ORIGINAL ARTICLE

Retrospective analysis of alpha-human papillomavirus (HPV) types in tissue samples from anogenital dysplasias – introduction of the RICH (Risk of HPV-related Carcinoma in HIV$^{+/-}$ patients) score

J.A. Hildebrand,[1,†] A.J. Fischbeck,[1,†] B. Hundsdoerfer,[1] B.M. Clanner-Engelshofen,[1] E. Marsela,[1] A. Wollenberg,[1] M. Flaig,[1] L.E. French,[1] M. Reinholz[1,*]

[1]Department of Dermatology and Allergology, University Hospital of Munich (LMU), Munich, Germany
*Correspondence: M. Reinholz. E-mail: markus.reinholz@med.uni-muenchen.de

Abstract

Background Chronic viral infections caused by highly contagious human papillomaviruses (HPVs) from the alpha genus are a substantial risk factor for tumour diseases.

Objectives The goal of this study was to compare the HPV infection pattern with histology in a patient group of immunocompromised HIV^{+} and non-immunocompromised patients with anal intraepithelial neoplasia.

Materials and Methods Tissue samples (n = 210) from the anogenital area of 121 patients underwent retrospective histological and molecular examination for HPV DNA prevalence by chip analysis. The study was part of a cancer screening from the Dermatology Department of the LMU Munich, Germany. All data were collected and processed anonymously.

Results HPV 6 or 11 are more abundant in tissue samples from histologically diagnosed condylomata acuminata (47.7%) compared to grade 1, 2, and 3 intraepithelial neoplasias (IN 1-3). Detection of high-risk (hr) alpha-HPV DNA was significantly higher in tissue samples from IN 3 (67.5%) compared to IN 1 and 2 (12.9%), and compared to condylomata acuminata (29.5%). No HPV types were detected in histologically unremarkable tissue samples. There was a significant association between the prevalence of HPV 16 and the classifications IN 1 to IN 3 (χ^2 (2) = 13.62, P = 0.001). We identified a significant correlation between the prevalence of high-risk and low-risk (lr) HPV types and HIV, especially mixed infections of different HPV types correlated with high-grade IN. Based on the present data, we suggest the risk of carcinoma in HIV$^{+/-}$ patients (RICH) score and test it in the 121 patients.

Conclusions hr alpha-HPVs, mainly HPV 16, are associated with increased oncogenic potential of premalignant lesions (IN 1-3), especially in HIV^{+} patients. Based on the combination of HIV/HPV-testing and histological analysis, we identified correlations that could potentially forecast the risk of malignant transformation and summarized them in the form of RICH score.

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Conflicts of Interest

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Introduction

Chronic viral infections are a major risk factor for neoplastic diseases and of high interest in clinical research.[1] Of the 2.2 million new cancer cases attributable to carcinogenic infections worldwide in 2012, 640 000 were caused by human papillomaviruses, thus constituting the second largest contributor to the carcinogenic infections after *Helicobacter pylori* (770 000 cases).[2] Human papillomaviruses belong to the ever-growing family of papillomaviridae, as defined by the International Committee for Taxonomy of Viruses (ICTV). They are non-enveloped DNA viruses with a small, annular, double-stranded genome consisting of about 6.800-8.000 bp.[3] Based on their DNA, five genera can be subdivided in more than 225 genotypes of papillomaviridae: alpha (α)-papillomavirus, beta (β)-papillomavirus, gamma (γ)-papillomavirus, mu (μ)-papillomavirus and nu (ν)-papillomavirus.[4]

Human papillomaviruses infect multi-layered epithelia like keratinocytes of the mucosa or cutaneous epithelium, and can also be

[†]Contributed equally.

subdivided into so-called 'low-risk' (lr) and 'high-risk' (hr) HPVs, based on their risk of inducing neoplastic transformation.[5,6]

Alpha-papillomaviruses mainly infect the anogenital tract and can cause various benign and malignant tumours according to their oncogenic potential.[7] The main risk factor for the development of anogenital squamous cell carcinoma (SCC) is the persistent infection with hr HPV.[8] In cervical carcinoma, hr HPV types were detected in 99.7 % of cervical cancers.[9] Furthermore, hr HPVs are associated with 43 % of vulvar cancers, with 50 % of penile carcinomas, with 70 % of vaginal carcinomas and with 88 % of anal carcinomas of both sexes.[10] The dominant hr HPV types in anogenital carcinomas are HPV 16 (range, 40.9–82.2 %) and HPV 18 (44.7 % of adenocarcinomas of the cervix, 2.6–18.1 % at other locations).[11]

The human pathogenic papillomaviruses are strictly epitheliotropic. Most HPV infections are transient infections, asymptomatic or subclinical and therefore not recognized. They usually heal in about 4 to 20 months and do not cause neoplasia,[12] whereas in some cases, for example in HIV^+ patients, HPV infections can increase the incidence of cancer.[13–15] The overall life expectancy of HIV^+ patients has increased in the last two decades due to the wide use of antiretroviral therapy (ART).[16–18] Additionally, anal intraepithelial neoplasia of grade 2 or 3 can be reduced by using quadrivalent HPV vaccines in men who have sex with men (MSM).[19]

Nowadays, the incidence of cervical cancer can be reduced by 83 % through specific precautionary vaccination against the HPV types 16,18, 31, 33, and 45.[20]

The aim of this study was to compare the HPV infection pattern with histology in immunocompromised and non-immunocompromised patients. Moreover, the histological grading was compared to the molecular analysis to distinguish the different entities. Based on the results, a predictive score was established and evaluated to determine the risk of developing IN in immunocompromised patients. This score is expected to improve clinical assessment.

Materials and methods

Patient population

The patient collective of this retrospective study consists of patients presenting at the Department of Dermatology of the Ludwig-Maximilian-University in Munich, Germany, from February 2010 to August 2015 who were treated according to the guidelines for prevention of anal cancer of the German AIDS Society. The study was independent of gender, age or nationality. All data were collected and processed anonymously.

Ethics committee

The ethics committee of LMU Munich reviewed and approved the ethical safety of the planned fully anonymized retrospective study of the prevalence of HPV types in diagnostic scans and tissue samples of cutaneous neoplasia in immunosuppressed and non-immunosuppressed patients.

Data collection

The data were collected from the electronic patient file. Additionally, for diagnostic purposes stored biopsies were re-analysed for HPV types.

Statistical evaluation

All data were statistically analysed with SPSS (Statistics® Version 23 IBM Inc, Armonk, NY, USA). The corresponding images were generated using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA/USA). Significance testing (significance level $\alpha = 0.05$) was performed using the Pearson chi-square test and Fisher's exact test. The strength of each effect was determined by Cramer's V test.

Patient group

Out of 150 patients, 29 patients (19.3 %) had to be excluded from this study because of incomplete data sets or insufficient tissue material. The gender distribution of the remaining 121 patients included 14 women (11.6 %) and 107 men (88.4 %).

The mean patient age (years ± SD) was 47.8 ± 14.3 years. The youngest patient was 24 years, the oldest 87 years old.

Tissue samples

Anogenital biopsy tissue samples were taken during clinical routine controls that included biopsy and were stored in the histological archive. For this study, they were retrospectively analysed and tested for HPV.

Samples were collected from February 2010 to August 2015, fixed with 10 % formalin (Merck KGaA, Darmstadt, Germany), paraffin (Merck KGaA) embedded and HE-stained (Merck KGaA, Darmstadt, Germany). Originally, only HIV^+ patients were considered. However, we did not find enough HIV^+ patients to test for HPV types. Therefore, we analysed samples from our histological database (ZOC-Database).

One or more tissue samples from each patient were analysed retrospectively. In total, 245 tissue samples were analysed; after the presence of tissue in the paraffin-embedded samples could be confirmed, a total of 210 (85.7 %) tissue samples underwent further histological and molecular genetic analysis.

From 59 patients (48.8 %) one tissue sample, from 39 patients (32.2 %) two tissue samples, from 20 patients (16.5 %) three tissue samples, from two patients (1.7 %) four tissue samples and from one patient (0.8 %) five tissue samples were collected.

Histological evaluation

All 210 tissue samples underwent histopathological examination by a board-certified dermatohistopathologist who assigned them to their diagnostic entities.

Localization distribution

Tissue samples were evaluated according to their localization and gender distribution. In male patients, 13 perianal (7.0 %), 20 anal (10.8 %), 32 penile (17.2 %) and 121 intraanal (65.0 %) tissue samples were collected. In female patients, one anal (4.2 %), one perianal (4.2 %), 8 intraanal (33.3 %) and 14 vulval (58.3 %) tissue samples were collected.

HIV status distribution depending on gender

The examined samples were divided in terms of gender and the presence of HIV disease. Of the 107 male patients, 59 (55.1 %) were HIV-positive, 22 (20.6 %) were HIV-negative, and in 26 (24.3 %) patients, the HIV status was unknown. Out of 14 female patients, three (21.4 %) were HIV-positive, seven (50.0 %) were HIV-negative, and four (28.6 %) had an unknown HIV status.

Chip analysis: Low-cost density (LCD) array HPV Type 3.5

The DNA chips from Chipron (Berlin/Germany) were developed for the identification and differentiation of clinically relevant human alpha-papillomaviruses, in total 32 different types (6, 11, 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 70, 72, 73, 81, 82, 83, 84, 90, 91). The kits consist of a primer system as well as HPV-chip arrays. The primer system 'MY09/11' is based on the sequences MY09 and MY11 and generates PCR amplifications of the size of about 450 base pairs. To prove the overall conditions (primer quality, PCR success), an agarose gel electrophoresis preceded the chip analysis. Besides, the additional PCR kit was performed according to the manufacturer's instructions and evaluated with the company's SlideReader and V12 software (Chipron).

Generation of the RICH (Risk of Carcinoma in HIV$^+$ patients) score

Based on the histological findings, chip analysis and serological HIV testing, the RICH score was generated and patients tested accordingly.

Results

Histological grading

Out of 114 (54.3 %) tissue samples with an IN, 15 samples displayed a grade 1 (IN 1), 16 samples a grade 2 (IN 2) and 83 samples a grade 3 (IN 3) neoplasia. Those tissue samples were obtained from the anal, the vulvar or the penile area. 88 of the 210 specimens (41.9 %) were histologically diagnosed as condylomata acuminata. Eight (3.8 %) samples were histologically inconspicuous.

Distribution of hr HPV, lr HPV and mixed infections in comparison with histological findings

Tissue samples were subdivided into four HPV status dependent groups: Without HPV, high-risk HPV (hr HPV) only, low-risk HPV (lr HPV) only and mixed infections (hr HPV and lr HPV). These groups were compared with respect to their histological findings ranging from unremarkable, IN 1, IN 2, IN 3, and condylomata acuminata. Of the 32 potentially detectable HPV types, DNA from HPV 35, 51, and 73 was never detected. All other types delivered type-specific results with incidences between approximately 1 % and 50 % (data not shown). No evidence for present HPV DNA could be found in any of the specimens that were histologically unremarkable. In the IN 1 group, the hr HPV type 16 was identified in one sample (6.7 %), while the remaining 14 samples were HPV negative or contained at least one lr HPV type. In the IN 2 group, at least one hr HPV type was identified in three samples (18.9 %). 13 samples were HPV negative or contained at least one lr HPV type. In the IN 3 group, at least one hr HPV type was identified in 56 samples, while 27 samples were HPV negative or contained at least one lr HPV type. This corresponds to a prevalence of hr HPV in 67.5 % of the IN 3 samples. Within the group of condylomata acuminata, four samples out of 88 in total were positive for at least one hr HPV type and 22 samples for at least one hr HPV type in combination with one or more lr HPV types. Hr HPV prevalence was 29.5 % among all condylomata acuminata. (Table 1)

Comparison of HPV detection using chip versus histological analysis

The detection of the HPV types in IN 3 showed the same sensitivity when the molecular analysis and the histological method were compared. For the HPV types IN 1 and IN 2, the molecular analysis was less sensitive compared with histology (Fig. 1).

Analysis of histological grading and molecular genetic HPV detection

Hr HPV and lr HPV were compared to their histological grading. There was a significant correlation between the presence of hr or lr HPV types and the histology (χ^2 (3) = 44.51, $P \leq 0.001$ / Fisher's exact test: $P \leq 0.001$) (Table 2).

Correlation analysis of condylomata acuminata and lr HPV type 6 and 11: Between condylomata acuminata, unremarkable, and IN 1 and 2, no significant difference in the prevalence of general lr HPV types could be determined. The effect is of medium strength with Cramer's V = 0.46.

70 out of 88 condylomata acuminata (79.5 %) samples were positive for lr HPV 6 or 11 with HPV 6 infection being the most prevalent type of single HPV infection (34.1 % of all condylomata samples), followed by single HPV 11 infection (13.6 %).

These two types were observed at an above-average frequency of 47.7 % in condylomata acuminata in comparison to IN 1, IN 2 and IN 3 (χ^2 (3) = 40.97, $P \leq 0.001$). This effect is of medium strength (Cramer's V = 0.44). Co-infection of HPV 6 and HPV 11 could not be detected in any of the analysed samples.

Table 1 Comparison of HPV combinations after CHIP analysis and histological grading

		Unremarkable *N* = 8 (3.8%)		Condyl. ac. (CA) *N* = 88 (41.9%)		IN 1 *N* = 15 (7.2%)		IN 2 *N* = 16 (7.6%)		IN 3 *N* = 83 (39.5%)		Total *N* = 210 (100.0%)	
		N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
no HPV	**No subtype detected**	**8**	**100**	**10**	**11.4**	**9**	**60**	**9**	**56.3**	**11**	**13.3**	**47**	**22.4**
hr HPV	16	–	–	2	2.3	1	6.7	1	6.3	29	34.9	33	15.7
	18	–	–	1	1.1	–	–	1	6.3	2	2.4	4	1.9
	18 + other hr	–	–	–	–	–	–	–	–	1	1.2	1	0.5
	16 + 18	–	–	–	–	–	–	–	–	3	3.6	3	1.4
	16 + other hr	–	–	–	–	–	–	–	–	2	2.4	2	1
	other hr only	–	–	1	1.1	–	–	–	–	8	9.6	9	4.3
lr HPV	6	–	–	30	34.1	3	20	–	–	7	8.4	40	19
	6 + other lr	–	–	5	5.7	1	6.7	–	–	2	2.4	8	3.8
	11	–	–	12	13.6	–	–	–	–	1	1.2	13	6.2
	11 + other lr	–	–	1	1.1	–	–	–	–	–	–	1	0.5
	6 + 11	–	–	–	–	–	–	–	–	–	–	–	–
	other lr only	–	–	4	4.5	1	6.7	4	25	6	7.2	15	7.1
hr + lr HPV	6 + other hr	–	–	2	2.3	–	–	–	–	2	2.4	4	1.9
	11 + other hr or other hr + other lr	–	–	4	4.5	–	–	–	–	1	1.2	5	2.4
	16 + other lr	–	–	2	2.3	–	–	1	6.3	1	1.2	4	1.9
	16 + other hr + other lr	–	–	–	–	–	–	–	–	1	1.2	1	0.5
	6 + 18 (+other hr + other lr)	–	–	2	2.3	–	–	–	–	–	–	2	1
	6 + 16 (+other hr + other lr)	–	–	2	2.3	–	–	–	–	3	3.6	5	2.4
	6 + 11 + other hr	–	–	1	1.1	–	–	–	–	–	–	1	0.5
	6 + 11 + 16	–	–	1	1.1	–	–	–	–	–	–	1	0.5
	11 + 16 (+ other hr)	–	–	5	5.7	–	–	–	–	3	3.6	8	3.8
	11 + 18 (+ other lr)	–	–	3	3.4	–	–	–	–	–	–	3	1.4

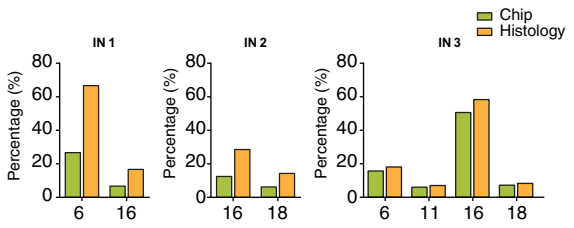

Figure 1 Frequency distribution of HPV types in entities IN 1-3 and their correlation and comparison of histology and molecular analysis in the distribution of the HPV types.

Correlation analysis of the histological grading and the molecular genetic analysis of hr HPV types: Overall, we observed a significant difference in hr HPV prevalence of the IN 3 group (67.5 %) compared with IN 1, IN 2 (12.9 %) and condylomata acuminata (29.5 %). Detection of any hr HPV type was observed in 65.1 % of examined IN 3 samples. HPV 16 was the most dominant type and was detected in 50.6 % of all IN 3 samples. There was a significant correlation between the prevalence of HPV 16 (but without HPV 18) and the classifications IN 1 to IN 3 (χ^2 (2) = 13.62, $P \leq 0.001$). This effect is of medium strength (V = 0.35). Moreover, in IN 3 samples 80.4 % of the total hr HPV infections were single hrHPV infections with HPV 16 being the most prevalent single infection (34.9 %). Only 18.2 % of hr HPV infections were single infections in the lesions histologically identified as condylomata acuminata. There was no significant correlation between the prevalence of HPV 18 (without HPV 16) and oncological grading (exact Fisher test: $P = 0.725$, V = 0.09).

Comparison of HIV status, molecular genetic results and histological findings

A significant correlation was observed between the prevalence of hr and lr HPV types and the oncological grading based on histology in all three groups (HIV$^+$, HIV$^-$, HIV unknown; $p \leq 0.01$). Accordingly, hr HPVs predominated the IN 3 samples of all three groups unaffected by the HIV status. However, a higher percentage of hr HPV types was detected in the IN 3 samples of patients of HIV$^-$/unknown status (82.4 % and 72.7 %, respectively) compared with those of HIV$^+$- patients (59.1 %). In contrast, in the condylomata acuminata and in the IN 1 and 2 of HIV$^+$-patients a higher percentage of hr HPV types was identified than in patients with HIV$^-$/unknown status. On the other hand, HIV$^+$ patients showed the highest percentage (40.9 %) of lr HPV types throughout the IN 3 samples compared with patients of HIV$^-$/unknown status (17.6 % and 27.3 %), while overall in the condylomata acuminata and in IN 1 and 2 samples of all patients the highest amount of lr HPVs could be detected. (Table 3)

Introduction of the RICH score

Based on our results, we introduce the RICH score that is calculated using the data received from histology, chip analysis and serological HIV testing combined (Table S1). Based on the histological finding, we suggest attributing 3 points to an IN 3 confirmation, two points to an IN 2 diagnosis and 1 point if IN 1 or condylomata acuminata are detected. A chip analysis detecting hr HPVs adds 2 points to the score, lr HPVs add 1 point, while the detection of both lr and hr HPVs adds 3 points. Moreover, HIV$^+$ patients receive additional 3 points, whereas HIV$^-$ patients are not additionally scored. Scores <3 are a reference point to proceed with normal screening procedures once a year. Patients with scores between 3 and 4 should pay particular attention and consult a doctor semi-annually or whenever they recognize signs of disease progression. Scores >4 recommend precise screening every 3 months, including high-resolution anal anoscopy and biopsy (Fig. 2). Given the case that only cytology/HPV status and HIV status are available, we suggest screening procedures every 3 months if patients receive a score >2.

Testing the efficacy of the RICH score in 121 patients

The data of the 91 fully assigned (with available HIV status) patients were used to assess their RICH score (Fig. 3). Of note, most patients were HIV$^+$ and therefore commonly had RICH scores >4, receiving recommendation for precise screening procedures every three months. Moreover, the RICH score could clearly separate patients that have a low risk of disease progression.

Discussion

Human alpha-papillomavirus infections are increasingly related to malignancy of tumours in female and male individuals.[7,21]

Table 2 HPV detection via histological grading and molecular analysis

		Unremarkable		Condyl. ac.		IN 1 + 2		IN 3		Total	
		N	%	*N*	%	*N*	%	*N*	%	*N*	%
lr HPV	All lr HPV types	8	0	62[a]	70.5	27[a]	87.1	27[b]	32.5	124	59
	HPV 6 + 11 only	0[a,b]	0	42[a]	47,7	3[b]	9,7	8[b]	9,6	53	25,2
hr HPV	All hr HPV types	0	0	26[a]	29.5	4[a]	12.9	56[b]	67.5	86	41

The superscript letters should be read line by line.
Different superscript letters indicate a significant difference ($P \leq 0.05$, correction of Bonferroni's significance level).

Table 3 Distribution of hr HPV and lr HPV according to HIV status and compared to histological grading

	P<0.001	Unremarkable		Condyl. ac.		IN 1+2		IN 3		Total	
		N	%	*N*	%	*N*	%	*N*	%	*N*	%
HIV –	Low-risk	$2^{a,b}$	100	22	84.6	$2^{a,b}$	100	3^{b}	17.6	29	61.7
	High-risk	$0^{a,b}$	0	4	15.4	$0^{a,b}$	0	14^{b}	82.4	18	38.3
HIV +	Low-risk	6^{a}	100	$21^{a,b}$	52.5	19^{a}	82.6	18^{b}	40.9	64	56.6
	High-risk	0^{a}	0	$19^{a,b}$	47.5	4^{a}	17.4	26^{b}	59.1	49	43.4
HIV?	Low-risk	–	–	19^{a}	86.4	6^{a}	100	6^{b}	27.3	31	62
	High-risk	–	–	3^{a}	13.6	0^{a}	0	16^{b}	72.7	19	38

The superscript letters should be read line by line.
Different superscript letters indicate a significant difference ($P \leq 0.05$, correction of Bonferroni's significance level).
Question mark indicates unknown HIV status.

While condylomata acuminata belong to HPV-induced benign tumours, long-lasting infection with oncogenic HPV types can cause IN in anogenital areas as well as anogenital carcinoma.[22–24] HPV types 16 and 18 are already prevalent in 60-70 % of premalignant cases.[8]

HIV/HPV co-infection

Immunocompromising diseases like HIV infections encourage HPV infection and strongly increase the risk of developing IN or anogenital carcinoma.[22,25] In accordance with this correlation, hr HPV types were detected in only 15.4 % of the condylomata acuminata of HIV^{-} patients. In contrast, hr HPV types were present in 47.5 % of HIV^{+} patients with condylomata acuminata. This indicates that HIV may also increase the risk of malignant transformation of condylomata acuminata.[26] Surprisingly, this correlation could not be confirmed in the entity of IN 3 which showed a decrease of hr HPV types in HIV^{+} samples, but an increase in lr HPV types. However, information about the duration of HIV infection as well as the HIV status in 23.8 % of the samples is missing. Another limitation is the heterogeneous group of samples (perianal, anal, penile, vulvar) and small sample numbers in some of the subgroups. Moreover, this study lacks a T-cell count correlating with immune function. Formerly, therapy derived immunosuppression was supposed to have no influence on the infection with HPV.[22] Literature about HPV status under immunosuppression is controversial, and our data clearly indicate a correlation between HIV prevalence and HPV infection.

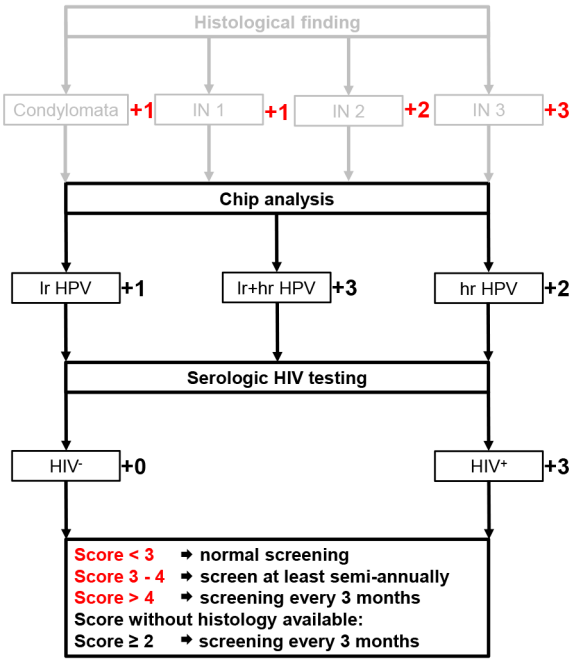

Figure 2 Dendrogramm for the evaluation of the RICH score. This screening score is calculated by going through the dendrogram from the top to the bottom, thereby building the sum of the numbers. The score can still be used if histology is not available (without the light grey part).

Chip testing vs. histology

In IN 1 and 2, the molecular analysis was more sensitive in detecting HPV types compared to histological examination. The detection sensitivity in IN 3 was however similar using both

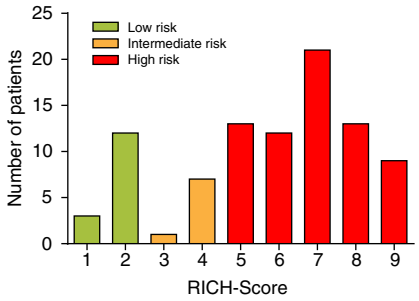

Figure 3 Distribution of patients among RICH scores from 1 to 9. Green bars for low-risk, orange bars for intermediate-risk, red bars for high-risk patients.

methods. We hereby conclude that molecular analysis is more precise in distinguishing HPV types in IN 1 and 2. This might be due to the sensitivity of this PCR based method, being able to detect low amounts of HPV DNA.

Correlation of hr HPV with the oncologic grade (IN 1-3)

We observed an increased hr HPV prevalence that correlates positively with a higher oncological grade: IN 1 (hr HPV = 41.9 %) compared to IN 3 (hr HPV = 86.7 %).

Among both entities an increase of hr HPV 16 (IN 1 = 6.67 %, IN 3 = 50.6 %) and HPV 18 (IN 1 = 0 %, IN 3 = 7.2 %) was detected.

In 13.3 % of IN 3 samples, no HPV was detected. We speculate that not enough DNA material was present. Another reason might be that the used chip detected only 32 of over 200 yet discovered HPV types. Based on our data, we speculate that the potentially not detected HPV types may still have oncogenic potential.

Mixed hr HPV infections as a major risk factor

Most hr HPV mixed infections were observed in IN 3, but not in condylomata acuminata. We speculate that mixed infections might increase the risk of malignant transformation. Moreover, we detected a variety of HPV types and mixed infections even in low-graded neoplasias (IN 1 and 2). We speculate that low-graded neoplasias with mixed infections are more likely to turn into IN 3. That is why even IN 1 and 2 patients with numerous HPV types should perform regular screening procedures.

Furthermore, it was shown that condylomata acuminata contained many different HPV types. Among these, 29.5 % of the cases showed hr HPV types (HPV 16 = 13.6 %, HPV 18 = 6.8 %, HPV 39 = 5.7 %). This explains why benign tumours like condylomata acuminata have oncogenic potential. Hence, new prevention procedures against HPV infections might be effectively preventive.

Preventing IN 1-3 by early HPV vaccination

The two vaccines Gardasil® (Merck Sharp & Dohme Corp, USA) and Cervarix® (GlaxoSmithKline, UK) revealed high efficacy and cross-protection to other HPV types.[20,27] Gardasil 9, for instance, targets the HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58.[27] After introducing a public vaccination programme in Australia, a significant decrease of HPV infections was observed.[28,29]

Furthermore, HPV vaccination is now recommended for boys between 9 and 14 (17) years old by the German Standing Committee on Vaccination (STIKO).[30]

As IN are the precursor lesions for anogenital carcinomas,[23] we find that screening programmes for the recognition and treatment of HPV infections should be promoted, especially for immunosuppressive patients.

The American STI-guidelines of the centre for disease control recommend a vaccination for men to produce herd immunity.[31] HPV is mainly sexually transmitted and fulfils the criteria for a global pandemic. Often young patients in the sexually active age are affected by HPV infection and therapy is difficult, long-winded and expensive. The vaccination is approved in many countries for girls and boys between the ages of 9 and 17 to prevent anogenital dysplasia.[32]

Introduction of the RICH score

The higher the histopathological grade of anal intraepithelial neoplasia the higher the risk of developing anal cancer, especially in HIV^+ patients.[33] Additionally, almost all precursor lesions and anal cancers are hr HPV positive, indicating that early screening procedures are beneficial.[23,34] Anal cancer screening should be performed in all HIV^+ patients and high-risk patients, which are marked by a history of condylomata acuminata, or IN, or HPV-associated cancer, or persistent hr HPV infection.[35] For example, IN 3 receiving topical treatment should be repeatedly screened with inspection, palpation, swab, cytology, anoscopy and if necessary biopsy.[35] This is also reflected in the RICH score which compared to other screening algorithms (like presented in [35]) facilitates the analysis and may therefore be suitable for clinical routine. We also included condylomata acuminata in the score since it lacks control and elimination of an HPV infection (similar to IN 1-3), and it is also associated with an increased prevalence of anal cancer in HIV^+ patients.[35] Condylomata is caused by lr HPV infection, indicating that even lr HPV types can also be sufficient to develop anal cancer after persistent infection. To improve the outcome of yet infected $HPV^{+/-}$/$HIV^{+/-}$ patients with anal intraepithelial neoplasia or condylomata acuminata, we generated the RICH score. This screening tool was generated based on our observations and literature and suggests differential examination intervals based on HIV status, molecular genetic results and histological findings, similar to studies performed for anal cancer.[36,37] Screening procedures should include high-resolution anal anoscopy and biopsy. Moreover, patients at low risk could clearly be separated from high-risk patients, while a large proportion was assigned to the area between high and low risk. Especially these patients may be regrouped at their semi-annual visits. This study though mainly includes HIV^+ patients, which automatically result in high RICH scores. We conclude that the RICH score offers an easy tool to assign patients to different risk groups.

However, further studies are required to show the outcome of HIV^+ and HIV^- patients using screening approaches based on recommendations of the RICH score.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Calculation of the RICH score from the 121 fully anonymized patients included in this study. Highlighted in yellow are the results based on histology, HPV and HIV testing that were used for Figure 3.