC(CI)
RNAm	0,63(0,5-0,7)
Cytology	0,62(0,51-0,73)
DNA	0,65(CI:0,55-0,76)

AUC	RNAm vs Cytology	RNAm vs DNA	DNA vs Cytology
p	0,756	0,664	0,577

Fig. 1. Receiver-operator-characteristics curve graph for the three tests and *P*-values.

screening test, as many patients without HSIL may be referred for high-resolution anoscopy, resulting in increased healthcare cost [Ho and Cranston, 2010].

On the other hand, primary screening with HR-HPV-DNA testing is also limited by its specificity as a consequence of the extraordinarily high prevalence of this infection in MSM infected with HIV [Berry et al., 2009].

The accuracy of anal cancer screening programs may be improved by biomarker assays that specifically highlight transforming HPV infections, improving patient management and allowing targeted high-resolution anoscopy in patients infected with HIV, who have a higher risk of progression [Wentzensen et al., 2012].

Numerous studies have shown that E6/7-mRNA testing has lower sensitivity but increased specificity for the detection of cervical HSIL compared to HR-HPV-DNA testing [Burger et al., 2011; Mockel et al., 2011]. However, there are few studies published, on the use of mRNA testing for the diagnosis of HSIL in MSM infected with HIV. Wentzensen et al. [2012] studied the use of various biomarkers as predictors of HSIL in a series of 363 MSM with HIV infection [Wentzensen et al., 2012]. In their study, detection of HR-HPV DNA showed the greatest sensitivity for the diagnosis of HSIL, followed by p16/ki67 and HPV E6/7 mRNA. Nevertheless, the greatest Youden index was obtained for E6/7 HPV mRNA. The sensitivity, specificity, PPV and NPV for E6/7 HPV mRNA for the diagnosis of HSIL was 79.8%, 62.4%, 50%, and 86% in their cohort.

Additionally, Silling et al. [2012] published a series of 289 MSM with HIV infection [Silling et al., 2012]. The sensitivity, specificity, PPV and NPV for the E6/7 mRNA test for the detection of HSIL was 95.3%, 46.0%, 98.1%, and 25.2%, respectively.

Finally, Phanuphak recently published a series of 123 HIV-positive and 123 HIV-negative MSM [Phanuphak et al., 2013]. They used a kit that covers the detection of E6/E7 mRNA from HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 69 (HPV OncoTect^{TM} E6, E7 mRNA Kit, IncellDx, Menlo Park, CA) Among all MSM at baseline, they found that E6/E7 mRNA was the most sensitive test (64.7%). Specificity was approximately the same for E6/E7 mRNA (57.9%), high-risk HPV DNA (54%), and p16 immunocytochemistry (56.5%).

The performance characteristics of the E6/7 HPV-mRNA test obtained in our study are very similar to those obtained in the series by Wentzensen et al. [2012]. Although the differences are not statistically significant in our study, the results indicate that the E6/7 mRNA test has greater specificity and PPV for the diagnosis of anal HSIL compared to HR-HPV-DNA and anal cytology, though it does have a lower

TABLE III. Discrepancies Between E6/E7 mRNA and HR-HPV DNA Testing

	HPV DNA+/RNA-	HPV DNA-/RNA+
HPV 16	4(3.9%)	7(6.9%)
HPV 18	3(2.9%)	3(2.9%)
HPV 31	28(27.7%)	4(3.9%)
HPV 33	3(2.9%)	3(2.9%)
HPV 45	1(0.9%)	7(6.9%)
Total	39(38.6%)	24(23.7%)

sensitivity. It should be noted that HRA is not widely available as it is offered in few centers. Therefore the use of a test with greater specificity is useful for avoiding unnecessary referrals. However, the lower sensitivity of the test requires exhaustive follow-up of patients with negative E6/7 mRNA results.

On the other hand, the E6/7 RNA test may be useful in the identification of the vast majority of men with lesions that are likely to progress, since the oncogenic potential of HPV infection depends on expression of E6/7 oncoproteins. Studies in cervix suggest that E6/7 HPV overexpression is a more specific marker of high-grade cervical lesions that are likely to become invasive and require intervention [Pierry et al., 2012]. Longitudinal studies will help to clarify if patients diagnosed of HSIL, that are positive for E6/7 mRNA have a worse prognosis than those that are negative for the test.

As reported in other series [Silling et al., 2012], we have found discrepancies between the mRNA and HRHPV-DNA detection for each individual high-risk genotype. Overall, there were 39 patients that were positive for DNA testing and negative for E6/7 mRNA testing of the same genotype. This may be explained as not all the infections are transcriptionally active for E6/7 expression, reflecting an episomal state. Conversely, there were 24 cases in which the CLART 2 assay detected no DNA from HR-HPV of the 5 genotypes studied, but the E6/7 mRNA assay yielded positive results. This may be explained by low viral load, high intratype variation or deleted sequences during viral integration.

Our study has several limitations. Although patients were consecutively included, our study population may not be representative of the general HIV-infected population. Also, the small size of the sample and the possibility of a selecting bias must be taken into account. Additionally, the NucliSENS EasyQ HPV assay lacks the detection and typing of all HR-HPV genotypes. Studies evaluating E6/7 mRNA tests that include 14 HR-HPV genotypes, have reported an increased specificity, but a similar sensitivity as HR-HPV-DNA testing for the detection of CIN2+/CIN3+, probably due to the larger number of HR-HPV-types covered by the assay [Dockter et al., 2009; Reuschenbach et al., 2010]. Even when sensitivity was moderate in Phanuphak series, larger studies are warranted to further explore the performance of this test. Finally, it is not possible to prospectively determine the PPV and NPV of the test in the development of HSIL and invasive anal carcinoma due to its cross-sectional design. As none of the three tests evaluated in our study performed at high level, additional studies investigating the potential role of repeating these tests over time, are warranted.

The strengths of this study include a consecutively recruited population of HIV-infected MSM, performing high-resolution anoscopy with a high biopsy rate on all study participants, and the use of liquid-based anal cytology.

In summary, our study demonstrate that the E6/7 mRNA test has a lower sensitivity but a higher specificity and PPV than anal cytology and HR-HPV DNA testing for the detection of anal HSIL in MSM infected with HIV. Although screening with E6/7 mRNA does not seem to improve the current approach with anal cytology, this test may be useful and cost-effective in settings were availability to high-resolution anoscopy is low, reducing the number of false positive cases.

Long-term studies will help to clarify if E6/7 HPV mRNA detection has a role as an early indicator of risk of progression to invasive cancer. Future studies on HPV-mRNA testing, should evaluate the addition of more HR-HPV genotypes, in order to improve the sensitivity of this marker to predict anal HSIL in high-risk populations.

REFERENCES

Arbyn M,Sanjosé De S, Saraiya M, Sideri M, Palefsky J, Lacey C, Gillison M, Bruni L, Ronco G, Wentzensen N, Brotherton J, Qiao YL, Denny L, BornstGiuliano A, Tommasino M, Monsonego J. 2012. EUROGIN 2011 roadmap on prevention and treatment of HPV-related disease. Int J Cancer 24:28–55.

Berry JM, Palefsky JM, Jay N, Cheng S-C, Darragh TM, Chin-Hong PV. 2009. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. Dis Colon Rectum 52:239–247.

Bower M, Powles T, Newsom-Davis T, Thirlwell C, Stebbing J, Mandalia S, Nelson M, Gazzard B. 2004. HIV-associated anal cancer: Has highly active antiretroviral therapy reduced the incidence or improved the outcome? J Acquir Immune Defic Syndr 37:1563–1565.

Burger EA, Kornør H, Klemp M, Lauvrak V, Kristiansen IS. 2011. HPV mRNA tests for the detection of cervical intraepithelial neoplasia: A systematic review. Gynecol Oncol 120:430–438.

Chiao EY, Giordano TP, Palefsky JM, Tyring S, El Serag H. 2006. Screening HIV-infected individuals for anal cancer precursor lesions: A systematic review. Clin Infect Dis 15:223–233.

Critchlow CW, Hawes SE, Kuypers JM, Goldbaum GM, Holmes KK, Surawicz CM, Kiviat NB. 1998. Effect of HIV infection on the natural history of anal human papillomavirus infection. AIDS 12:1177–1184.

Dockter J, Schroder A, Hill C, Guzenski L, Monsonego J, Giachetti C. 2009. Clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. J Clin Virol 45:S55–S61.

Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE. 2007. Prevalence of HPV infection among females in the United States. JAMA 297:813–819.

Fox P. 2009. Anal cancer screening in men who have sex with men. Curr Opin HIV AIDS 4:64–67.

Ho KS, Cranston RD. 2010. Anal cytology screening in HIV-positive men who have sex with men: What's new and what's now? Curr Opin Infect Dis 23:21–25.

Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. 2004. Anal cancer incidence and survival: The surveillance, epidemiology, and end results experience, 1973-2000. Cancer 101:281–288.

Kreuter A, Potthoff A, Brockmeyer NH, Gambichler T, Swoboda J, Stücker M, Schmitt M, Pfister H, Wieland U. 2010. Anal carcinoma in HIV-positive men: Results of a prospective study from Germany. Br J Dermatol 162:1269–1277.

Kurdgelashvili G, Dores GM, Srour S, Dores GM, Srour SA, Chaturvedi AK, Huycke MM, Devesa SS. 2013. Incidence of potentially human papillomavirus-related neoplasms in the United States, 1978 to 2007. Cancer 119:2291–2299.

Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, Hillman RJ, Petoumenos K, Roberts J, Tabrizi SN, Templeton DJ, Grulich AE. 2012. Anal human papillomavirus infection and

associated neoplastic lesions in men who have sex with men: A systematic review and meta-analysis. Lancet Oncol 22:1–14.

Management of STIs in HIV-Infected Patients. Human Papillomavirus (HPV). New York State Department of Health AIDS Institute. Available at: http://www.hivguidelines.org/clinical-guidelines/adults/management-of-stis-in-hiv-infected-patients/human-papillomavirus-hpv/ (Accessed 10/03/2014).

Möckel J, Quaas J, Meisel H, Endres AS, Schneider V. 2011. Human papillomavirus E6/E7 mRNA testing has higher specificity than liquid-based DNA testing in the evaluation of cervical intraepithelial neoplasia. Anal Quant Cytol Histol 33:311–315.

Nayar R, Solomon D. 2004. Second edition of 'The Bethesda System for reporting cervical cytology' - atlas, website, and Bethesda interobserver reproducibility project. Cytojournal 21:1–4.

Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. 1997. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol 14:415–422.

Palefsky JM, Holly EA, Efirdc JT, Da Costa M, Jay N, Berry JM, Darragh TM. 2005. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. AIDS 19:1407–1414.

Phanuphak N, Teeratakulpisarn N, Keelawat S, Pankam T, Barisri J, Triratanachat S, Deesua A, Rodbamrung P, Wongsabut J, Tantbirojn P, Numto S, Ruangvejvorachai P, Phanuphak P, Palefsky JM, Ananworanich J, Kerr SJ. 2013. Use of human papillomavirus DNA, E6/E7 mRNA, and p16 immunocytochemistry to detect and predict anal high-grade squamous intraepithelial lesions in HIV-positive and HIV-negative men who have sex with men. PLoS ONE 12:e78291.

Pierry D, Weiss G, Lack B, Chen V, Fusco J. 2012. Intracellular human papillomavirus E6, E7 mRNA quantification predicts CIN 2+ in cervical biopsies better than Papanicolaou screening for women regardless of age. Arch Pathol Lab Med 136: 956–960.

Reuschenbach M, Clad A, von Knebel Doeberitz C, Wentzensen N, Rahmsdorf J, Schaffrath F, Griesser H, Freudenberg N, von Knebel Doeberitz M. 2010. Performance of p16INK4a-cytology, HPV mRNA, and HPV DNA testing to identify high grade cervical dysplasia in women with abnormal screening results. Gynecol Oncol 119:98–105.

Silling S, Kreuter A, Hellmich M, Swoboda J, Pfister H, Wieland U. 2012. Human papillomavirus oncogene mRNA testing for the detection of anal dysplasia in HIV-positive men who have sex with men. J Clin Virol 53:325–331.

Stanley MA, Winder DM, Sterling JC, Winder DM, Sterling JC, Goon PK. 2011. HPV infection, anal intra-epithelial neoplasia (AIN) and anal cancer: current issues. BMC Cancer. 2012;8:12-398. Swedish KA, Lee EQ GS. The Changing Picture of High-grade Anal Intraepithelial Neoplasia in Men Who Have Sex With Men: The Effects of 10 Years of Experience Performing High-resolution Anoscopy. Dis Colon Rectum 54:1003–1007.

Wentzensen N, Follansbee S, Borgonovo S, Tokugawa D, Schwartz L, Lorey TS, Sahasrabuddhe VV, Lamere B, Gage JC, Fetterman B, Darragh TM, Castle PE. 2012. Human papillomavirus genotyping, human papillomavirus mRNA expression, and p16/Ki-67 cytology to detect anal cancer precursors in HIV-infected MSM. AIDS 13:2185–2192.

zur Hausen H. 2002. Papillomaviruses and cancer: From basic studies to clinical application. Nat Rev Cancer 2:342–350.