High-risk human papillomavirus type does not predict grade of cervical intraepithelial neoplasia

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Summary

Purpose of Investigation: The aim of this study was to examine whether HPV testing specificity for cervical intraepithelial neoplasia (CIN) grades 2 or 3 could be improved by restricting the range of HPV types classified as 'high-risk'.

Methods: DNA was extracted from 28 CIN I, nine CIN II and 13 CIN III formalin-fixed, paraffin-embedded biopsies. HPV type was determined by General Primer mediated 5+/6+ PCR assay.

Results: The prevalence of specific HPV types among the different grades of CIN and the relationship to the referral smear diagnosis was examined. HPV type-16 alone was more highly associated with CIN grade ($p < 0.0001$; Specificity = 0.93; Sensitivity = 0.68) than was the group of HPV types collectively classed as high-risk ($p = 0.025$; Specificity = 0.23; Sensitivity = 1.00).

Conclusions: These data suggest HPV testing specificity could be improved simply by including a separate test for HPV-16. In conjunction with previous studies, the data also suggests redefinition of the high-risk HPV category to take into account the differing degrees of oncogenicity of high-risk HPV types.

Key words: Human papillomavirus; Cervical intraepithelial neoplasia; ASCUS; Cervical screening.

Introduction

Human papillomaviruses (HPV) have been identified as a necessary cause of cervical cancer and virtually 100% of cervical carcinomas are HPV-positive suggesting HPV testing could have a useful role in improving cervical screening program sensitivity [1]. However the relationship between HPV infection and cervical carcinogenesis is not straightforward and HPV testing remains controversial.

It is estimated that 80% of women will acquire an HPV infection at some point during their lifetime [2]. However the majority of these infections are transient and it is suggested only approximately 20% ever result in recognizable cervical intraepithelial neoplasia (CIN) [3]. HPV has also been established as a necessary cause of CIN [4], however a meta-analysis of over 15,500 CIN lesions (all grades) indicates only 2% may progress to invasive carcinoma if left untreated [5]. This demonstrates that HPV is not a sufficient cause of cervical cancer and this is perhaps the central difficulty in defining a useful HPV test.

Over 100 HPV types have been identified and over 40 have been isolated from genital lesions. For clinical purposes these HPV types are currently broadly classed as 'low-risk', 'high-risk' or of unknown risk. HPV 6, 11, 40, 42, 43, 44 are examples of low-risk types. The high-risk types the Hybrid Capture 2™ (HC 2) assay is designed to recognize include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Application of HC 2 to cytologically defined low-grade squamous intraepithelial lesions (LSIL) found 83% were infected with a high-risk HPV type [6]. Additionally, it has been found that the majority of transient HPV infections are high-risk type positive [7]. Clearly these data raise questions about the usefulness of high-risk HPV type as a marker for CIN grades II or III, invasive carcinoma or for lesions that will progress.

The aims of this study were: 1) determination of the distribution of HPV types individually, or collectively classed as low-risk or HR among CIN lesions; 2) analysis of the relationship between the cytologic diagnosis (abnormal-squamous cells of uncertain significance [ASCUS], LSIL or HSIL), HPV type and biopsy diagnosis, and; 3) determination of the potential for improving HPV test specificity by re-classification of the high-risk HPV category.

Materials and Methods

Samples: Twenty-eight CIN I (four condylomatous), nine CIN II, and 13 CIN III formalin-fixed paraffin-embedded (FFPE) lesions were collected from Fletcher Allen Health Care Pathology archives. The referral smear diagnosis that preceded the biopsy samples was obtained by a retrospective search of patient records and in accordance with Institutional Review Board regulations.

DNA Extraction: Three 10 μm sections were cut into tubes from each FFPE tissue block, dewaxed with xylene, washed with ethanol and air-dried. Tissues were digested overnight at 55°C with 400 μg/ml proteinase K (Life Technologies) in 200 μl – 400 μl 50 mM Tris-HCl pH 8.0. Supernatant, obtained following tissue debris pelleting by centrifugation, was used directly for PCR amplification.

Determination of HPV Type: An adaptation of the general primer mediated 5+/6+ PCR method was used to determine HPV type as described elsewhere [8]. The method as used

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enabled the detection of 22 HPV types (HPV types 6, 11, 16, 18, 31, 33, 34 35, 39, 40, 42, 43, 44, 45, 51, 52, 54, 56, 58, 59, 66, 68).

Statistics: Chi-square analysis was used to test for linear trends in the proportions of high-risk HPV types and HPV 16 with increasing CIN grade. For other analyses CIN II/III were considered as one group and the sensitivity, specificity, odds ratio (OR), and Fisher's Exact test were calculated to assess the association of HPV 16 and other high-risk types with CIN grade. Analyses were performed using GraphPad InStat software (San Diego, CA).

Results

The results are summarized in Tables 1, 2 and 3. Fourteen HPV types were positively identified among the 50 samples. The HPV type found in two of the 28 CIN I samples could not be determined using the panel of 22 oligoprobes. Twenty-one women were referred for biopsy on the basis of an ASCUS smear test diagnosis, 11 women after an LSIL diagnosis and 16 women after an HSIL diagnosis. Statistical data are summarized in Tables 4 and 5.

HPV type and biopsy diagnosis

High-Risk Type and CIN Grade: Including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66 as high-risk [9], then 20 (77%) CIN I lesions and 22 (100%) CIN II/III lesions were high-risk HPV type positive (Table 1), indicating a statistically significant association of high-risk HPV with lesion grade ($p = 0.0248$). Sensitivity of high-risk HPV for CIN II/III was 100%, however specificity was poor with just 23% of CIN I lesions being high-risk HPV type negative. The OR indicates CIN II/III will occur more than 14 times as often in women exposed to high-risk HPV as in women positive for low-risk HPV (Table 4).

HPV 16 and CIN Grade: Using HPV 16 as a marker of lesion grade, a stronger association with CIN II/III is seen ($p < 0.0001$). Sensitivity is lower (68%) since other high-risk HPV types are associated with CIN II/III but specificity is good with 92% of CIN I lesions testing negative for HPV 16. The OR suggests CIN II/III will occur nearly 26 times as often in HPV 16-positive women as in women positive for another HPV type. The chi-square trend test indicates a highly significant correlation of HPV 16 across CIN grades I, II, and III ($p < 0.0001$).

Table 1. — *Distribution of HPV types among CIN I, II and II lesions. *One sample was positive both for HPV 6 and HPV 43.*

HPV type positivity	CIN I (n = 26)	CIN II (n = 9)	CIN III (n = 13)
Low-risk			
6	2*	–	–
11	2	–	–
43	3*	–	–
Percent low-risk	6/26 (23%)	0	0
High-risk			
16	2	3	12
18	1	1	–
31	1	–	2
33	1	1	–
35	1	–	–
39	3	–	–
45	1	1	–
51	3	–	–
52	2	–	–
56	2	1	–
58	–	1	–
66	3	–	–
Percent high-risk	20/26 (77%)	8/8 (100%)	14/14 (100%)
Percent HPV 16	2/26 (7.5%)	3/8 (37.5%)	12/14 (86%)

Table 2. — *Distribution of HPV types (determined from biopsy) relative to the LSIL, ASCUS and HSIL referral smear diagnosis. *One sample was positive both for HPV 6 and HPV 43.*

HPV type positivity	LSIL (n = 11)	ASCUS (n = 21)	HSIL (n = 16)
Low-risk			
6	–	2*	–
11	–	2	–
43	2	1*	–
Percent low-risk	2/11 (18%)	4/21 (19%)	0
High-risk			
16	–	7	10
18	–	–	2
31	1	1	1
33	1	1	–
35	–	–	1
39	1	2	–
45	1	–	1
51	3	–	–
52	–	2	–
56	1	2	–
58	–	–	1
66	1	2	–
Percent high-risk	9/11 (82%)	17/21 (81%)	16/16 (100%)
Percent HPV 16	0	7/21 (33%)	10/16 (62.5%)

Table 3. — *Relationship between HPV type and CIN I, II or III biopsy diagnosis for cases referred with an ASCUS smear diagnosis (n = 21). * One sample was positive both for HPV 6 and HPV 43.*

HPV type positivity	CIN I (n = 14)	CIN II (n = 2)	CIN III (n = 5)
Low-risk types			
6	2*	–	–
11	2	–	–
43	1*	–	–
Percent low-risk	4/14 (28.5%)	0	0
High-risk types			
16	2	1	4
31	–	–	1
33	1	–	–
39	2	–	–
52	2	–	–
56	1	1	–
66	2	–	–
Percent high-risk	10/14 (71%)	2/2 (100%)	5/5 (100%)
Percent HPV 16	2/14 (14%)	1/2 (50%)	4/5 (80%)

Table 4. — *Statistical relationships of high-risk HPV and HPV 16 to CIN II/III biopsy diagnosis. (ND – not done due to zero value in denominator.*

HPV type and CIN II/III	High-risk type	HPV 16
Sensitivity	1.00	0.68
Specificity	0.23	0.93
Odds Ratio (95% CI)	ND	27.86 (5.112 to 151.81)
p value	0.0248	< 0.0001

Table 5. — *Statistical relationships of high-risk HPV and HPV 16 to ASCUS cytology referrals (n = 21) subsequently diagnosed with CIN II/III. (ND – not done due to zero value in denominator).*

HPV type and ASCUS/CIN II/III Diagnosis	High-risk type	HPV 16
Sensitivity	1.00	0.71
Specificity	0.29	0.857
Odds Ratio (95% CI)	ND	15.000 (1.628 to 138.23)
Fishers exact p value	0.255	0.0107

HPV type, referral smear diagnosis and biopsy diagnosis

All 16 women referred with HSIL were positive for high-risk HPV types, ten (62.5%) were HPV 16 positive. Fourteen (87.5%) of these HSIL samples were diagnosed with CIN II/III on biopsy and two with CIN I (one HPV 18 positive and one HPV 35 positive). Ten of 11 (91%) women referred with LSIL were diagnosed with CIN I after biopsy and nine (82%) were positive for a high-risk HPV type but none were HPV 16 positive. One LSIL sample was diagnosed CIN II (HPV 33 positive) on biopsy. Seventeen of 21 (81%) women referred with an ASCUS smear were high-risk HPV type positive and seven of these (41%) were positive for HPV 16 (Table 2). Seven (33%) ASCUS referrals were diagnosed with CIN II/III on biopsy, each of these was positive for a high-risk type, and five (71%) were HPV 16 positive (Table 3).

High-Risk Type, ASCUS and CIN II/III: Among the cases of ASCUS, high-risk HPV type did not significantly distinguish those women who were subsequently diagnosed with CIN II/III (p = 0.255). Ten of 17 (59%) ASCUS referrals were CIN I on biopsy and high-risk HPV-type positive. No low-risk HPV-types were found among ASCUS/CINII/III cases so sensitivity was 100% however specificity was poor (41%). HPV 16 did distinguish the CIN grade in ASCUS cases; p = 0.0107, sensitivity=71%, specificity = 88%, OR = 15.000 (Table 5). A significant chi-square trend in the association of ASCUS referrals diagnosed as CIN I, II or III (HPV 16 positive) was also indicated (p = 0.008).

Discussion

The present study is the first to the authors' knowledge to determine HPV type on histologically defined pre-invasive lesions and relate this data to referral smear diagnoses. This approach has advantages over determining HPV type from smear cell samples and relating these data to a subsequent biopsy diagnosis. In particular, HPV assays of smear samples indicate multiple infections are common [6]. However studies of CIN lesions indicate only single HPV-type infections are found associated with a given CIN lesion [10]. Determining which of the HPV types identified from smear cells is causally associated with a given CIN grade cannot be determined without analyzing the biopsy tissue itself. Therefore the relationship between HPV type, smear diagnosis and biopsy diagnosis is best determined on the basis of HPV type detected in the CIN lesion.

The main findings of this study are that high-risk HPV is found in the majority of CIN I lesions and that HPV 16 is relatively rare in CIN I but common among CIN II/III lesions. Further, the majority of biopsies taken following an ASCUS smear diagnosis were high-risk HPV type positive but only a minority of these biopsies was diagnosed with CIN II/III. HPV 16 was the most common HPV type found among these biopsies. The majority of biopsies obtained after an LSIL referral were also high-risk HPV type positive but HPV 16 was not found among them. These findings have significant implications for HPV testing.

In this study 77% of CIN I were high-risk HPV-type positive and 82% of LSIL referrals were also high-risk HPV positive which is similar to the ALTS study estimate that 83% of LSIL are high-risk HPV positive [6]. Given that meta-analysis of over 4,500 (non-clinically treated) CIN I lesions indicated 60% of CIN I lesions regress, 30% persist and just 10% progress to CIN II or above [5], it follows that only a minority of high-risk HPV positive CIN I lesions progress. The significantly uneven distribution of HPV 16 between lesion grades (p < 0.0001) is consistent with the possibility that CIN I lesions positive for HPV 16 may have a much higher potential for progression than CIN I lesions positive for other high-risk types. This suggests there may be a case for a separate test specifically for HPV 16.

High-risk HPV sensitivity for CIN II/III and for ASCUS referrals CIN II/III on subsequent biopsy could not have been better, however the poor specificity due to the commonality of high-risk HPV in CIN I lesions limits high-risk HPV testing utility. It has been suggested that high-risk HPV testing could be useful in the management of the ASCUS diagnosis [11]. High-risk HPV-negative ASCUS could be excluded from the need for colposcopic referral. In the present study 17/21 (81%) of ASCUS were high-risk HPV positive suggesting the reduction in number of referrals may not be that significant.

There is a need to improve the specificity of HPV-based testing regimens for ASCUS and persistent LSIL diagnoses. It has been suggested that specificity could be improved by such measures as restricting testing according to patient age or increasing the time from (ASCUS) diagnosis to HPV testing [11]. The data from the present study suggest reappraisal of the high-risk category definition as an important additional means to improving specificity.

The high-risk HPV category is largely based on the findings of studies such as Bosch *et al.* [12] who collected HPV data from 932 cervical carcinomas gathered from around the world. Overall, approximately 50% of the carcinomas were HPV 16 positive, 14% were HPV 18 positive, 8% were HPV 45 positive and 5% were HPV 31 positive. Other HPV types currently classed as high-risk were identified in 1% to 3% of the carcinomas. Some ten years ago, an analysis of over 2,500 cytology samples (with diagnoses ranging from normal to invasive cancer) for 15 different HPV types found HPV types 16, 18, 45 and 56 were especially high-risk for invasive disease [13]. A classification of HPV types as high-risk (HPV types 16, 18, 45, 56), intermediate-risk (HPV Types 31, 33, 35, 51, 52), and low-risk (HPV types 6, 11, 42, 43, 44) has been suggested [12].

Conclusion

The data strongly support re-defining the high-risk category as a means to improving HPV testing specificity. High-risk HPV infections (as currently defined) are common in the population; only a minority of these results in a CIN lesion and only a minority of lesions progress to require clinical intervention. It would be preferable to have a test(s) that took into account differing relative associations of the high-risk HPV types with grade of CIN and/or invasive carcinoma. The development of such tests is not beyond current technology. Possibly the high-risk and intermediate-risk classification should be reconsidered and/or HPV 16 testing should be included as a separate test. Replacing the current high-risk test with two or more others would between them enable the same sensitivity as the present test. Large-scale studies of the specific HPV types found among histologically defined lesions ranging from CIN I to invasive cancer diagnosis with referral (preferably ThinPrep™) cytological diagnosis are required. Such studies will further help refine the HPV-risk definition and determine the usefulness of having a separate test for HPV 16, especially in cases of persistent ASCUS and LSIL.

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