# The agreement between cervical abnormalities identified by cytology and detection of high-risk types of human papillomavirus

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*Objectives and design.* Human papillomavirus (HPV) is causally associated with cervical cancer. Using the Digene Hybrid Capture 2 high-risk HPV test (HC2), we investigated the prevalence of high-risk HPV in cervical specimens, and compared results with those of Papanicolaou (Pap) smears taken concurrently.

*Subjects and setting.* Cervical specimens were obtained from women attending hospitals/community health centres in the Western Cape province of South Africa. They were participating in a case-control study of the association of hormonal contraceptives and invasive cervical cancer.

*Results.* Of 1 491 women tested, 254 (17%) were HPV DNA positive. The age-specific prevalence of HPV was 36/97 (37.1%) in those aged < 30 years, 78/369 (21.1%) in those aged 30 - 39 years, 78/603 (12.9%) in those aged 40 - 49 years and 62/422 (14.7%) in those aged 50 - 59 years. In women with normal cytology the prevalence of HPV was 10.9% (138/1 264); in those with abnormal squamous cells of unknown significance (AS-CUS) it was 30.8% (36/117); in those with low-grade squamous intraepithelial lesions (LSIL) it was 63.2% (36/57), and in those with high-grade squamous intraepithelial lesions (HSIL) it was 83% (44/53). The odds ratio between HPV and HSIL in women aged 40 - 59 years was 57.1 (confidence interval 22.4 - 170.7).

*Conclusions.* HC2 detected a high prevalence of HPV (17%) in this population. Most women with HSIL (83%) were positive, indicating that HPV testing of AS-CUS women may aid in management. When costs decrease, HC2 could be introduced as an adjunct to Pap smears in identifying women at risk for high-grade cervical disease and could be useful in the maintenance of cervical health in those who remain Pap smear negative.

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Human papillomavirus (HPV) is a common sexually transmitted virus and is causally associated with cervical cancerous and pre-cancerous lesions.[1] HPV types infecting the genital tract are broadly divided into high- and low-risk types on the basis of their association with cervical cancer. The low-risk types are causally associated with genital warts. In an analysis of pooled data from 11 case-control studies, Munoz *et al.*[2] compared HPV types in cancer cases with control women and showed that 15 types, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, were classified as high-risk types and a further three types, HPV 26, 53 and 66, were probable high-risk types. The Digene Hybrid Capture 2 HPV test (HC2) (Digene, Gaithersburg, MD, USA) is the only commercially available assay system for HPV detection and the only test approved by the US Food and Drug Administration (FDA). The test detects 13 high-risk HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 low-risk types.

The Papanicoloau (Pap) smear test is the standard cytology screening method and is used worldwide because it is a comparatively inexpensive and simple procedure. The test is, however, only moderately accurate[3] and additional methods are being sought in order to improve screening strategies. A multi-centre, randomised clinical trial was done in the USA to study the management of abnormal squamous cells of unknown significance (AS-CUS) and low-grade squamous intraepithelial lesions (LSIL) by one of three methods: immediate colposcopy, cytological follow-up and triage by HPV.[4] The findings of this trial are summarised as follows: HPV testing was not useful in the triage of women with LSIL, as the prevalence of HPV was too high. However, in women with AS-CUS cytology, HPV triage proved useful, as it was as sensitive as immediate colposcopy and halved the number of referrals for colposcopy.[4]

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An ideal HPV test to facilitate a cervical screening/cervical cancer prevention campaign in less-developed and resource-poor countries would be one that is simple, quick and cost-effective. HPV testing has the potential to improve the negative predictive value of cytology (proportion of women without cervical abnormalities who test Pap smear negative), thus allowing screening intervals to be increased.[5] However, HPV tests can show a high false-positive rate for cervical disease (the labelling of those with normal cytology as diseased), which could result in large numbers of women with cervical HPV infection being referred for colposcopy; an entirely unacceptable situation, especially in low-resource settings. This study therefore compared HC2 with Pap smear cytology for the detection of cervical disease in South African women.

## Methods

### Subjects and specimens

For cytology (Pap smear) and HPV testing (cervical brushings), specimens (concurrently sampled) were obtained from women attending hospitals and community health centres in the Western Cape province of South Africa between January 1998 and December 2001. These women were recruited as controls in a case-control study to investigate associations between hormonal contraceptives and invasive cervical cancer.[6] The study was confined to coloured and black women; we were unable to study white women because invasive cervical cancer is uncommon in this ethnic group. To be eligible for inclusion, the women had to be less than 60 years of age and had to have lived within 150 km of Cape Town during the preceding 6 months. Of the 1 654 women approached, 107 refused to participate (38 because they had recently had a Pap smear) and 1 sample was mislaid. A further 55 were excluded, 7 because they were found to have cervical cancer, and 48 because of unsatisfactory Pap smear results. The final 1 491 women were aged between 22 and 59 years (median age 44 years).

Pap smear results were obtained from archived records (Table I). Cervical abnormalities were defined into four categories: negative (no abnormal cytology), AS-CUS, LSIL and high-grade squamous intraepithelial lesions (HSIL). Normal cytology was found in 1 264 (84.8%) of the 1 491 women, AS-CUS in 117 (7.8%), LSIL in 57 (3.8%) and HSIL in 53 (3.6%).

### HPV testing

Cervical scrapings were assayed for high-risk HPV types using the HC2 test. This enabled the detection of high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The assay denaturation protocol was modified: after mixing the cervical specimen for 5 seconds, 70 µl of the fluid was removed from the specimen tube and mixed with 35 µl of denaturation agent. The remainder of the specimen was stored at –70°C for future use. Test results were classified as positive according to the manufacturer's instructions.

### Data analysis

The correlations between HPV infections and Pap smear-defined cervical abnormalities were calculated using odds ratios (ORs) (with confidence intervals (CIs)) and kappa values. ORs and the Cornfield and Exact 95% CIs for ORs were calculated using Epi Info Version 5 (Centers for Disease Control, Epidemiology Program Office, Atlanta, Ga, USA). The test for a linear trend between increasing severity of cervical lesions and increasing prevalence of HPV infection was calculated using Epi Info Version 5 and text information provided by Altman.[7] Kappa values were calculated according to text information provided by Fleiss.[8]

## Results

Of the 1 491 women examined by the hybrid capture test, 254 (17%) were found to be high-risk HPV DNA positive. The black women had a significantly ($p = 0.004$) higher prevalence, 82/376 (21.8%), than the coloured women, 172/1 115 (15.4%). The age-specific prevalence of high-risk HPV was 36/97 (37.1%) in those aged less than 30 years, 78/369 (21.1%) in those aged 30 - 39 years, 78/603 (12.9%) in those aged 40 - 49 years and 62/422 (14.7%) in those aged 50 - 59 years (Fig. 1). The prevalence of high-risk HPV declined with age but then increased slightly in those aged 50 and older.

The prevalence of high-risk HPV increased with the severity of cervical abnormality, being 10.9% (138/1 264) in women with normal cytology, 30.8% (36/117) in those with AS-CUS, 63.2% (36/57) in those with LSIL and 83.0% (44/53) in those with HSIL (Fig. 2). Of the 254 HC2-positive women, 54.3% (138) were graded as having normal cervical cytology, 14.2% (36) as AS-CUS, 14.2% (36) as LSIL and 17.3% (44) as HSIL.

**Table I. Age distribution of Pap smear results**

| Age (yrs) | Negative (% (*N*/total)) | AS-CUS (% (*N*/total)) | LSIL (% (*N*/total)) | HSIL (% (*N*/total)) |
|---|---|---|---|---|
| 21 - 39 | 82.8 (386/466) | 7.9 (37/466) | 6.0 (28/466) | 3.2 (15/466) |
| 40 - 59 | 85.7 (878/1 025) | 7.8 (80/1 025) | 2.8 (29/1 025) | 3.7 (38/1 025) |
| Totals | 84.8 (1 264/1 491) | 7.8 (117/1 491) | 3.8 (57/1 491) | 3.6 (53/1 491) |

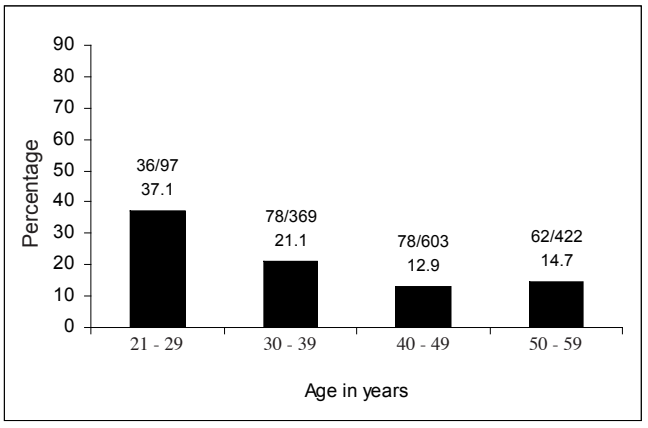


*Fig. 1. The prevalence of high-risk HPV in women according to age.*

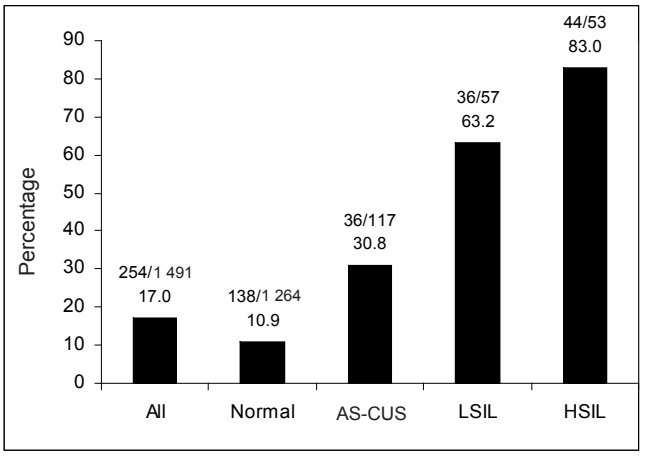


*Fig. 2. The prevalence of high-risk HPV in all women, in women with normal cytology, and in women with AS-CUS, LSIL or HSIL.*

The OR for an association between high-risk HPV and AS-CUS was 4.4 (CI 2.1 - 9.3) and 3.3 (CI 1.8 - 6.1) in women aged 21 - 39 and 40 - 59 years, respectively, for LSIL 22.6 (CI 7.9 - 78.3) and 10.0 (CI 4.4 - 22.9), respectively, and for HSIL 20.5 (CI 5.3 - 115.3) and 57.1 (CI 22.4 - 170.7), respectively (Table II). The $x^2$ test for linear trend showed a relationship between increasing severity of disease of cervical lesions and increasing prevalence of HPV ($p \geq 0.00001$). Calculated kappa values of associations between the Pap smear and hybrid capture (with comparisons stratified according to age) showed low agreement beyond chance ($\kappa < 0.40$) in all categories of Pap smear cytology, except for women with HSIL in the 40 - 59-year age group ($\kappa = 0.40$) (Table III).

## Discussion

Pap smears have been instrumental in reducing cervical cancer incidence worldwide, but they nevertheless have documented limitations. The accuracy of the Pap smear test is variable, with the suggestion that it is less efficient at discriminating between the diseased and those without disease than is generally believed.[3] However, screening with the Pap smear test requires little in terms of consumables and equipment, and it is an established and widely practised method of screening for cervical abnormalities. By comparison, HC2 assays are grounded in a clearly defined and stringently standardised testing system. HC2 specimen collection is a relatively simple procedure, specimen transport and storage parameters are robust, assay results across testing centres are designed to be comparable, and test findings can easily be used in concert with other cervical screening tests.

However, there are several reasons why the use of HC2 in cervical disease diagnosis has limitations. Firstly, HC2 assays provide evidence of HPV presence and not of cervical disease. Furthermore, while high-risk HPV infection is necessary for the development of cervical neoplastic disease, severe neoplasia generally develops only after a lengthy period of time and only in a small proportion of HPV-infected women. The results presented here emphasise the latter in the prevalence figures of high-risk HPV in all women, namely 17% (254/1 491), compared with the prevalence of LSIL/HSIL, namely 7.4% (110/1 491) (Fig. 2). Furthermore, a single HC2 sample assay cannot determine whether high-risk HPV infection is of a persistent nature, persistence being a necessary factor in the development of cervical neoplasia.[9]

A further limiting feature of this particular study was that HC2 specimens (testing for risk factor), and Pap smears (testing for outcome), were taken simultaneously, with conclusions being drawn from what was essentially a cross-sectional study design, where findings are only intended to be exploratory and suggestive by nature. Further, in comparing HC2 with the Pap

**Table II. Odds ratios showing the association of high-risk HPV and Pap smear results, according to age**

| Age (yrs) | AS-CUS | | LSIL | | HSIL | |
|---|---|---|---|---|---|---|
| | OR | CI | OR | CI | OR | CI |
| 21 - 39 | 4.4 | 2.1 - 9.3 | 22.6 | 7.9 - 78.3* | 20.5 | 5.3 - 115.3*† |
| 40 - 59 | 3.3 | 1.8 - 6.1 | 10.0 | 4.4 - 22.9 | 57.1 | 22.4 - 170.7*† |

*CIs according to Exact method, otherwise according to Cornfield.
†CI excessively wide owing to the small number of values in the relevant cell.
OR = odds ratio; CI = confidence interval.

**Table III. Kappa values showing the agreement beyond chance between HC2 and Pap smear results, according to age**

| Age (yrs) | AS-CUS | LSIL | HSIL |
|---|---|---|---|
| 21 - 39 | 0.19 | 0.32 | 0.22 |
| 40 - 59 | 0.15 | 0.20 | 0.40 |

smear, which is known to be an imperfect measure of cervical neoplasia, sensitivity, specificity and positive predictive values for HC2 might tend to be lower than expected compared with HC2 comparisons against a true gold standard, namely colposcopically mediated biopsy/histology. Lastly, the cost of the essential laboratory equipment, trained laboratory staff and HC2 consumables needed, might prove prohibitively high for public health service HPV screening initiatives. HC2 too, unlike the Pap smear test, is a relatively novel assay in this country, with few laboratory nodes available for the testing of specimens or for the training of assay staff.

Despite these limitations, the findings presented here, using a novel technique, have much in common with findings from a variety of similar and more stringently designed international studies. Age was shown to be a factor in cervical disease and in HPV infection, with a high prevalence of HPV in the younger age group, then a steady decline until the age of 50, then a slight increase in those older than 50 years. This rise in older women is in keeping with findings from other studies.[10] It was therefore clearly illustrated that there are strong relationships between age, HPV prevalence and severity of cytological disease. ORs (Table II) of the association between HPV, cervical abnormalities and age provide an indication of what is perhaps the true relevance of HPV DNA testing, namely that HPV findings are a measure of the risk of current or future cervical abnormalities and any risk assessment must be based on a personal/medical history profile of the woman concerned, in which age and lifestyle are important factors. For instance, in women older than 30 years with AS-CUS/LSIL, a positive HPV test can be regarded as a credible measure of the risk of cervical disease. In this study the prevalence of HPV as measured by HC2 was 30.8% in cases of AS-CUS and 63.2% in LSIL. It has been reported that only 5 - 10% of women with AS-CUS harbour underlying serious cervical disease, but of all HSILs identified in screening populations, one-third are identified following further investigations from AS-CUS results.[11] For women with AS-CUS cytology, HPV triage showed a sensitivity equal to immediate colposcopy, and resulted in a halving of colposcopic referrals.[4] Following these and other findings, the US FDA has approved HC2 for use in AS-CUS triage.

With regard to HPV tests and the management of LSIL, 63.1% of LSILs in this study were HC2 positive, which, as shown in similar studies, limits its use in management decisions.[12] Generally it is accepted that HPV-positive status in a person aged above 30 years or who is immune compromised or HIV positive[13] would be an association requiring further investigation.

The prevalence of HSIL in this study rose with age, whereas that of HPV decreased. In women aged over 40 years, therefore, HPV-positive status raises the measure of risk. Yet kappa values calculated on data stratified according to age and cervical condition (Table III) only yielded a value of 0.40 in the HSIL category for women between the ages of 40 and 59 years (moderate agreement beyond chance). These results mean that although there was a strong association between HPV positivity and the degree of abnormality of the Pap smear, the kappa results at best indicate an imprecise agreement between these two tests. A positive HC2 test in an older (and especially a previously unscreened) person should therefore initiate vigorous further investigations. Conversely, the high rates of negative HC2 findings show a diagnostically useful association between cervical health and the absence of HPV. The results from older women showed there was a high probability that a negative HC2 test result accurately reflected absence of HSIL. The results of this study are in keeping with other findings in that where women routinely test both HPV negative and cytologically normal, the number of screening tests could be reduced according to the recommended management policy guidelines. Moreover, negative HC2 tests after treatment for cervical neoplasia also indicate a good probability that there has been no resurgence of cervical disease.[14] It is worth noting that since the time of progression from detectable LSIL to preclinical invasive cancer is generally 12 - 13 years, Meijer *et al*.[15] have proposed re-screening every 8 years in cytologically normal and HPV-negative women. To summarise, negative HC2 and Pap smear test surveillance has important implications in the assurance of continuing cervical health and in the prevention of resurgence of cervical disease after treatment.

## Conclusion

Many of the findings in this study mirror those from similar studies.[16] The strong association between cervical high-risk HPV infection, cervical abnormality and age indicates that HC2 is a useful addition to screening for HPV-associated cervical disease. Aside from its use in conjunction with the Pap smear, HC2 might have a use in increasing the resolving power of other diagnostic and management options, such as direct inspection and immediate treatment techniques, especially under busy clinic conditions. The development of vaccines showing protection against incident and persistent HPV-16 and HPV-18 infections, and incident HPV-31 and HPV- 45 infections, is advanced.[17] The prevalence of oncogenic HPV types in women with normal cytology in this study was 10.9% (Fig. 2). These results indicate that cervical oncogenic HPV prevalence is significant in South Africa. The current HC2,

together with other HPV tests, will be useful in monitoring the epidemiological impact of a vaccination programme. Clinically, HC2 will be used to test for oncogenic HPV types in women who are already infected, before vaccines become available.[18] Conversely, the analysis presented here of the differences between HPV prevalence, that of Pap smear cytology and the dynamics of cervical disease, would rule out the use of HC2 in its current form as an alternative primary screening method for cervical abnormalities. Moreover, until the cost of HC2 testing is substantially reduced, it is not practical to introduce the test into the public sector as part of a primary screening strategy. Currently HC2 testing would be of immediate clinical use for the triage of women with AS-CUS smears and in the follow-up surveillance of treated cervical disease.

HC2 presents an additional procedure that, in combination with other methods, can aid in the risk assessment of current and future cervical disease. In South Africa there is a pressing need for additional viable, practical and innovative screening aids for cervical cancer and pre-cancer, which could include HC2 testing.

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