

# Papanicolaou Smear and HPV Testing for Cervical Screening

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**Background.** Since the adequacy of screening for pre-cancer and cervical cancer with Papanicolaou (Pap) smear alone has been questioned, a number of adjunctive tests have been evaluated. This study determined whether human papillomavirus (HPV) testing can improve cervical screening when performed coincident with cytologic sampling.

**Methods.** Patients were evaluated by Pap smear, HPV testing, and colposcopy at two study centers. Screening with either Pap smear alone or in combination with HPV testing (Pap and HPV testing) was evaluated by colposcopy-directed biopsy as the highest diagnostic standard.

**Results.** The Pap smear alone detected 8/27 (29.6%) cases of women with significant pathology (precancerous lesion and HPV infection) on biopsy, whereas the combination of Pap and HPV testing detected 24/27 (88.8%) cases. ( $p < 0.001$ ). Patients for whom both test results were normal (negative Pap and HPV testing) were extremely unlikely to harbor significant pathology (less than 1% of those screened). Pap smear and HPV testing together was especially helpful in the detection of low-grade cervical lesions compared to the detection rate with Pap smear alone.

**Conclusions.** These data indicate that HPV testing combined with Pap smear exam increases the accuracy of cervical screening. HPV testing appears to be particularly useful as a triage instrument in women with otherwise negative Pap smears. Further studies to evaluate the cost effectiveness of this combined screening protocol are needed. (*Mid Taiwan J Med* 2002;7:21-7)

## Key words

cervical screening, HPV testing, papanicolaou smear

## INTRODUCTION

For over 50 years, gynecological screening for pre-cancer and cervical cancer has relied entirely on the Papanicolaou (Pap) smear. Although in most studies, the Pap smear has retained a reasonably high degree of specificity, the false negative rate of the Pap smear has been reported to range from 6% to 55% [1,2]. This apparent lack of sensitivity takes on even greater significance when one considers the growing number of women

with potentially premalignant conditions [3].

Many have questioned the reliability of screening for cervical neoplasia using the Pap smear alone and have suggested using additional exams to improve the accuracy of cervical screening [4,5]. Among the techniques proposed are cervicography, speculoscopic tests, DNA hybridization tests, and Hybrid Capture HPV tests for human papillomavirus (HPV) [6].

Although colposcopy is the most accurate method of visually examining the presence or absence of cervical pathology [7], it is time consuming, costly, requires considerable expertise, and is not available to all providers of gynecologic al health care

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screening. Thus, it is widely accepted that colposcopy is not a practical cervical screening tool in most areas of the world.

Although a wealth of knowledge about the molecular biology and epidemiology of HPV was gained in the 1980s, the 1990s will be noted for efforts by researchers to evaluate the clinical applicability of testing for the presence of oncogenic viruses. Studies of the clinical utility of HPV testing include its use for primary screening [8], secondary screening in the triage of low-grade Pap smear abnormalities [9], for the clarification of equivocal biopsy results and noncorrelating colposcopies (when colposcopy or biopsy fails to identify the source of an abnormal Pap) [10], and in laboratory quality assurance [11].

Ideally, the addition of HPV DNA testing to conventional cytology would improve the detection rate of high-grade cervical intraepithelial neoplasia (CIN) as well as reduce the referral rate by safely allowing HPV-negative women with borderline or mild dyskaryosis to be monitored with cytology at normal intervals. However, a very large trial involving several hundred thousand women will be necessary to fully evaluate this. As a preliminary step, we have conducted a smaller study to evaluate the usefulness and effectiveness of hybrid capture II tests as an adjunct to the Pap smear.

## PATIENTS AND METHODS

### Study Centers

A gynecologic oncologist from the medical center and a gynecologist from a private gynecological clinic served as the examiners during this study. The gynecologic oncologist was well-trained in colposcopy. The clinical centers at which the women were studied were: the China Medical College Hospital, Taichung and Lee Womens' Clinic, Taichung, Taiwan.

### Patients

From October 1997 to April 2001 both centers were engaged in studies designed to evaluate the usefulness and effectiveness of

the hybrid capture II HPV DNA test as an adjunct to the Pap smear.

In order to be classified as a screening patient and be included in the study group, it was required that women undergo routine annual examinations, that they did not have a history of any therapy for cervical or vaginal pathology, and that their previous Papanicolaou smear, (previously obtained within 2 years), was either class I or normal. There were 488 women who met this criteria, and they ranged in age from 21 to 61 years (mean: 32.6 years). These women represented an outpatient population reporting for regular annual screening evaluations. None of these women were pregnant and only 10 were menopausal. None of these patients were recruited from a dysplasia or STD clinic.

### Study Protocol

After informed consent, a Pap smear was performed and cells from both the ectocervix and endocervical canal were obtained. Following the smear, a sterile Dacron-tipped applicator was used to collect cells for HPV DNA analysis. This applicator was a part of a transport kit provided by Digene Diagnostics [Silver Spring, Maryland, USA]. The cells were collected from the cervical os and the transformation zone. The applicator was then placed in transport medium with care being taken not to contaminate the specimen. Colposcopy was then performed. Abnormalities, if present, were noted on the study data form. If abnormalities were noted on colposcopy, punch biopsies were recommended.

The Digene Hybrid Capture II system is a sandwich capture molecular hybridization assay that uses chemiluminescent detection. Samples containing the target DNA hybridize with a specific ribonucleic acid probe. The resultant hybrid is captured onto the surface of a tube coated with an antiribonucleic acid/DNA hybrid antibody. The immobilized hybrid then reacts with an antihybrid antibody conjugated to alkaline phosphatase and detected with a chemiluminescent substrate. As the substrate is cleaved by the

bound alkaline phosphatase, light is emitted, which is measured as relative light units on a luminometer. The intensity is proportional to the amount of target DNA in the patient's specimen. A relative light unit measurement greater than or equal to the cut-off value indicates the presence of HPV sequences in the patient's specimen, whereas a relative light unit measurement less than the cut-off value indicates the absence of HPV sequences.

The Digene Hybrid Capture II HPV DNA assay is a comprehensive HPV test that identifies the presence of the 14 common anogenital HPV types. The assay distinguishes low-risk HPV types (6, 11, 42, 43, and 44) from intermediate- and high-risk HPV types (16, 18, 31, 33, 35, 45, 51, 52, and 56). In this study population we were interested only in identifying the intermediate- and high-risk types, and thus, a positive test in this study for HPV indicated that the patient was positive for intermediate- and high-risk HPV types.

#### Cytologic Studies and Histopathology

Cytologic and histopathologic findings were described according to the usual format. Abnormal cytologic findings were confirmed by a cytopathologist in all cases. Pap smears were adequate, containing both ectocervical and endocervical samples. Those that were read as normal or class I, and smears showing only inflammatory cells or inflammatory atypia without condylomatous features were considered negative. Pap smears showing atypia with condylomatous features (both koilocytosis and perinuclear halos), CIN, carcinoma in situ (CIS) or invasive cancer (IC) were considered positive. Biopsy results showing metaplasia, nonspecific (inflammatory) atypia not exhibiting features of HPV, or

cervicitis alone were considered negative. Biopsy results showing HPV, CIN, CIS, or IC revealed significant pathology.

#### Data Analysis

Both the Pap smear alone, and in combination with the HPV DNA test (Pap and HPV testing) were independently evaluated as screening tests. If either the Papanicolaou smear or HPV test was positive, the Pap and HPV screening result was considered positive. Only if both the Pap smear and HPV tests were negative was this screening protocol considered negative. Statistical significance was determined by the  $\chi^2$  test. A  $p$  value  $< 0.05$  was considered significant.

### RESULTS

Of the 488 women who met the aforementioned criteria, 442 had both a negative Pap smear and no abnormal lesion visualized on subsequent colposcopy. Because biopsies were not obtained from this group of patients, and because all colposcopies were performed by an experienced colposcopist, these women were presumed to be free of any significant disease, provided that the colposcopy was adequate. Of the remaining 46 women with either a positive Pap smear, a positive colposcopy, or both, the Pap smear was positive in only 10 of them, while Pap and HPV testing together was positive in 30 of the patients.

The relationship between a positive screening test result and the corresponding biopsy result is given in Table 1. Of the 27 women with significant pathology noted on subsequent biopsy results or ECC (endocervical curettage), only 8 (29.6%) had a positive Pap smear, while 24 (88.8%) tested

**Table 1. Correlation of positive screening test results with specific cervical histology**

Biopsy result	No. of patients	Pap smear positive	Pap or HPV positive
CIN III	2	2	2
CIN II	4	2	4
CIN I	9	3	7
HPV	12	1	11
Total significant pathology	27	8	24
Chronic cervicitis	14	2	3
Normal	5	0	3

**Table 2. Correlation of screening test result with presence or absence of cervical pathology**

Screening result	Biopsy results	
	Significant pathology present	Significant pathology absent
Pap smear positive	8	2
Pap smear negative	19	460
Sensitivity, 8/27 = 30%		
Specificity, 460/462 = 99%		
Positive predictive value, 8/10 = 80%		
Negative predictive value, 460/479 = 96%		
Pap or HPV positive	24	6
Pap and HPV negative	3	455
Sensitivity, 24/27 = 89%		
Specificity, 455/461 = 98%		
Positive predictive value, 24/30 = 80%		
Negative predictive value, 455/458 = 99%		

positive with combined Pap and HPV testing. The difference is statistically significant ( $p < 0.001$ ). The combined Pap smear and HPV testing was particularly more sensitive (18/21 = 86%) than the Pap smear alone (4/21 = 19%) in the detection of low grade, early cervical lesions (CIN I and HPV). This improved sensitivity for the combined screening protocol was seen in all age groups.

The data from these 488 women are shown in a contingency table, which allows for the computation of sensitivity, specificity, and positive and negative predictive values. The screening protocol of Pap and HPV testing was significantly more sensitive than the Pap smear alone for the detection of significant pathology ( $p < 0.001$ ). Furthermore, this screening protocol had a negative predictive value of 99%, reflecting the fact that in the face of a negative Pap and HPV test, less than 1% of the women had any significant pathology (1 woman with CIN I and 2 women with HPV) (Table 2).

The specificity of the Pap smear as a screening test is similar to the specificity of the Pap and HPV testing protocol.

## DISCUSSION

Concern about the accuracy of Pap smear screening for cervical neoplasia has led to the development of techniques that may improve screening sensitivity, either by substituting for, or adding to the Pap smear. This study demonstrates that a screening

protocol that combines the Pap smear with HPV testing may enhance the sensitivity of cervical screening.

In the patient population studied, 80% of the women with a positive Pap smear had significant pathology. Even when the HPV testing was negative, nearly all of the patients with a positive Pap smear had significant pathology, indicating that the Pap smear provides important information that is independent of and additive to the information provided by HPV testing.

The greatest value of HPV testing appears to be in the evaluation of women with negative Pap smears. Of the women with a negative Papanicolaou smear and a positive HPV test who underwent a biopsy, nearly half had significant pathology, with 40% of these biopsies showing CIN. The data indicated that physicians screening with the Papanicolaou smear alone would have missed 19 of the 27 (70%) women with significant pathology. The addition of HPV testing resulted in 16 of these 19 women being correctly identified as positive, allowing for the institution of management and/or follow-up protocols. Although Pap smear screening was more sensitive (4/6 = 66.7%) for high-grade CIN than for all lesions, including low-grade CIN and HPV infection (8/27 = 29.6%), a better sampling tool other than a cotton swab, as used in this study, should improve the screening sensitivity.

The data obtained also indicate that of the 46 women who underwent biopsy, 3

women with positive Pap and HPV testing had only inflammation, while 3 women appeared to have a normal cervix. It is noteworthy that all 6 of these women had cervical lesions that seemed to be abnormal in the opinion of an experienced colposcopist.

One explanation for the apparent discordance between the colposcopist's diagnostic opinion and punch biopsy results could be an overcall phenomenon on the part of the colposcopist [12]. It has been noted in prior studies that screening examinations based on visual data (i.e., colposcopy or cervicography) may suffer from overcall when the presence or absence of disease is based on histologic evaluation [13]. Alternatively, undercall on the part of the pathologist interpreting the punch biopsy specimen could also lead to biopsies showing a lack of significant pathology in lesions deemed to be abnormal on colposcopy.

A third possible explanation for this discrepancy is based on data which shows that colposcopically directed punch biopsies may not accurately reflect overt cervical pathology. This has been reported in studies describing histologic findings obtained after loop electrosurgical excision procedures (LEEP) of the entire lesion [14]. These data have disclosed not only the presence of microinvasive cancer in women with punch biopsies showing only HPV or mild CIN, but in one study, 24 of 51 women with punch biopsy results showing either no active epithelial disease or atypical cells characteristic of HPV infection, had CIN of varying degrees of CIS on LEEP specimens [15]. Furthermore, it has been demonstrated that cytologic evidence of inflammation or atypia may predict cervical dysplasia in some circumstances [16]. Thus, it is possible that a woman whose punch biopsy results show only various stages of reactive and reparative change may actually be harboring significant pathology adjacent to the area biopsied. Under such circumstances, only women with biopsy results revealing normal (or metaplastic) cells should be considered truly normal.

The data in this study indicate that women with a negative Pap smear and negative HPV testing are unlikely to have significant pathology; less than 1% of such women who underwent both tests in this study had CIN. From these data, we conclude that triage to routine (yearly) follow-up is appropriate for this subset of women. On the other hand, women with either a positive Pap smear or positive HPV testing deserve either closer follow-up or immediate colposcopy.

The HPV test can be performed on material collected at the time the smear is taken and requires no additional discomfort for the women being screened. It is readily automated and should cost around NT\$1000 per test. Thus, in addition to a potential role in reducing the incidence and mortality of cervical cancer, the addition of HPV testing may also improve the efficiency and reduce the cost of screening. For example, physicians will be able to immediately refer women who have lesions destined to persist, and will be able to allow longer intervals between smears and cessation of screening at an earlier age in women who are truly negative according to both tests.

The success of any screening program depends on the accuracy of the test employed. We suggest that the addition of HPV testing to cytology can substantially increase the detection rates of low-grade CIN with an acceptable positive predictive value. The HPV test may be important in preventing the increasing proportion of invasive cancers among women with apparently adequate screening histories [17]. It has also been suggested that the maximum benefits of cytology have already been achieved in well-screened populations, and new methods are needed if further progress is to be made [18]. However, it is also possible that many of the low-grade CIN lesions detected only by HPV positivity would have spontaneously regressed or would have been detected by subsequent cytologic screening before progressing to invasive disease. Thus, even though a high detection rate for CIN II/III is a

requirement for a good screening test, a large randomized trial needs to be done to determine the value of HPV testing in reducing invasive cancer rates before it can be recommended for routine screening. Such a trial will require hundreds of thousands of women, and to be cost-effective it will have to be restricted to women over 30, among whom invasive cancer is more common.

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#### REFERENCES

1. Coppleson LW, Brown B. Estimation of the screening error rate from the observed detection rates in repeated cervical cytology. *Am J Obstet Gynecol* 1974;119:953-8.
2. Morell ND, Taylor JR, Snyder RN, et al. False-negative cytology rates in patients in whom invasive cervical cancer subsequently developed. *Obstet Gynecol* 1982;60:41-5.
3. Eddy DM. Screening for cervical cancer. [Review] *Ann Intern Med* 1990;113:214-26.
4. August N. Cervicography for evaluating the "atypical" Papanicolaou smear. *J Reprod Med* 1991;36:89-94.
5. Reid R, Greenberg MD, Lorincz A, et al. Should cervical cytologic testing be augmented by cervicography or human papillomavirus deoxyribonucleic acid detection? *Am J Obstet Gynecol* 1991;164:1461-71.
6. Hatch KD, Schneider A, Abdel-Nour MW. An evaluation of human papillomavirus testing for intermediate-and high-risk types as triage before colposcopy. *Am J Obstet Gynecol* 1995;172:1150-7.
7. Kohan S, Beckman EM, Bigelow B, et al. Colposcopy and the management of cervical intraepithelial neoplasia. *Gynecol Oncol* 1977;5:27-39.
8. Cuzick J, Szarewski A, Terry G, et al. Human papillomavirus testing in primary cervical screening. *Lancet* 1995;345:1533-6.
9. Cox JT, Lorincz AT, Schiffman MH, et al. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 1995;172:946-54.
10. Nuovo GJ, Moritz J, Walsh LL, et al. Predictive value of human papillomavirus DNA detection by filter hybridization and polymerase chain reaction in women with negative results of colposcopic examination. *Am J Clin Pathol* 1992;98:489-92.
11. Luthi TE, Burk RD. Human papillomavirus and cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1993;85:1868-70.
12. Lozowski MS, Mishriki Y, Talebian F, et al. The combined use of cytology and colposcopy in enhancing diagnostic accuracy in preclinical lesions of the uterine cervix. *Acta Cytol* 1982;26:285-91.
13. Buxton EJ, Luesley DM, Shafi MI, et al. Colposcopically directed punch biopsy: a potentially misleading investigation. *Br J Obstet Gynaecol* 1991;98:1273-6.
14. Prendiville W, Cullimore J, Norman S. Large loop excision of the transformation zone (LLETZ). A new method of management for women with cervical intraepithelial neoplasia. *Br J Obstet Gynaecol* 1989;96:1054-60.
15. Chappatte OA, Byrne DL, Raju KS, et al. Histologic differences between colposcopic-directed biopsy and loop excision of the transformation zone (LETZ): a cause for concern. *Gynecol Oncol* 1991;43:46-50.
16. Noumoff JS. Atypia in cervical cytology as a risk factor for intraepithelial neoplasia. *Am J Obstet Gynecol* 1987;156:628-31.
17. Slater DN, Milner PC, Radley H. Audit of deaths from cervical cancer: proposal for an essential component of the National Screening Program. *J Clin Pathol* 1994;47:27-8.
18. van Wijngaarden WJ, Duncan ID, Hussain KA. Screening for cervical neoplasia in Dundee and Angus: 10 years on. *Br J Obstet Gynaecol* 1995;102:137-42.

# 人類乳突瘤病毒檢測合併傳統抹片在婦女子宮頸篩檢的應用

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**背景** 因為傳統抹片篩檢子宮頸癌或癌前病變仍有疏漏不足，所以許多輔助檢查都被研究或採用。本研究分析人類乳突瘤病毒去氧核糖核苷酸的檢測併用傳統抹片有否改善篩檢的功效。

**方法** 本研究共有來自2個不同單位的488位婦女同時接受傳統抹片，人類乳突瘤病毒取樣及最後陰道鏡檢和必要的切片檢查，依病理切片報告分析評估傳統抹片，傳統抹片合併人類乳突瘤病毒檢測在篩檢應用上的差異。

**結果** 傳統抹片對於子宮頸癌前病變(子宮頸上皮內贅生瘤)的篩檢敏感度為8/27(29.6%)，而若合併人類乳突瘤病毒檢測，其敏感度提升為24/27(88.8%)，有統計學上意義( $p < 0.001$ )。如果併用的兩種檢查均為正常，則子宮頸暗藏病變的只佔篩檢婦女的不到1%。合併病毒以及抹片檢查對於低度的子宮頸病灶的發現也格外有幫忙。

**結論** 本研究證實合併人類乳突瘤病毒檢測能夠提升子宮上皮內病變篩檢的敏感度，對於傳統抹片正常的可疑群婦女有再加強篩檢的好處，但是應用在大量篩檢時的經濟效益仍值得評估。(中台灣醫誌 2002;7:21-7)

## 關鍵詞

篩檢，人類乳突瘤病毒DNA 檢測，子宮頸抹片

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