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Research article

Evaluation of adjunctive HPV testing by Hybrid Capture II® in women with minor cytological abnormalities for the diagnosis of CIN2/3 and cost comparison with colposcopy

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Abstract

Background: As a proportion of high grade cervical intraepithelial neoplasia (CIN2/3) are associated with equivocal cervical smears, which show borderline or mild dyskaryosis, follow up with repeat smears, colposcopy and biopsy is required. Since infection with oncogenic Human Papilloma Virus (HR HPV) has been found to be associated with the development of cervical cancer, HRHPV testing appears to be an alternative.

Objective: The present study assesses if HRHPV testing can predict CIN2/3 in women referred for mild dyskaryosis and borderline cytological changes in an health authority with a referral policy to colposcopy after one single mild dyskaryotic Pap smear.

Study design: The HPV DNA Hybrid Capture II (Digene/Abbott, Maidenhead) was evaluated on 110 consenting women with mild dyskaryosis and 23 women with persistent borderline changes, who were referred for colposcopy between May and November 2001. A cost comparison between two referral policies was performed.

Results: CIN2/3 was diagnosed histologically in 30 of 133 women (22%) with minor cytological abnormalities. As the Receiver Operator Characteristics plot suggested a cut-off of 3 pg/ml the HRHPV HCII was evaluated at 3 RLU (relative light units) and at the manufacturer's recommendation of 1 RLU. At both cut-offs sensitivity and negative predictive value were high at 97%. Specificity was low at 37% at a cut-off of 1 pg/ml and 46% at a cut-off of 3 RLU. To remain cost neutral in comparison to immediate colposcopy the costs for one HR HPV HC II must not exceed £34.37 per test at a cut off of 3 pg/ml.

Conclusion: The negative likelihood ratio (NLR) was of good diagnostic value with 0.089 at 1 RLU and 0.072 at 3 RLU, which reduces the post-test probability for CIN2/3 to 2% in this population. Women with minor cytological disorders can be excluded from colposcopy on a negative HR HPV result.

Specificity can be improved by restricting HR HPV testing to women with persistent borderline cytological changes or to women over 30 years.

Background

Oncogenic Human Papilloma Virus (HR HPV) genome could be found in 99.7% of 942 histologically proven cervix carcinoma [1]. HPV gene product E6 is thought to degrade the tumour suppressor protein p53 destabilizing the cell cycle, which leads to cervical cancer in susceptible individuals [2]. Cytological screening has reduced the annual incidence of cervical carcinoma from 17/100000 to 8.5/100000 in the last 20 years in the UK [3]. However up to 30% of high grade cervical intraepithelial neoplasia (CIN2/3) are associated with equivocal cervical smears, which show borderline or mild dyskaryosis [3]. This requires follow up of women with minor cytological abnormalities with repeat smears, colposcopy and biopsy.

Adjunctive HRHPV testing could triage women for colposcopy. Liquid based cytology, which is presently assessed in some UK laboratories, allows adjunctive HPV testing using a commercial gene probe HPV DNA Hybrid Capture II (Digene/Abbott, Maidenhead) without the need for an additional specimen. NHS Cancer Screening Programme (NHSCSP) guidelines stipulate referral after two mild dyskaryotic smears or three borderline smear, whereas the health authority in North West London recommends referral after one mild dyskaryosis in order to improve coverage and follow-up in a population with a high mobility [4]. Despite of this different referral policy the CIN2/3 prevalence is similar to other colposcopy clinics with 20% [3].

We evaluated the performance of the Hybrid Capture HPV DNA test on liquid based cytology for the diagnosis of CIN2/3 in women referred to colposcopy clinic of West Middlesex University Hospital (WMUH) after three borderline or one mild dyskaryosis smear. A cost comparison between the conventional referral strategy and triage for colposcopy by positive HPV test was performed.

Materials and methods

Patients

Between May and November 2001 146 women were referred to the colposcopy clinic of West Middlesex University Hospital for minor cytological abnormalities in their cervical smear. The time period between abnormal Pap smear and colposcopy did not exceed three months (median = 4 weeks). All women gave their consent. We had received ethical approval from the Hounslow District Research Ethics committee to perform the study. The smear was collected by rotating a cervical broom (Papette® Wallach Surgical, Orange CT) ten times around the cervical os and rinsing it into ten ml of PreservCyt® (Cytyc corporation, Boxborough, MA), from which a Thin prep Pap slide was prepared and the HPV test performed. Colposcopy was performed on all women and a punch biopsy was taken from suspicious areas of the cervix. If colposcopic findings were normal, no biopsy was taken and the case was classified as absence of CIN.

Cytology and histology

Cytopathologists at Quest Diagnostics classified Pap smears into borderline, mild, moderate and severe dyskaryosis. Punch biopsies were classified by two consultant histopathologists at West Middlesex University Hospital as CIN2/3 and CIN1 and HPV typical changes. The histopathologists were blinded to HPV results as were the microbiologists to the histology results at the time of HPV testing.

Hybrid capture HPV DNA test

The Hybrid capture II HPV DNA test (Digene/Abbott, Maidenhead) was performed on liquid based cytology specimens. The RNA probe mix for the detection of high risk HPV types 16,18,31,33,35,39,45,51,52,56, 58,59 and 68 was used according to the manufacturer's instructions. Signal amplification is based on immunocapture of DNA/RNA hybrids and subsequent EIA with a chemiluminescent reporter system. The results are given as relative light unit (RLU) ratio. As the RLU ratio is the ratio of light emitted by the specimen to the light emitted from the mean RLU of triplicate positive control specimens containing 1 pg/ml of HPV DNA (5000 copies of HPV genome)it is a semiquantitativ estimate of viral load in the specimen.

Cost comparison

The cost of the conventional strategy to perform colposcopy on all women with minor cytological disorders were compared with triaging women to colposcopy only if they had a positive HRHPV in addition to their minor cytological abnormality.

Costings are specified on NHS health care resource group (HRG) level, which defined the costs for gynaecological outpatient procedures, according to which colposcopy including biopsy amounts to £93.28 per procedure [5]. The cost neutral price for one HRHPV HCII test was calculated by assessing the savings which are achieved by not performing colposcopy in women who test HRHPV negative.

As liquid based cytology has been introduced into the colposcopy clinic few years ago, no additional costs for cervical swabs accrued. Six monthly follow up Pap smears are required after a negative HRHPV test as well as after colposcopy, which makes cytological follow up cost neutral on comparison.

Statistical methods

For statistical analysis the Excel (Microsoft Windows 97) compatible Analyse-it program (ANALYSE-IT SOFTWARE LTD) was used.

Table 1: Characteristics of colposcopy population

Histology	Referral cytology	Age mean ± SD	Median of high-risk HPV load in liquid based cytology (RLU)	Mean high-risk HPV load (±SD) in liquid based cytology (RLU)	HRHPV DNA > 1RLU	HRHPV DNA > 3RLU
64 CIN absent	17 BL 47 MD	33y ± 11y	1.37	208 ± 509 RLU	N = 35 (55%)	N = 31 (48%)
39 CIN1	5 BL 34 MD	31y ± 11y	94	446 ± 782 RLU	N = 30 (77%)	N = 26 (66%)
30 CIN2/3	1BL 29 MD	30y ± 8y	174	568 ± 765 RLU	N = 29 (97%)	N = 29 (97%)

Continuous variables were compared by one-way analysis of variance and Mann-Whitney U-test. Difference between proportions was analysed by Normal test. Association between categorical variables was assessed by Chi-square test. Test indices were given with the 95% confidence intervals.

For sample size calculations we assumed that 40% of women without CIN2/3 were infected by HRHPV. Thus to detect a minimum odds ratio of 17 and to give the study a power of 80% 11 CIN2/3 cases and four times as many controls (n = 40) would be required at a confidence level of 95%. If 70% of women without CIN2/3 were infected 23 cases and 92 controls are necessary.

The Bayes theorem was applied to arrive at the post test probability.

Results

Over five months 146 women gave their consent for an HPV test, of these six were excluded for previous LLETZ, two for an inadequate biopsy specimen and five for a low cell count in the liquid based cytology, which does not allow an additional HPV test. The mean age of the remaining 133 women was 31 years (SD = 10y and median = 30 years). One hundred and eight women underwent colposcopically directed punch biopsy and in 25 women the cervix did not show any abnormalities, which would require biopsy.

Although the median of the high-risk HPV load rose with increasing CIN grade, the difference of means between different grades of CIN or between presence and absence of CIN was not significant in parametric tests ($p > 0.05$, one way analysis of variance), but the medians differed significantly ($p < 0.02$, Mann-Whitney U-test) (table 1).

To determine a cut-off for a positive result a receiver-operator characteristics (ROC) plot was constructed (see figure 1). At 1 RLU, which is the cut-off recommended by the manufacturer, the sensitivity amounted to 97% but the false positive rate yielded 63%. At 3 RLU the sensitivity was still 97% and specificity improved to 46%. At higher concentrations sensitivity dropped below 90%. As HPV testing is a screening test the sensitivity should be high in order not to miss any precancerous lesions. False positive results will be corrected by biopsy results. Thus the assay performance was evaluated at the manufacturer's recommended threshold of 1 RLU and at the apparent optimal cut-off of 3 RLU.

At a cut-off of 1RLU the sensitivity of the HCII for high-risk HPV in this population amounted to 97% (CI 91%-103%) and the negative predictive value was 97% (CI 92%-102%). Specificity and positive predictive value ranged low with 37% (CI 28%-46%) and 30% (CI 21%-39%) (see table 2). The odds ratio of a positive HCII test for CIN2 and worse was 17.1 (CI 2.46–686). Detection of high-risk HPV types was strongly associated with CIN2 or worse ($p < 0.001$, Chi-square test).

The pre-test prevalence of CIN2/3 in this population was 22.6% (CI 15%-29%). The negative likelihood ratio (NLR) was 0.089 (CI 0.02–0.35), which means that a negative HCII test for high-risk HPV types is 11 times less likely in patients with CIN2/3 than in patients without high grade CIN. This means a negative HPV result by the HPV DNA HCII test can reduce the probability that a woman with mild dyskaryosis or borderline smear harbours CIN2 or worse from 22.6% to 2.6%.

At a cut-off of 3 RLU performance improved in terms of specificity, NPV and PPV (see table 2). The negative likelihood ratio (NLR) dropped to 0.072 (CI 0.022–0.24), which reduces the pre-test probability of 22% for CIN2/3 to 2% post-test probability.

Specificity was higher in women referred for borderline and in women over 30 years. Sixty-six women in the cohort were older than 30 years (mean 39y ± 8y). HRHPV infection was less common in women over 30 years

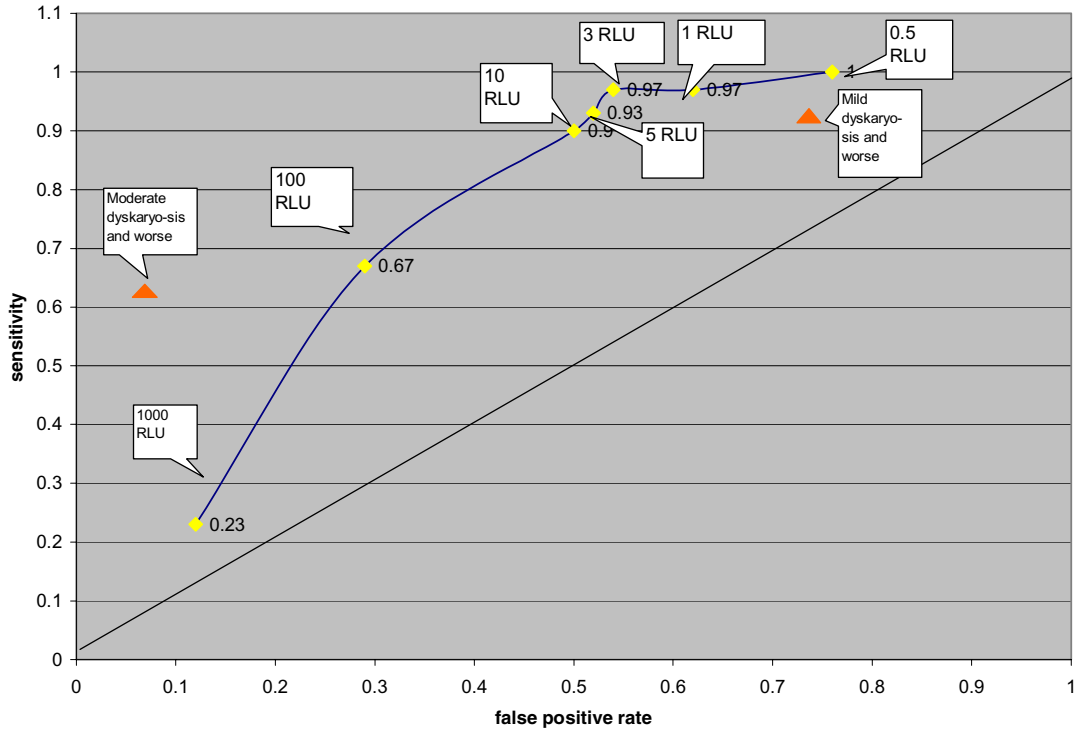

Figure 1
Receiver Operator Characteristic curve of high risk HPV HC II for the diagnosis of CIN2/3 in women with minor cytological abnormalities (red triangles show performance of Pap smears according to KC61 part C, RLU = ratio of relative light units of specimen to positive controls)

Table 2: HRHPV Hybrid Capture II performance for the diagnosis of CIN2/3

referral smear	cut off	n	sensitivity	specificity	NPV	NLR	OR
MD & BL	1 RLU	133	97%	37%	97%	0.089	17
MD & BL	3 RLU	133	97%	46%	98%	0.072	26
1xMD	1 RLU	110	97%	36%	97%	0.096	17
1xMD	3 RLU	110	97%	41%	97%	0.083	19
3xBL	1 RLU	23	100%	52%	100%	NA	NA
3xBL	3 RLU	23	100%	64%	100%	NA	NA
MD & BL in woman >30y	1 RLU	66	100%	47%	100%	NA	NA
MD & BL in woman >30y	3 RLU	66	100%	58%	100%	NA	NA

MD = mild dyskaryosis, BL= borderline, NA = non applicable due to 0 in 2 × 2 table

(56%) than in younger women (77%). However the difference was not significant ($p > 0.05$, Normal test). Specificity reached 58% in women over 30 years.

The expenses for the HRHPV HCII test were weighed against the savings achieved by less colposcopies. As costings for colposcopy are defined by the health care resource group within the NHS, it is possible to calculate the cost neutral price for one HRHPV HCII test from these savings. Colposcopy could have been omitted only in 39 to 49 of 133 patients who tested HPV negative for the expense of 133 HPV tests and would miss one CIN2/3 case (see table 3). To be cost neutral the price of the HRHPV HCII test at a cut-off of 3 RLU must not exceed £34.37 inclusive test reagents, overheads and staff costs.

If only women over 30 years would be triaged by HRHPV HCII at a cut-off of 3RLU, the cost neutral price for one HRHPV HCII test would amount to £41.

Discussion

The present cohort is representative of women referred to our colposcopy clinic when compared with national audit figures of the past two years [6,7]. The indication for referral was in 85% mild dyskaryosis, which reflects the referral policy after one single mild dyskaryotic Papanicolaou smear [7]. The CIN2/3 prevalence of 22% was in concordance with 18% in the year 2000 and with 27% in 2001[6,7].

Though the HRHPV viral load rose with increasing grade of CIN the means did not differ significantly. Natural history studies showed that the HPV load fluctuates during the course of infection being low in the beginning and at the end [8]. To convert the continuous variable into a HRHPV positive and negative result a ROC plot was constructed. The ROC plot suggested an optimal cut-off at 3RLU at which the sensitivity remained high and the specificity rose to 46%. Two other validation studies of the HRHPV HCII in women with LSIL also observed that from 1RLU to 2.5RLU the sensitivity remained stable with a strong increase of specificity in women with LSIL [9,10].

Sensitivity and negative predictive value were very high with both 97%, and thus a negative HRHPV test can exclude high grade CIN with a higher frequency than repeat cytology as shown in a randomised trial [11]. A quantitative measure to exclude disease is the negative likelihood ratio (NLR). The NLRs of 0.089 at 1RLU or of 0.074 at 3RLU reduce the pre-test probability of 22% to a post-test probability of 2%, which means that women referred for a minor cytological abnormality and a negative HRHPV HCII test have a chance of 2% to harbour CIN2/3. If used to triage women with minor cytological abnormalities for colposcopy, who test HRHPV positive at a cut off of 3RLU, HRHPV testing would reduce colposcopy referrals by 36%. This strategy would be cost neutral in the NHS if the costs for HR HPV testing do not exceed £34.37 per test.

The specificity of 37% at 1RLU and 46% at 3 RLU resulted in a poor positive predictive value. The high false positive rate is due to the high proportion of women who are infected by HRHPV but will clear the virus and will not progress to high grade CIN. Low specificity was found in other studies on women referred to colposcopy for mild dyskaryosis. Rebello et al. [12] found a specificity of only 39% in mild dyskaryosis but of 76% in borderline referrals. Also Wright et al. [10] could demonstrate at a cut-off of 1 RLU a specificity of 25% in LSIL referrals in contrast to 75% in ASCUS. The ALTS trial arm of LSIL was closed early due to the high HPV positive rate of the HRHPV HCII test [13]. CIN is more prevalent in women with mild dyskaryosis than with ASCUS or borderline changes.

Table 3: Cost comparison of different management strategies and cut offs

	Colposcopy in all women with minor cytol. disorders without HPV testing	Colposcopy only in HPV positive (≥1RLU) women with minor cytol. disorders	Colposcopy only in HPV positive (≥3RLU) women with minor cytol. disorders	Colposcopy in women over 30 y with minor cytol. disorders without HPV testing	Colposcopy only in HPV positive (≥3RLU) women over 30 y with minor cytol. disorders
No. of colposcopies	133	94	84	66	37
No. of HPV tests	0	133	133	0	66
Costs of colposcopies	£12407	£8768	£7835	£6156	£3451
Savings by omitting colposcopies in women who test HRHPV negative	0	£3639	£4572	0	£2705
Cost neutral price for 1 HRHPV HCII test (savings/No. of HPV tests)		£27.35	£34.37		£41
No of CIN2 or worse detected	30	29	29	16	16

To reduce the false positive rate HRHPV testing could be confined to women with borderline changes, which are more frequently due to reactive changes than to CIN. The specificity rose to 63% in this subgroup. This trend is consistent with results of other evaluation studies in women with ASCUS in which the specificity figures ranged between 64–75% [14,15]. However, as borderline changes are only less than 20% for gynaecological referral in our setting, this strategy will not serve the majority of women referred to the colposcopy clinic at WMUH.

Previous studies could show that the performance of HRHPV testing for the diagnosis of CIN2/3 is better in women over 30 years, because women at this age should have cleared primary infection and if still HRHPV positive may be at risk of persistent infection with progression. Fifty percent of women referred to colposcopy were over 30 years old with a mean age of 39 years. At a cut-off of 3 RLU specificity reached 58% and thus 44% of women over 30 years who tested HPV negative could have been excluded from colposcopy. This is in concordance with Cuzick's [9] previous large UK evaluation studies in women over 35 years (mean age = 46 years).

Conclusion

The diagnostic value of the HRHPV HCII is based on a negative result predicting absence of CIN2/3 with an excellent negative predictive value of 97%, which reduces the probability of CIN2/3 from 22% to 2% in women with minor cytological disorders. As the majority of HRHPV infected women will clear the virus, the HRHPV HCII test is not specific for progression of infection to high grade CIN in women with minor cytological abnormalities. However persistence of HRHPV infection after the age of thirty years has a moderate positive predictive value and could reduce gynaecological referrals in this subgroup by 44% on the basis of a negative HRHPV test result. In future susceptibility to cervical cancer such as HLA type or cellular progression markers may be more specific and predictive for progression to high grade CIN than HRHPV testing in women with minor cytological disorders.

List of Abbreviations

HRHPV, oncogenic Human Papilloma Virus; CIN2/3, high grade cervical intraepithelial neoplasia; OR, odds ratio; LLETZ, laser loop excision of the transformation zone; Pap, Papanicolaou; ASCUS, atypical squamous cells of undetermined significance; HSIL, high grade squamous intraepithelial lesion; LSIL, low grade squamous intraepithelial lesion: ROC plot, Receiver-Operator Characteristics plot, NLR, negative likelihood ratio; WMUH, West Middlesex University Hospital, RLU, ratio of relative light units of specimen to relative units of positive control

Competing interests

None declared.

Authors' contributions

AG, MK, JF, SK participated in the study design. JF performed the study in the colposcopy clinic. SK provided the liquid cytology specimens for the HPV test and access to referral smear and history. AG carried out the HPV HCII test. AG and MK drafted the manuscript.

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