



Human Papillomavirus Type 16 L1 Serological Test Shows High Concordance with Anal Cytology in People Living with HIV

JID Innovations (2022);2:100124 doi:10.1016/j.xjidi.2022.100124

TO THE EDITOR

The past decades have seen a steady rise in the incidence of squamous cell carcinoma of the anus, which is now one of the fastest accelerating causes of cancer incidence and mortality in the United States (Deshmukh et al., 2020). Up to 80% of anal cancer cases are caused by infection with human papillomavirus type 16 (HPV16) (Alemany et al., 2015).

In this study, we report a significant association between HPV16 L1 antibodies and anal dysplasia, with potential implications for anal cancer screening in people living with HIV (PLWH).

PLWH live with an increased risk of developing human papillomavirus-induced cancers, and in particular anal squamous cell carcinoma (Silverberg et al., 2012). This led several regional societies to issue recommendations for regular anal cancer screening in this population (Brown, 2020; Esser et al., 2015). Current recommended screening methods include rectal examination and anal cytology. However, these methods are subject to several disadvantages. For instance, the prospect of a rectal examination presents a barrier for patients deciding whether to attend screening. Cost effectiveness and shortage of proctologists also need to be considered. Blood-based screening methods would therefore be highly desirable and could be easily integrated into the regular medical check-ups of PLWH, which often include routine collection of blood.

We used a competitive immunoassay (Prevo-Check, Abviris Deutschland GmbH, Ahrensburg, Germany) for the detection of HPV16 L1 antibodies in serum. In a recent case-control study, this method proved highly sensitive for HPV16-induced oropharyngeal (95%) and anal cancers (90%), while maintaining a high degree of specificity (>99%) (Blatt et al., 2021; Weiland et al., 2020). The goal of this pilot study was to investigate the relationship between the HPV16 L1 seromarker and anal cytology in PLWH attending anal cancer screening.

A total of 63 HIV-seropositive patients (61 male) with a mean age of 53 years (range: 26–78) were recruited at our outpatient clinic. Written informed consent was obtained from all participants before enrolment. The study complied with the Declaration of Helsinki and was approved by the ethics committee of Ludwig Maximilian University (Munich, Germany) under approval number 17-557. All patients underwent anal swabs for cytological analysis, graded according to the Bethesda system.

Abnormal cytology was observed in 43% (n = 27) of patients: 6% (n = 4) had atypical squamous cells of undetermined significance, 22% (n = 14) had low-grade squamous intraepithelial lesions (LSIL), and 14% (n = 9) had high-grade squamous intraepithelial lesions (HSIL). In 57% (n = 36), anal swabs were negative for intraepithelial lesions or malignancy (Table 1). Quantification of serum HPV16 L1 antibodies was achieved by colorimetric

readout (ESEQuant LR3, Qiagen Lake Constance, Stockach, Germany) of a lateral flow rapid test (Prevo-Check).

HPV16 L1 antibodies were found in 56% (n = 35) of patients (concentration range: 50–2,900 ng/ml). Patients with abnormal cytology (atypical squamous cells of undetermined significance, LSIL, or HSIL) had significantly higher antibody levels than patients with normal cytology (mean ± SEM: 750 ± 144 ng/ml vs. 214 ± 61 ng/ml, $P = 0.002$; Figure 1a inset). This effect was independent of current CD4⁺ cell count (no significant main effect of, or interaction with, CD4 group, $P > 0.45$; Figure 2a). More detailed analysis revealed significant increases in antibody concentration with increasing severity of pathological grade ($P = 0.002$; Figure 1a), measuring significantly higher titers in HSIL cases (mean ± SEM: 1,090 ± 225.6 ng/ml; median: 1,060 ng/ml) than in LSIL cases (mean ± SEM: 575 ± 204.5 ng/ml; median: 400 ng/ml; posthoc test [Fisher's least significant difference], $P < 0.05$). Of the eight patients with HPV16 L1 antibody titers of >1,000 ng/ml, 87.5% (n = 7) had abnormal anal cytology (LSIL, HSIL, or atypical squamous cells of undetermined significance). The proportion of cases with abnormal cytology dropped to 52% in patients with titers in the range of 50–1,000 ng/ml and to 21% in patients without any detectable HPV16 L1 antibodies (Figure 1b).

Overall, these data show a positive relationship between antibody titer and both the probability and the severity of an abnormal cytological finding.

Because antibody production can be affected by the status of the immune system, we also investigated the relationship between recent CD4⁺ T-cell counts and HPV16 L1 antibody titers. We did not find any significant correlations between recent CD4⁺ cell count

Abbreviations: CI, confidence interval; HPV16, human papillomavirus type 16; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion

Accepted manuscript published online XXX; corrected proof published online XXX

Cite this article as: *JID Innovations* 2022;2:100124

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Table 1. HPV16 L1 Serology Results by Varying Positivity Cut Offs

Cytology	Total Cases	1,280 ng/ml Cut Off (Maximum Specificity)				1,000 ng/ml Cut Off (Previously Described by Weiland et al. [2020])				200 ng/ml Cut Off (Maximum HSIL Sensitivity)			
		Serologically positive		Serologically negative		Serologically positive		Serologically negative		Serologically positive		Serologically negative	
		n	%	n	%	n	%	n	%	n	%	n	%
NILM	36	0	0	36	100	1	3	35	97	9	25	27	75
ASCUS	4	1	25	3	75	1	25	3	75	2	50	2	50
LSIL	14	1	7	13	93	1	7	13	93	9	64	5	36
HSIL	9	1	11	8	89	5	56	4	44	9	100	0	0

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HPV16, human papillomavirus type 16; HSIL, high-grade squamous intraepithelial lesions; NILM, negative for intraepithelial lesions or malignancy.

and HPV16 L1 antibody levels among cytologically positive patients ($r = 0.02$, $P = 0.94$), cytologically negative patients ($r = -0.02$, $P = 0.91$; Figure 2b), or when considering the entire study population ($r < 0.01$, $P = 0.97$; not shown). Overall, our data suggest that HIV-related immunological parameters can be ruled out as confounding factors (Figure 2).

One frequent challenge when considering in vitro diagnostic tests is in defining the optimal cut-off value above which a test is considered positive. A previous study using the same immunoassay reported 1,000 ng/ml as the appropriate cut off for the detection of HPV16-induced oropharyngeal cancer. Applying this cut off to the present dataset resulted in a positive predictive value for abnormal cytology of 88% (95% confidence interval [CI] = 47.8–98.2%) and specificity of 97.2% (95% CI = 85.5–99.9%), while enabling the detection of over half of HSIL (55.6%; 95% CI = 21.2–86.3%), but only 7.1% (95% CI = 0.2–33.9%) of the LSIL cases.

Because this study presents the assessment of the seromarker in a population with precancerous pathology, we also explored test performance characteristics at alternative cut offs. Receiver operating characteristic curve analysis performed on HSIL versus negative cytology cases yielded an area under the curve of 0.92 (95% CI = 0.84–1.00; Figure 1c), suggesting a high concordance between serology and high-grade anal cytology. Maximum specificity (100%) was achieved at antibody concentrations above 1,280 ng/ml (Youden's J = 0.33). Youden index, maximizing both sensitivity and specificity, was highest at an antibody concentration of 200 ng/ml (Youden's J = 0.75). Application of this concentration cut off to the dataset enabled the detection of 100% (95% CI = 66.4–100.0%) of HSIL cases and 64% (95% CI = 35.1–87.2%) of LSIL cases, at an overall specificity for anal dysplasia of 75% (95% CI = 57.8–87.9%) and a positive predictive value of 69% (95% CI = 54.7–80.3%).

Taken together, we show a significant association between the HPV16 L1 seromarker, and both the severity and the probability of an abnormal anal cytology finding. Although the

results of this study provide encouragement that a blood-based strategy has desirable properties for anal cancer screening in HIV-positive patients, this study is subject to several limitations which need to be addressed in future studies. Most importantly, further studies with a large sample of histologically verified anal intraepithelial neoplasia are necessary to provide a direct assessment of the sensitivity and specificity of this marker in detecting neoplasia.

Although this study does not allow us to make direct comparisons between serum testing and histology, a high concordance between HSIL from anal swabs and histologically confirmed high-grade anal intraepithelial neoplasia (anal intraepithelial neoplasia II-III) has been reported in previous studies, including one performed in our own setting (Dietrich et al., 2015; Repiso Jiménez et al., 2014). Furthermore, given that anal cytology is the method recommended by current guidelines, we believe that the present data are a valuable step in evaluating serology in the context of anal cancer screening.

Our data show a high concordance between anal cytology and serology, which may be a significant, valuable addition to current anal cancer screening methods, with a referral for more detailed rectal examination indicated for patients with high levels of antibodies. Practice settings where resources for such examination (e.g., by high-resolution anoscopy) are limited may prefer using a stringent cut off (e.g. >1,000 ng/ml or 1,280 ng/ml) to ensure that only patients with high-grade dysplasia are referred. On the contrary, a lower cut off of 200 ng/ml may be more appropriate for settings that prioritize sensitivity and detection of low-grade dysplasia in addition to high-grade dysplasia.

PLWH attend quarterly check-ups that include routine collection of blood samples. Serum testing could be easily integrated into these routine visits without the need for additional equipment, invasive procedures, or specialist training. Blood-based testing would be particularly beneficial in settings where regular proctologic assessments are either not feasible or are refused by the patient. A rapid test for HPV16 L1 antibodies could help to

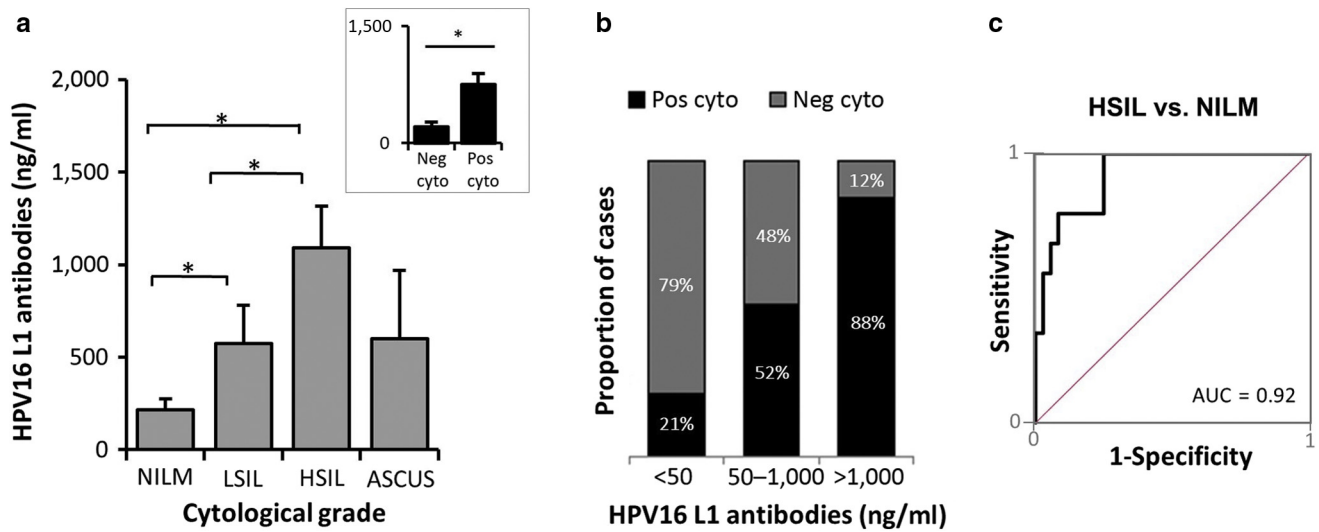


Figure 1. Association between HPV16 L1 serology and anal cytology. (a) Antibodies against HPV16 L1 were measured using a competitive immunoassay. Antibody titers were significantly higher in patients with positive cytology (ASCUS, LSIL, or HSIL) compared with patients with normal cytology (inset, $*P < 0.05$). There was a significant effect of cytological grade, with titers significantly higher in patients with HSIL than patients with LSIL (one-way ANOVA and posthoc tests, $*P < 0.05$). Means + SEM. (b) Proportion of cases with positive and negative anal cytology as function of HPV16 L1 antibody titers. (c) Receiver operating characteristic curve for the serological detection of anal HSIL. The AUC was 0.92. Youden index was highest at the antibody cut offs 200 ng/ml ($J = 0.75$). ASCUS, atypical squamous cells of undetermined significance; AUC, area under the curve; cyto, cytology; HPV16, human papillomavirus type 16; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesion; neg., negative; NILM, negative for intraepithelial lesions or malignancy; pos., positive; vs. versus.

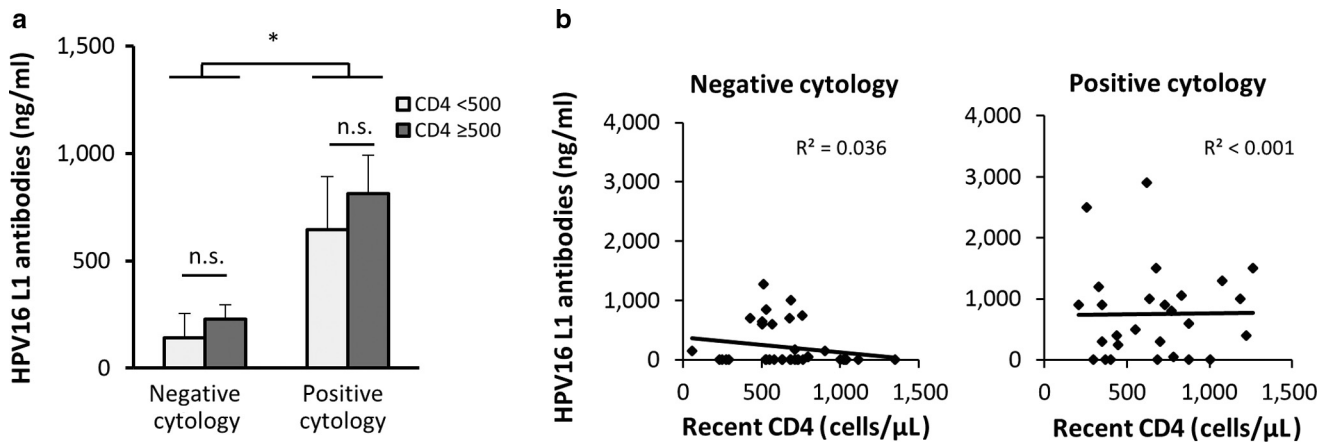


Figure 2. No significant association between HPV16 L1 antibodies and recent CD4⁺ T-cell count. (a) HPV16 L1 antibody titers were significantly associated with positive anal cytology (univariate analysis, main effect, $*P < 0.05$) but not CD4 group (recent CD4⁺ T cells < 500 or ≥ 500 cells/ μ L, no main or interactive effects, all $P > 0.45$). (b) No significant correlation between HPV16 L1 antibodies and recent CD4⁺ T-cell count in patients with negative or positive anal cytology (Pearson's correlation, all $r < 0.02$, $P > 0.90$), or when considering the entire study population ($r = 0.005$, $P = 0.97$; not shown). HPV16, human papillomavirus type 16; n.s., nonsignificant.

identify patients in most urgent need of more detailed assessments (e.g., high-resolution anoscopy), thereby contributing both to increased patient compliance and to a more efficient use of limited resources.

In summary, our study shows high concordance between anal cytology and HPV16 L1 serology in an anal cancer screening setting. Should the results of this pilot study be confirmed in larger trials, the HPV16-specific rapid test used in this study has the

potential to offer a noninvasive, cost-effective, and easy-to-use additional tool in the fight against human papillomavirus-induced anal cancer in PLWH.

Data availability statement

Datasets related to this article can be found at:

<https://data.mendeley.com/datasets/gnsfxwwtdj/draft?a=9b857a09-9cf3-4804-8f42-5723143572fe>, an open-source online data repository hosted at

Mendeley Data (Chen and Maloof, 2017). Additional data are available on request from the corresponding author.

ORCIDiS

Sara Ecke: <http://orcid.org/0000-0002-5300-0093>

Anna Huber: <http://orcid.org/0000-0003-1411-8256>

Ralf Hilfrich: <http://orcid.org/0000-0002-4342-2495>

Lars French: <http://orcid.org/0000-0002-4629-1486>

Markus Reinholz: <http://orcid.org/0000-0002-0465-3506>

AUTHOR CONTRIBUTIONS

Conceptualization: SE, AH, RH, LF, MR; Data Curation: SE, LF, MR; Formal Analysis: SE, AH, RH, LF, MR; Funding Acquisition: SE, AH, RH, LF, MR; Investigation: SE, MR; Methodology: SE, AH, RH, LF, MR; Resources: SE, AH, RH, LF, MR; Supervision: LF, MR; Validation: SE, AH, RH, MR; Visualization: SE, AH, RH, MR; Writing - Original Draft Preparation: SE, LF, MR; Writing - Review and Editing: SE, AH, RH, LF, MR

CONFLICT OF INTEREST

MR has received honoraria as a speaker for Abviris Deutschland GmbH, MSD, Abviris, and Mylan/Viartis and has served on the Advisory Board for MSD. AH is an employee of Abviris Deutschland GmbH. RH is inventor of the assay, was an employee of Abviris Deutschland GmbH at the time of study, and is a founder and shareholder of Abviris. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Florian Bergauer for performing the cytological analysis and Gertraud Kaiser-Darofß for assistance in our department. The patients in this manuscript have given written informed consent to publication of their case details.

**Sara Ecke¹, Anna Huber²,
Ralf Hilfrich³, Lars French^{1,4} and
Markus Reinholz^{1,*}**

¹Department of Dermatology and Allergy, University Hospital, Ludwig Maxmilian University of Munich, Munich, Germany;

²Abviris Deutschland GmbH, Ahrensburg, Germany; ³Cytoimmun Diagnostics GmbH, Pirmasens, Germany; and ⁴Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Miami, Florida, USA

*Corresponding author
e-mail: markus.reinholz@med.uni-muenchen.de

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