Human Papillomavirus Research on the Prevention, Diagnosis, and Prognosis of Cervical Cancer in Taiwan

Angel Chao, MD, PhD; Huei-Jean Huang, MD; Chyong-Huey Lai, MD

Cervical cancer is third in incidence and fourth in mortality among cancers of women worldwide. Epidemiological studies have shown that human papillomavirus (HPV) is necessary, if not sufficient, to cause nearly 100% of cervical cancers. HPV testing is useful in primary screening for cervical neoplasms. The value of HPV detection or genotyping is potentially useful in triage of borderline or low-grade abnormal cervical cytology, follow-up after treatment of cervical intraepithelial neoplasia, assessment of prognosis and treatment planning for invasive cervical cancer. Studies from Chang Gung Memorial Hospital have defined the genotype distribution of cervical cancer in Taiwan and confirmed the independent prognostic value of the HPV genotype in cervical cancer. The cost-effectiveness of using HPV testing in prevention and management of cervical neoplasms depends on the medical and public health infrastructure of the individual country. The population-based HPV prevalence and genotype distribution as well as longitudinal follow-up studies have established strong support for incorporating HPV testing with cervical cytology and for future comparisons of HPV epidemiology before and after implementation of HPV prophylactic vaccines in Taiwan. Future directions in HPV research are discussed. (Chang Gung Med J 2012;35: 297-308)

Key words: cervical neoplasm, human papillomavirus, cancer screening, prognosis

Cervical cancer is third in incidence and fourth in mortality among cancers of women worldwide. (1) zur Hausen first suggested that human papillomavirus (HPV) was likely to be a sexually transmitted agent in the mid-1970s. (2) More recent molecular and epidemiological studies have shown that HPV is the main causative agent of cervical neoplasms. HPV contributes to the development of cervical intraepithelial neoplasia (CIN) and subsequent invasive carcinomas. (3) To date, approximately 120 different HPV types that infect humans have been identified. The 15 leading HPV types (HPV16, 18, 58, 33, 52, 39, 45, 31, 51, 56, 59, 35, 68, 73, and 82) that have been linked to cervical cancer constitute high-risk types. (4) Characterization of the HPV types according to phylogenetic species resulted in their assignment to the following groups: α1/α8/α10 (HPV6, 11, 40, 32, 42, 43, 44, 55, and 74), α3/α15 (HPV61, 62, 71, 72, 81, 83, 84, and 85).

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83, and 84), α5/α6 (HPV26, 51, 53, 56, 66, 69, and 82), α7 (HPV18, 39, 45, 59, 68, and 70), and α9 (HPV16, 31, 33, 35, 52, 58, and 67). Although the rate of cervical cancer has declined since 1960, this disease is still a major burden in the Asia-Pacific region. According to statistics from the Department of Health of Taiwan, the incidence of cervical cancer was 12.2/100,000 in 2007. Research on the role of HPV in cervical cancer is active in Taiwan. The purpose of this review is to summarize the literature on methods of HPV detection and genotyping, HPV molecular epidemiology in cervical neoplasms, the roles of HPV testing in diagnosis and management of CIN, HPV genotypes in the prognosis for cervical cancer, and perspectives on HPV vaccines with emphasis on the most significant findings of those from Taiwan.

**Diagnostic efficacy of HPV detection**

Over recent decades, many studies worldwide have attempted to identify specific HPV DNA types. Initially, Southern blot analysis was the gold standard for HPV DNA analysis. However, this technique is specific but laborious, and its sensitivity is low. A commercial kit called the Vira Pap/ Vira Type kit, which involves the use of the dot blot test based on radiolabeling, was developed. In 1993, the presence of HPV DNA could be detected by the hybrid capture assay, which utilizes a chemiluminescence substrate and is marketed as ViraType Plus by Digene Diagnostics, Beltsville, Md, U.S.A. The hybrid capture second generation (HC2) assay, approved by the U.S. Food and Drug Administration, is currently available for the detection of 13 carcinogenic HPV types, which include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Several important studies have used HC2, however, the threshold of detection is approximately 10000 copies of the virus.

With the advancement of technologies such as the polymerase chain reaction (PCR), the detection limit of HPV DNA is as low as 50-100 copies/cells of the virus in a sample. However, a negative result may be related to a technical error, a sampling error, or unspecified/untested HPV genotypes, so the existence of HPV-negative cervical cancer is certainly doubtful. General (consensus) PCR primer sets, such as MY09/MY11, GP5+/6+, SPF1/2, CUT primers, and type-specific PCR primers have been developed. Huang et al. created a modified set of primers (SPF1/GP6+) that amplify a 184-bp DNA fragment followed by reverse blotting of 38 types of HPV DNA with a macroarray gene chip (EasyChip® HPV Blot; King Car, Taiwan) in a single reaction. Different methods of HPV detection are shown in Table 1. The SPF1/GP6+ primer set is sensitive in detecting HPV in paraffin-embedded tissues obtained from patients with cervical cancer. The performance of HPV Blot has been shown to be comparable to that of HC2 in cervical swab samples as well. Discrepancies between the results of the two assays were investigated by direct sequencing and type-specific PCR. In an analysis of 354 swab samples, the concordance of HPV Blot and HC2 was 80.8%, with substantial agreement between the methods. A multicenter study of 468 patients compared PCR using a modified MY11/GP6+ -based HPV blot assay with L1 type-specific PCR for 20 genotypes. Hence HPV Blot was certified as an alternative diagnostic medical device in Taiwan.

The list of possible causes underlying false-negative molecular diagnoses includes low viral load (below the threshold required for detection), insufficient DNA extracted from the samples, inefficient PCR amplification, or weak alkaline phosphatase substrate activity. Efforts have been made to improve the diagnostic performance of HPV DNA testing. Chao et al. demonstrated that reliable determination of HPV status is crucially dependent on the amount of input genomic DNA (for example, 25 ng for SPF1/GP6+ PCR plus HPV Blot).

**HPV testing for follow-up after treatment of CIN**

Lin et al. used the HC2 assay to evaluate 75 patients with grade 3 CIN who underwent hysterectomy following conization. Those with negative results for HPV after conization were all (23/23) free of residual disease in the uterus (a 100% negative predictive value). All those who had residual disease (27/27) had positive results for HPV at the time of hysterectomy (100% sensitivity). This prospective study confirmed the excellent sensitivity and negative predictive value of HPV DNA testing after conization in predicting residual cervical neoplasia. Follow-up analyses of a consecutive series of 2,154 patients who had received conization revealed that HPV follow-up status was a significant predictor.
of residual/recurrent high-grade CIN.\(^{(26)}\) This result was confirmed by other prospective studies on the addition of HPV testing to conventional methods in follow-up after conization.\(^{(27,28)}\)

**HPV genotype distribution in the general population, as represented by a Taoyuan County-Chang Gung Memorial Hospital cohort**

Primary screening of cervical cancer by Papanicolaou (Pap) smear or monolayer cytology provides sensitivity rates ranging from 30% to 87%.\(^{(29)}\) The additional value of HPV DNA testing (SPF1/11 PCR plus HPV Blot) as a complementary method to the Pap smear to improve the sensitivity in detecting ≥ CIN2 was investigated in 10014 women (> 30 years old) who resided in Taoyuan, Taiwan who had intact uteri and no history of treatment for preinvasive or invasive cervical neoplasms.\(^{(30)}\) Informed consents were obtained, and cervical swab samples were collected from women at a mobile examination booth or outpatient clinics by gynecologists from Taoyuan County-Chang Gung Memorial Hospital (CGMH), local practitioners in their offices, or public health nurses at their health stations. The overall HPV prevalence in the study population was 10.8% (95% confidence interval [CI] 10.5%-11.4%). A total of 37 types of HPV were identified; the leading three were HPV52, 18, and 58. The sensitivity of the Pap smear was 81.9%, which improved to 97.2% with combined Pap and HPV testing. Adding HPV testing identified an additional 11 cases and achieved a 15.3% improvement in sensitivity.

There was a significant positive correlation of HPV prevalence with older age, postmenopausal status, current use of oral contraceptives, and no history of hormone replacement therapy use. Past users of oral contraceptives and those who had never undergone Pap smears demonstrated a higher risk of abnormal Pap smears, whereas women aged 40-49 years old had a reduced risk. No specific subgroup was found to benefit most from the combined strategy.\(^{(30)}\) The value of adding an HPV test to the conventional Pap smear must be evaluated after longer follow-up of this population-based cohort.

Recently, a report on a community-based study of 10602 participants (age 30-65 years) from Taiwan showed that the overall HPV prevalence was 16.2% (using MY11/GP6+ PCR plus HPV Blot), and for cytologically normal participants was 13.8%. The

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**Table 1. Common HPV Typing Methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Author</th>
</tr>
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<tbody>
<tr>
<td>ViraType Plus</td>
<td>Digene corporation, FDA approved, signal amplification to detect and quantitate viral nucleic acid</td>
<td>Brown et al(^{(31)})</td>
</tr>
<tr>
<td>HC2</td>
<td>Digene corporation, semi-quantitative, detects 13 high-risk HPV types in a cocktail, no type determination</td>
<td>Cavuslu et al(^{(32)})</td>
</tr>
<tr>
<td>MY09/11 PCR</td>
<td>Amplimer 450 bp, reverse line blot assay, detects 37 genotypes</td>
<td>Bauer et al(^{(33)})</td>
</tr>
<tr>
<td>SPF1/2 PCR</td>
<td>Amplimer 65 bp, followed by direct sequencing, sequences compared with known types</td>
<td>van Muyden(^{(34)})</td>
</tr>
<tr>
<td>GP5+/6+ or GP11/12 PCR</td>
<td>Amplimer 140-150 bp, dot blot or restriction fragment length polymorphism, detects 11 genotypes</td>
<td>Snijders(^{(35)})</td>
</tr>
<tr>
<td>CUT primer PCR</td>
<td>Amplimer 370 bp, followed by direct sequencing, detects 69 types</td>
<td>Chouhy et al(^{(36)})</td>
</tr>
<tr>
<td>Genpoint kit (Dako cytation, Kyoto, Japan)</td>
<td>Unamplified target, in situ hybridization with biotin-labeled HPV16 probe on paraffin-embedded sections</td>
<td>Fujii et al(^{(37)})</td>
</tr>
<tr>
<td>SPF1/GP6+ PCR EasyChip® HPV Blot (Kingcar, Taiwan)</td>
<td>Amplimer 184 bp, hybridization with (HPV Blot), detects 38 types</td>
<td>Huang et al(^{(38)})</td>
</tr>
</tbody>
</table>

**Abbreviations:** PCR: polymerase chain reaction; HC2: hybrid capture second generation; HPV: human papillomavirus.
most common carcinogenic types were, in order, HPV52, 16, 56, and 18. The prevalence of abnormal Pap smears at baseline in this study was 3.9%, including cytologically atypical squamous cells of undetermined significance (ASCUS) (1.3%), low-grade squamous intraepithelial lesion (LSIL) (1.1%) and high-grade squamous intraepithelial lesions (HSIL+) (1.5%). The prevalences of Taoyuan County- CGMH cohort of HSIL and ASCUS/LSIL were 0.45% and 1.53%, respectively. The rate of abnormal Pap smears reflects the study population, which partially explains the differences between these two large studies from Taiwan. In general, hospital-based studies tend to detect higher HPV positivity among study subjects. The HPV prevalence in a hospital-based study in northern Taiwan (n = 215) was 20.9%. Similarly, in a hospital-based study performed in southern Taiwan (n = 4383), Lin et al. reported that the overall prevalence was 19.3%, with the leading HPV types being HPV16, 52, and 58. The age-specific curves of HPV prevalence were U-shaped, and the prevalence of HPV in women > 30 years old (32.6%) was higher than that in women > 30 years old (17.2%). However, in the Dutch Population Based Screening Study Amsterdam trial (n = 44102), the prevalence of high-risk HPV decreased from 12% at age 29-33 years to 2.4% at 59-61 years.

Three-year follow-up of HPV testing to complement Pap smears in cervical screening

Incorporating HPV testing into cervical cancer screening has been advocated. Although the specificity of the test would be decreased, an expected increase in the sensitivity of early detection of high-grade cervical neoplasms is predicted. The identification of a high-risk HPV infection causes anxiety in patients. Because of lack of adequate knowledge in predicting the outcome, the appropriate approach to management is unclear, except for the need for periodic follow-up care.

Several host factors, such as older age, hormone replacement therapy, concurrent other lower-genital tract infections, a generalized decrease in immune responsiveness, parity, and viral variables, such as HPV genotype, multiple HPV infections, and multiple HPV variants, have been reported to be associated with HPV persistence/decreased clearance.

Women ≥ 30 years old with HPV-positive status but normal cytology and a negative colposcopy were recruited into a 3-year longitudinal follow-up study. Of the 626 eligible women, 526 (median age 47 years, range 29-75 years) were enrolled, and 412 returned at least once for follow-up. The median follow-up time of the enrolled subjects was 23 months (range 6.8-39). The 3-year cumulative total HPV clearance was 49.0% (95% CI: 43.3%-54.7%). The median 3-year cumulative type-specific HPV clearance rate was 50.0% (range 0%-100.0%), with a median time to clearance of 12.4 months (range 6.4-24.5). Older age and high viral load were associated with significantly decreased total HPV clearance. After adjusting for confounding variables, the hazard ratio for developing ≥ CIN2 in baseline HPV-positive women was 34.0-fold (95% CI: 15.5-74.7) that in HPV-negative women. Therefore, careful follow-up is important in HPV-positive women with normal cytology. The above study is ongoing, involving an additional 3 years of periodic long-term follow-up and an expanded number of subjects to obtain a comprehensive clinical profile.

In a 5-year follow-up study of 1708 women treated at ten hospitals in seven cities around Taiwan, 108 cytology-negative and HPV-positive women and 1202 cytology- and HPV-negative women were analyzed. The cumulative incidences of high-grade CIN and cervical cancer in HPV-positive women were 5.6 and 3.7%, respectively, whereas those in HPV-negative women were 0.3 and 0%, respectively. HPV-positive women had a 24.9-fold higher chance of developing ≥ CIN2 than HPV-negative subjects.

To analyze women who were Pap- and HPV-negative at baseline, a nested cohort of subjects (n = 8825) from a population-based cervical cancer screening study who were followed for 3 years was investigated. The incidence of novel acquisition of HPV was 4.2 per 100 woman-years. The 3-year cumulative total HPV acquisition rate was 11.1% (95% CI: 8.1-14.1). Multivariate analysis revealed that an increased number of sexual partners (≥ 2 vs. 1) was associated with an increased risk of HPV infection (odds ratio OR: 5.0, 95% CI: 2.0-12.6). Three cases of ≥ CIN2 were identified among active HPV-negative subjects at the 3-year follow-up. The HPV genotypes in the dysplastic tissues were revealed to be present in the baseline samples of two of these three cases after reanalysis. In the passive
HPV-negative group, only one case progressed to CIN2, probably after HPV acquisition. It was concluded that negative Pap and HPV tests conferred an extremely low risk of developing ≥ CIN2 within 3 years despite incident HPV infection. A screening interval of 3 years is safe and recommended.\(^{(45)}\)

**HPV testing as triage for borderline or low-grade abnormal cervical cytology**

The Taiwan Cooperative Oncology Group initiated an epidemiologic HPV prevalence study of 1264 women with abnormal cervical cytology visiting gynecologic clinics of 11 major medical centers in 2005.\(^{(46)}\) The prevalences of HPV in the atypical squamous/glandular cells with undetermined significance (suspicious) (n = 316) and low-grade intraepithelial lesions (n = 474) were 36.1% and 74.7%, respectively. The main correlates of HPV prevalence were lifetime number of sex partners ≥ 2, vaginal douching after intercourse, vitamin supplementation, and performance of Pap smear tests. The risk for vaginal douching was augmented by the promiscuity of sex partners and smoking, whereas vitamin supplementation had some protection in reducing HPV infection. The results of this study provided data on the epidemiologic correlates of HPV in borderline and LSIL.\(^{(46)}\) To predict the progression of LSILs with HPV infection, Ho et al. analyzed 65 LSILs with HPV DNA types 16, 18, 52, or 58 among 294 women with baseline LSILs. They found that women with LSILs whose viral loads increased between baseline and the 6-month follow-up had a 45% risk of developing HSIL (OR = 7.6, 95% CI = 1.9-29.4, \(p < 0.01\)) as evaluated by real-time PCR and a 44% risk (OR = 6.1, 95% CI = 1.6-22.7, \(p < 0.01\)) as evaluated by HC2. The authors concluded that evaluation of viral load changes (increased or not increased) through repeat HPV DNA testing could predict disease progression in LSIL cases with HPV types 16, 18, 52, and 58.\(^{(47)}\)

To determine the effects of age, Lin and associates analyzed 119 women over 50 years old with ASCUS or LSIL. Those who had positive HPV infections were at increased risk for developing ≥ CIN2.\(^{(48)}\) The ASCUS-LSIL Triage Study recruited 3488 women with ASCUS and randomly assigned them to the following three groups: immediate colposcopy arm (referral to colposcopy regardless of enrollment test result), HPV triage arm (referral to colposcopy if positive for HPV), and conservative arm (referral to colposcopy if cytology was HSIL).\(^{(49)}\) The results showed that the cumulative diagnoses of CIN3 did not vary significantly among the three groups. The conservative strategy of repeat cytology at the HSIL threshold referred 12.3% of women to colposcopy while detecting 54.6% of cumulative CIN3. The HPV triage strategy referred 55.6% of women and detected 72.3% of cumulative cases of CIN3. By halving the number of women referred for colposcopy, HPV testing triage is considered cost-effective in the United States. The situation is different in Taiwan, where immediate colposcopy is cheaper than HPV testing. However, with HPV genotype information, the decision to perform a biopsy could be different for different gynecologists performing the colposcopy.

**HPV genotype distribution in CIN and invasive cervical cancer in Taiwan**

In another study from Taiwan, HPV genotyping was performed on 1086 paraffin-embedded, formaldehyde-fixed CIN 2/3 specimens excised between 1999 and 2001 at CGMH.\(^{(49)}\) HPV DNA was detected in 91.6% of the specimens, and multiple HPV types were identified in 19.3%. The most common HPV types were HPV16 (24%), 52 (20%), 58 (20%), 33 (13%), 31 (8%), and 18 (4.6%). The leading 6 types accounted for 87.6% of the cases, and HPV16 or 18 accounted for only 30.9%. In women older than 50 years, HPV16 and 18 accounted for 21.3%, and HPV52, 58 and 33 represented 55.5%. In women younger than 50 years, HPV16 and 18 accounted for 32.1% of cases (\(p < 0.0001\)), and HPV52, 58 and 33 accounted for 47.9% (\(p = 0.02\)). The rates of HPV16, 18, 39, and 45 infection were significantly higher in cervical cancer than in CIN 2/3 (\(n = 2118\)). Comparison of HPV typing in cancer and CIN 2/3 between Taoyuan and other geographical regions in a meta-analysis is shown in Table 2.\(^{(49,50)}\) Interestingly, HPV16 was the most common HPV type in cervical cancer in all geographical regions in the world. Therefore, an effective vaccine against the most common HPV types could prevent a significant proportion of the cervical cancer cases that occur in Taiwan.

In a community-based cohort of 10602 participants, HPV16 was found in 48.2% of 56 cases of invasive and in situ cervical cancer.\(^{(31)}\) In a study of
40 patients with cervical cancer in Hualien, the HPV16 and 18 genotypes were found in 70% of the specimens analyzed. In the early 1990s, National Taiwan University Hospital reported an HPV positive rate of 79% in 433 cervical cancer samples of stage I-II fresh-frozen tissues from radical hysterectomies. The authors found that pelvic lymph node metastases were significantly more prevalent in HPV-positive (24.3%) than HPV-negative (11%) women with stage I disease. Furthermore, the incidence of positive lymph nodes was significantly associated with squamous cell carcinoma (*p* < 0.01), but not with adenocarcinoma. Interestingly, the authors did not find any HPV genotype that exhibited prognostic significance. However, researchers at Tri-Service General Hospital analyzed 94 cases of stage I-IV fresh-frozen specimens and found that HPV58-related types (types 58, 33, and 52) were prevalent in the older age group (> 52 years) and predicted a favorable outcome. In a large series reported by CGMH, 2118 stage I-IV paraffin-embedded archival tissues collected between 1993 and 2001 were analyzed. HPV was found in 96.6% of patients, with 18% having multiple infections. HPV16 was noted in 50% of the HPV-positive cases, followed by HPV18, 58, 33, and 52. Type 58-related HPVs (33, 52, or 58; 30.3%) were more prevalent in older patients, those with stage III and IV cancer, and those who had received radiation or concurrent chemoradiation. The 1067 patients with stage I-IIA cancer who underwent radical hysterectomy were further analyzed for prognostic outcome.

### Table 2. Geographical Distribution of Prevalence of HPV Types in Cervical Intraepithelial Neoplasia Grades 2 and 3 and Squamous Cell Carcinoma of the Cervix

<table>
<thead>
<tr>
<th>HPV types</th>
<th>SCC</th>
<th>CIN2/3</th>
<th>Prevalence ratio</th>
<th>SCC</th>
<th>CIN2/3</th>
<th>Prevalence ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>1836</td>
<td>99.4%</td>
<td>995</td>
<td>91.6%</td>
<td>1.09 (1.07-1.11)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>973</td>
<td>52.7%</td>
<td>262</td>
<td>24.1%</td>
<td>2.18 (1.95-2.45)</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>325</td>
<td>17.6%</td>
<td>216</td>
<td>19.9%</td>
<td>0.89 (0.76-1.03)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>282</td>
<td>15.3%</td>
<td>50</td>
<td>4.6%</td>
<td>3.33 (2.49-4.44)</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>171</td>
<td>9.3%</td>
<td>142</td>
<td>13.1%</td>
<td>0.71 (0.57-0.87)</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>136</td>
<td>7.4%</td>
<td>220</td>
<td>20.3%</td>
<td>0.36 (0.30-0.44)</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>44</td>
<td>2.4%</td>
<td>81</td>
<td>7.5%</td>
<td>0.32 (0.22-0.46)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HPV: human papillomavirus; CIN2/3: cervical intraepithelial neoplasia grades 2 and 3; SCC: squamous cell carcinoma; *: Data in the meta-analysis (50) gained from Africa, Asia, Europe, North America, Oceania, and South /Central America. Bold indicates significance between Taiwan and the world in SCC or CIN2/3.; *p*-values: b < 0.001, c < 0.001, d < 0.0037, e < 0.001, f < 0.001, g < 0.0034, h < 0.001, i < 0.001, j < 0.001, k < 0.0045, l < 0.001, m < 0.001.

In a large series reported by CGMH, 2118 stage I-IV paraffin-embedded archival tissues collected between 1993 and 2001 were analyzed. HPV was found in 96.6% of patients, with 18% having multiple infections. HPV16 was noted in 50% of the HPV-positive cases, followed by HPV18, 58, 33, and 52. Type 58-related HPVs (33, 52, or 58; 30.3%) were more prevalent in older patients, those with stage III and IV cancer, and those who had received radiation or concurrent chemoradiation. The 1067 patients with stage I-IIA cancer who underwent radical hysterectomy were further analyzed for prognostic outcome. Bootstrap resampling using R software (http://www.r-project.org) was randomly performed to construct new data sets followed by Cox regression analysis, which was repeated 1000 times; the counts of selection considered to be significant for each variable were recorded. The median follow-up of the surviving patients was 77 months. Outcome-predicting models were constructed using seven significant covariates for either overall survival (OS) or recurrence-free survival (RFS). The prognostic score was 0 for FIGO stage I and 1 for stage II; 0 for depth of stromal invasion less than one third and 1 for stromal invasion greater than or equal to one third; 0 for no lymph node metastasis and 1 for lymph node metastasis; 0 for no parametrial extension and 1 for the presence of parametrial extension; 0 for differentiation of grade 1 and 1 for...
grade 2 or 3; 0 for HPV18 negativity and 1 for HPV18 positivity; 0 for age < 45 years and 1 for age < 45 years. The probability of dying was highest in the high-risk group (prognostic score = 4 to 7), followed by the intermediate-risk group (prognostic score = 3), and the low-risk group (prognostic score = 0 to 2). The five-year OS rates of the high-, intermediate-, and low-risk groups were 97.4%, 87.6%, and 77.9%, respectively. The five-year RFS rates of the high-, intermediate-, and low-risk groups were 95.9%, 86.7%, and 72.7%, respectively. The predicting models for death and relapse in early-stage cervical cancer could be useful for counseling patients before treatment and stratifying study subjects in future clinical trials.\(^{(54)}\)

In another cohort of 1100 cervical cancer patients treated by primary radiotherapy, the leading types were the alpha-7 and -9 species.\(^{(56)}\) The high-risk group consisted of patients with HPV infection or those infected with the alpha-7 species only. Patients coinfected with the alpha-7 and alpha-9 species belonged to the medium-risk group, and the others were included in the low-risk group.

### Methylation in the carcinogenesis of cervical cancer

In addition to HPV testing, DNA methylation has been investigated in the context of CIN and cervical cancer.\(^{(57-59)}\) Investigating the methylation status of CpG dinucleotides found within the long control region (LCR), Ding et al. reported that methylation of the LCR of HPV16 is strongly associated with the severity of the cervical neoplasm.\(^{(59)}\) Among cervical squamous cell carcinoma specimens, 50% were found to be methylated, whereas only 5% were methylated in LSIL.\(^{(59)}\) Lai et al. demonstrated that six genes (SOX1, PAX1, LMX1A, NKX6-1, WT1 and ONECUT1) were differentially expressed using a CpG island (CGI) microarray containing 8,640 CGI tags; this finding was further validated in cervical cancer tissues. The parallel testing of HPV and PAX1 methylation in 22 cervical swabs generated improved sensitivity compared with HPV testing alone (80% vs. 66%, respectively) without compromising specificity (63% vs. 64%, respectively) for HSIL/SCC. When testing the PAX1 methylation marker alone, the specificity for HSIL/SCC was 99%.\(^{(58)}\) In ASCUS, the odds ratios (95% CI) for CIN 3 or more severe neoplasms in women who tested positive for methylation of three novel methylation-silenced genes (PAX1, WT1, and PCDH10) were 26.4 (9.0-77.3), 18.1 (6.9-47.2), and 10.3 (4.1-25.9), respectively.\(^{(57)}\) In triage for ASCUS, each methylation test resulted in fewer colposcopy referrals and lower false-positive rates but higher false-negative rates than the HPV tests.\(^{(57)}\) The potential use of DNA methylation as a marker for complementing cervical cancer detection warrants studies with larger sample sizes.

### HPV vaccines

HPV vaccines are divided into prophylactic and therapeutic vaccines. The prophylactic vaccines are commercially available as Gardasil® (HPV6, 11, 16, and 18) and Cervarix® (HPV16 and 18). The impact of a prophylactic vaccine on the incidence of cervical neoplasms can only be observed over the course of decades.\(^{(60-62)}\) A decrease in the incidence of high grade cervical abnormalities within 3 years after the implementation of a population-wide HPV vaccination programme in Australia has been reported.\(^{(63)}\)

An effective therapeutic vaccine is still urgently needed because a prophylactic vaccine does not impact HPV clearance rates of existing infections.\(^{(64)}\) Currently, a significant number of patients have HPV-related lesions that need to be abolished. Therapeutic vaccines mainly require the generation of T cell-mediated immunity and include live vector-based, peptide- or protein-based, nucleic acid-based, and cell-based vaccines that target the HPV E6 and/or E7 antigens.\(^{(65)}\) DNA-based vaccines are stable, easy to produce and may be repeatedly administered. However, DNA-based therapeutic vaccines are limited in their ability to amplify and spread in vivo. Given that dendritic cells are key players in the generation of antigen-specific immune responses, strategies to modify the properties of DNA-transfected dendritic cells are being developed.\(^{(64)}\)

Methods to enhance DNA vaccines include (1) encoding IL-2 linked to the HPV-16 E7 antigen or pretreatment with cisplatin to generate enhanced E7-specific cytotoxic T lymphocytes;\(^{(66,67)}\) (2) improving antigen expression in dendritic cells by developing a codon-optimized HPV-16 E6 DNA vaccine (pNGVL4a-E6/opt);\(^{(68)}\) and (3) linking HPV-16 E7 to endoplasmic reticulum chaperone molecules (ER-60, tapasin, and calnexin)\(^{(69)}\) to generate E7-specific T cell-mediated immune responses and antitumor
effects in vaccinated mice. This led to a more potent DNA vaccine when tested in vaccinated mice compared to untreated mice. CD4 (+) T cells are also known to exert long-term antitumor effects. CD4 (+) T cells were shown to assist in the generation of E7-specific CD8 (+) T cell immune responses in mice vaccinated with SINrep5-E7/HSP70 and boosted with vac-E7/HSP70. Several therapeutic HPV DNA vaccine trials have been completed. For instance, a microencapsulated DNA vaccine encoding HPV-16 and -18 E6- and E7-derived epitopes, termed ZYC-101a (MGI Pharma), was tested in a multicenter, double-blind, randomized, placebo-controlled trial. The results were significant in a group younger than 25 years old in resolving CIN2/3 lesions compared with patients receiving a placebo. The therapeutic HPV vaccine is foreseen as a significant approach that could be combined with existing modes of therapy, such as chemotherapy or radiation.

Future directions
Epidemiological studies have shown that HPV is necessary, if not sufficient, to cause virtually 100% of cervical cancers. The question of whether HPV-negative cervical cancer exists warrants further investigation. Other pressing issues that warrant further research include the following: (1) In addition to methods such as HPV Blot with an L1 consensus primer, E6- and E7-type specific PCR, real-time quantitative PCR, and in situ hybridization that have been employed to improve HPV detection in these specimens, the next generation of sequencing technologies may allow resequencing the entire human genome to identify the binding site(s) for HPV. (2) The prevalence of high-risk HPV in vulvar, penile, and anal cancer is worthy of investigation. (3) The impact of the introduction of prophylactic HPV vaccines on the type distribution and incidence of cervical cancer and the cost-effectiveness need to be established. (4) In combination with the currently available treatment modalities, a therapeutic HPV vaccine is anticipated to play an important role in treating HPV-related diseases.

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人類乳突狀病毒在台灣的子宮頸癌預防，診斷和預後之研究

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子宮頸癌的發病率在全世界婦女癌症排名第三，而死亡率排名第四。流行病學研究顯示，人類乳突狀病毒 (HPV) 是造成近 100% 的子宮頸癌的主要因素。HPV 檢測在初級篩檢子宮頸腫瘤的用途是被肯定。HPV 基因分型檢測用於邊緣或低度子宮頸細胞學異常，治療後續子宮頸宮內腫瘤病變 (CIN) 及評估子宮頸癌預後及擬定治療計劃有潛在的價值。長庚醫院的研究已證實子宮頸癌的 HPV 基因型在台灣的分佈及獨立預後價值的確認。使用 HPV 檢測在預防和處理子宮頸腫瘤的決策依個別國家的醫療衛生基礎設施而有不同的成本效益。在台灣人口的盛行率和 HPV 基因型的分佈以及縱向追蹤研究提供堅強的支持台灣將 HPV 檢測納入與子宮頸細胞學做子宮頸腫瘤篩檢；以及未來在 HPV 預防性疫苗實施前後 HPV 流行病學的比較。在文中也會討論未來的 HPV 研究方向。(長庚醫誌 2012;35:297-308)

關鍵詞：子宮頸腫瘤，人類乳突狀病毒，癌症篩檢，預後