RESEARCH COMMUNICATION

Performance of High-Risk Human Papillomavirus Testing in the Triage of Abnormal Cervical Cytology among Chinese Younger Women in Shanghai, China

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Abstract

Objective: This study aimed to evaluate the role of high risk HPV DNA testing in identifying Chinese younger women with abnormal cytology at risk of harboring cervical intraepithelial neoplasia at grade 2 (CIN2) or worse so as to popularize an effective triage strategy for younger women. Methods: A total of 246 younger women aged 25 - 36 years old with abnormal cytology were recruited in our study. All were assessed by liquid-based cytology, high-risk HPV DNA test, and colposcopy with directed biopsy and endocervical curettage as necessary. Residual specimens from liquid-based cytology were subjected to real-time PCR testing to identify the presence of 10 high-risk HPV types that are prevalent in China. Results: Among the 246 abnormal cytology samples, 97 (39.4%) were found to be positive for high risk HPV. A clear association was observed between cytological findings and the proportion of patients with positive high risk HPV DNA: namely 29.8% HPV positivity in the ASCUS group; 43.5% in LSIL group; and 90.0% in HSIL group (p<0.01). Overall, high risk HPV test achieved a high specificity (79.8%) and PPV (86.5%) for an endpoint of CIN2+, and higher sensitivity (91.3%) and NPV (98.7%) for an endpoint of CIN3+. For younger women with ASCUS+ cytology, high risk HPV test achieved a higher NPV for CIN2+ and CIN3+ (96.0%, 99.0%). For LISL+ cytology, high risk HPV testing had a high sensitivity with LSIL (90.0%) and HSIL (100%), but there was also a corresponding decrease in specificity. Conclusions: The results indicate that high risk HPV DNA testing is highly sensitive and moderately specific for CIN grade 2 or worse in women younger than 36 years. LBC primary testing followed by high risk HPV DNA triage improved sensitivity and the false-positive rate for cervical cancer screening and are suitable for developed regions in China.

Keywords: Human papillomaviurs - cervical dysplasia - liquid-based cytology - PCR

Introduction

The mortality of cervical cancer in the developed countries has decreased greatly because of the introduction of the conventional Papanicolaou (Pap) smear test (Saslow et al., 2002). However, cervical cancer is vastly more common in developing nations than in developed nations, and it is fairly usual in China with about 132,300 new cases each year (Kim et al., 2009). Moreover, cervical cancer is often diagnosed in younger women, which is the second most common cancer in women aged younger than 35, after breast cancer. A study of Chinese women revealed that cervical cancer was not only more common in China but also developed at a younger age than developed countries (Kim et al., 2009). It has been observed that women with minor cytological abnormalities in their screening history are at an increased risk of cervical cancer, which indicates that follow-up and treatment of these women has been insufficient (Silfverdal et al., 2009).

Although cervical cytology screening reduces the incidence and mortality from cervical cancer, many women are diagnosed as having equivocal cytological abnormalities, such as atypical squamous cells of undetermined significance (ASC-US). The management of the abnormal cytological result is controversial and clinical practice patterns range from performing immediate colposcopy to repeating cervical cytology at specified intervals. The majority of abnormal cytology represents minor changes in the cervical cells that are more common in younger women and tend to regress. However, despite a masked low-grade cytological phenotype, as many as 28% of women with cytologic ASC-US harbor cervical intraepithelial neoplasia (CIN) grade 2 or 3 (Cox et al., 2003), these women are at risk of developing high-grade lesions. On the other hand, women referred immediately to colposcopy could be overtreated with

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potential adverse pregnancy outcomes (Kyrgiou et al., 2006, Arbyn et al., 2008). A randomized controlled trial showed that, although the detection rate of CIN grade 2 or worse (CIN2+) is higher at baseline for women referred immediately to colposcopy, there is almost no difference in its cumulative incidence by three years compared to cytological surveillance (2009).

Human papillomavirus (HPV) infection is associated with virtually all cases of cervical cancer. Long term infection with high-risk strains of HPV can lead to the development of cervical dysplasia and cancer (Khan et al., 2005). HPV-DNA testing was recommended as the primary screening method for to cervical cancer by International Agency for Research on Cancer (IARC). In epidemiological research, numerous case-control studies have reported strong associations between cervical cancer and infection with HPV-16, HPV-18, and other "high-risk" HPVs. To date, about 20 different HPV types have been classified as high-risk or probably high-risk types. Among them, HPV 16 and 18 are the most predominant high-risk HPV types,(Munoz et al., 2003; Kulkarni et al., 2011) and HPV 58 and 52 were the priority HPV types in Chinese women.(Bao et al., 2008a) In Asian women, the most eight common HPV types were 16, 18, 58, 33, 52, 45, 31, and 35 that accounted for additional 20% of cervical cancer cases(Bao et al., 2008b). A meta-analysis report has showed that high-risk HPV DNA test has a role in the triage of atypical squamous cells of undetermined significance (ASCUS) smears (Arbyn et al., 2004); however, its value in the triage of abnormal cytology for women of reproductive age is questionable.

Much of the evidence on the clinical usefulness of primary screening by testing for HPV-DNA is based on research with the Digene High-Risk HPV HC2 DNA Test (QIAGEN) assay and reference to the women from the developed countries (Lorincz, 1996; Ogilvie et al., 2005). Given the economic status and different prevalent HPV types in China, here, we explored a new HPV-DNA test expressly designed for low-resource setting and the new test is a signal-amplification assay that detects target HPV-DNA from 10 different high-risk HPV types (16, 18, 31, 33, 35, 45, 52, 53, 56, and 58). The purpose of this study is to investigate the effect of different criteria of referral, based on results from high-risk HPV and liquid-based cytology testing, to define the best methods in the triage strategy for Chinese younger women.

Materials and Methods

Patient population

A total of 258 sexually active women aged 23 - 36 years old with abnormal cytology were interviewed and examined between July 2009 and June 2011. Health specific and sexual behavior data were also collected from all subjects using a structured questionnaire with an informed consent clause. Women included in the pooled analysis all concurrently received HPV DNA testing and colposcopy. The final clinical diagnosis of the women was made by histological evaluation of biopsy samples obtained at colposcopy. All possibly abnormal histological slides were reviewed independently by an experienced pathologist. Ethical approval for this study was granted by the ethics committee of Xinhua hospital and informed consents were obtained.

Liquid-based cytology (LBC)

The cervical cell scrapings were collected with a cytobrush from the ecto- and endocervix of the uterus of each woman. Cervical cells were collected with a cytobrush and dispersed in a standard liquid solution. After obtaining smeared cell slides for ThinPrep liquid-based cytology test, the remaining cell samples on the cytobrush were suspended in PBS and stored at -80°C until DNA extraction. All patients underwent LBC (ThinPrep, USA), and results were graded according to the Bethesda system(Solomon et al., 2002), but did not use the subcategories of atypical cells of undetermined significance (ASCUS). The cytological classifications were: within normal limits (negative); atypical squamous cells of undetermined significance (ASCUS); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma (SCC); adenocarcinoma in situ (AIS); or adenocarcinoma. The results below ASCUS (including normal and inflammatory cells) were defined as negative in cytology; the results of ASCUS or more severe were defined as abnormal in cytology.

Detection of high risk HPV DNA

The amplification was performed on ABI 7500 PCR system (Applied Biosystems, USA). Real-time PCR is the sensitive method for HPV DNA detection. We use the real-time PCR method to detect HPV genotypes (HPV16, 18, 31, 33, 35, 45, 52, 53, 56, 58) with the high-risk human papillomavirus detection kit (Fosun Diagnostics, China), which is approved by Chinese State Food and Drug Administration (SFDA), following the protocol recommended by the manufacturer. GAPDH gene was amplified in each sample as a control of specimen adequacy to prevent false negative result. The accuracy is more than 97.5%, compared with DNA sequencing. Infection with low-risk HPV types was not evaluated.

Colposcopy and histology

The colposcopist then took one or two colposcopically directed biopsies of the area with the worst colposcopic impression according to standard of practice, and one or two biopsies of squamous and columnar epithelium from an area of normal appearance. If the overall colposcopic impression was normal, biopsies were obtained from one or two normal sites and included both types of cervical epitheliums. All biopsies were submitted to pathologists for sectioning and reading. We used the histology result as the criterion standard of diagnosis.

Statistical analysis

Analysis of the data was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, USA). We assessed the accuracy parameters, such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of high risk HPV on the basis of detecting abnormal cytology samples

Table 1. Characteristics of Baseline Results of Cytology, HPV Result, and Reviewed Histological Outcome

Variables	Liquid-Based Cytology			
	ASCUS	LSIL	HSIL+	Total
High risk HPV	141	85	20	246
Negative	99 (70.2%)	48 (56.5%)	2 (10%)	149 (60.6%)
Positive	42 (29.8%)	37 (43.5%)	18 (90%)	97 (39.4%)
Histology				
Normal or Benign	70 (49.6%)	10 (11.8%)	1 (5%)	81 (32.9%)
CIN 1	50 (35.5%)	39 (45.9%)	3 (15%)	92 (37.4%)
CIN 2	19 (13.5%)	26 (30.6%)	5 (25%)	50 (20.3%)
CIN 3	2 (1.4%)	10 (11.8%)	10 (50%)	22 (8.9%)
Carcinoma	0 (0.0%)	0 (0.0%)	1 (5%)	1 (0.4%)

Table 2. Prevalence of Human Papollomavirus (HPV) Testing in the Subjects with Abnormal Cervical Cytology

Variables	Cases	Histology				
		Normal or Benign	CIN1	CIN2	CIN3	Carcinoma
High risk HPV (-)	149	68	70	9	2	0
ASCUS		58	37	2	1	0
LSIL		9	31	7	1	0
HSIL+		1	1	0	0	0
High risk HPV (+)	97	13	22	41	20	1
ASCUS		12	12	17	1	0
LSIL		1	8	19	9	0
HSIL+		0	2	5	10	1

harboring CIN2+ or CIN3+ as confirmed by histology biopsies. The $\chi 2$ test was used to assess the statistical significance of differences between the various tissue specimens. Receiver operating characteristic curves (ROC) curves analysis was performed using MedCalc software. Differences were considered to be statistically significant at p< 0.05.

Results

Patient characteristics

A total of 258 Chinese younger women with ASCUS or worse cervical cytology in Shanghai were recruited in our study. With 12 cases lost, the final effective sample size was 246. The age of participants was from 23 to 36 years old, without the history of pregnant. The average age ± standard deviation of the subjects was 29.4 ± 3.1 years. The proportion of Han nationality people was 98.9%. A total of 204 (83.0%) women reported having used contraception. 1.2% of the women (3 of 246) had gone through HPV detection before. A total of 24 of the

246 (9.8%) women had experienced cervical cytology detection. Overall, high-risk HPV infections were not statistically significant difference among women <30 years of age (41.7%) and >30 years of age (38.3%) in this study (p>0.05). When patients were grouped according to their marital status, level of education, age of first sexual intercourse and numbers of sexual partners, percentage of high-risk HPV infection cases were not significantly different for each attribute (p>0.05). Table 1 shows the baseline results from cytology, HPV tests, and reviewed histology.

Correlation of cytology with histology

All 246 subjects underwent cervical cytology tests, high-risk HPV-DNA tests, and colposcopy. According to categories of abnormal cytology, ASCUS accounted for 57.3% (141/246), LSIL for 34.6% (85/246), and HSIL+ for 8.1% (20/246). As shown in Table 1, we present the correspondence between cytology and the relevant histologic diagnosis in all 246 patients. The majority of AUSCS samples (49.6%) corresponded to normal and benign (No-CIN or less), the majority of LSIL samples (45.9%) corresponded to low grade histology (CIN1 or less), and the majority of HSIL or more severe samples (75%) corresponded to high grade histology (CIN2 or worse).

Prevalence of high risk HPV-DNA in abnormal cytology and histology

Among the 246 abnormal cytology samples, 97 (39.4%) were found to be positive for high risk HPV by Real-Time PCR test (Table 1). We recorded positive HPV results in 42 (29.8%) women with ASCUS, 37(43.5%) with LSIL, and 18 (90%) with HSIL+ (Table 1). The HPV distribution in different cytology is statistical significantly difference (p<0.01). The HPV prevalence was higher among women with a diagnosis of LSIL (43.5%) and with HSIL (90%) than among 42 women with ASCUS cytological findings (29.8%) (p<0.01). The HPV prevalence was 16.0% (13/81) among women with cytological abnormalities that were not histologically confirmed. The distribution of final histology and its relationship to high risk HPV status is shown in Table 2, which 62.9% (61/97) patients has histologically confirmed CIN2 or CIN3. 84.7% (61/72) patients with histology of CIN2 or CIN3 are positive of high risk HPV. Most women with histology results of CIN grade 2 or worse tested positive by HPV DNA testing. There were two patients with confirmed high-grade dysplasia (CIN3) had a negative high risk HPV test. Only one patient with confirmed cervical carcinoma had both the HISL of cytology and a positive high risk HPV test.

Table 3. Accuracy Parameters of High Risk HPV Testing for Histology-Confirmed CIN2+ and CIN3+ in Abnormal Cytology Cases

Cytology	Endpoint CIN2+				Endpoint CIN3+			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Abnormal LBC	83.6%	79.8%	86.5%	45.3%	91.3%	66.4%	21.9%	98.7%
ASCUS	81.0%	80.0%	41.5%	96.0%	50.0%	71.2%	2.4%	99.0%
LSIL	77.8%	81.6%	75.7%	83.3%	90.0%	62.7%	24.3%	97.9%
HSIL	100%	50.0%	88.9%	100%	100%	22.2%	61.1%	100%

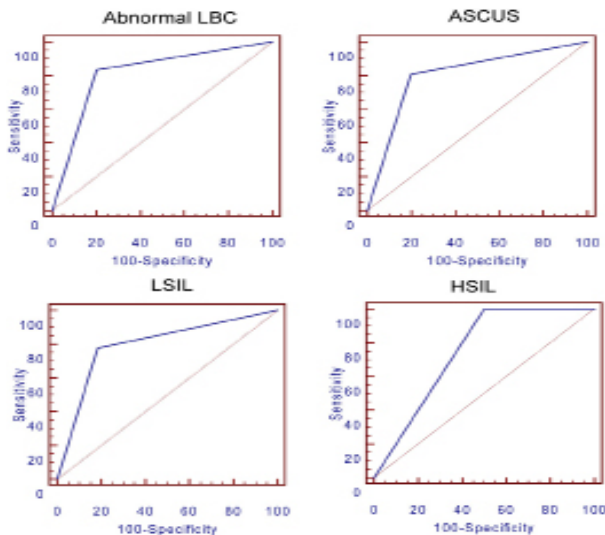PPV, positive predictive value; NPV, negative predictive value.

Figure 1. Receiver Operating Characteristic Curves of High Risk HPV DNA Using Cervical nItraepithelial Neoplasia at Grade 2 (CIN2) as the Disease Threshold in Younger Women with Abnormal Cytology

High risk HPV test promote the triage of abnormal cytology for younger women

To explore the capacity of high risk HPV DNA test in triage of abnormal cytology in younger women, we subsequently assessed the accuracy parameters of high risk HPV DNA test in detecting those abnormal cytological samples that harbor CIN2+ or CIN3+ histology (Table 3). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the high risk HPV DNA tests were then evaluated. Overall, for CIN2+, high risk HPV DNA test achieved a higher specificity (79.8%) and PPV (86.5%). On the contrary, high risk HPV DNA had a higher sensitivity (91.3%) and NPV (98.7%) for CIN3+ (Table 3). Receiver operating characteristic curves (ROC) of high risk HPV DNA using cervical intraepithelial neoplasia at grade 2 (CIN2) as the disease threshold in younger women with abnormal cytology was shown in Figure 1. The area under the curve for all abnormal cytology is 0.817. In detail, the area under the curve for ASCUS is 0.81, which is higher than that for LSIL (0.79) and for HSIL+ (0.75), but there were no found to be statistically significantly different (p>0.05) (Figure 1). We compared the area under the ROC curve for high risk HPV DNA and liquid-based cytology for the detection of CIN2+ and CIN3+. The results showed that there was no statistically different between high risk HPV DNA and LBC (p>0.05). When analysis was restricted to all younger women with ASCUS+ cytology, high risk HPV test achieved a higher NPV for CIN2+ and CIN3+ (96.0%, 99.0%).

For younger women with LISL+ cytology, high risk HPV testing had a high sensitivity (90.0%), but low specificity for the detection of histologically confirmed CIN3+, giving it a high negative predictive value of 97.9%. For detecting cases with HSIL+ cytology, high risk HPV DNA tests achieved 100% sensitivity and 100% NPV for CIN2+ or CIN3+, but there was also a corresponding decrease in specificity (Table 3, Figure1).

Discussion

Clinical applications of HPV DNA testing were used for primary screening and triage of equivocal cytology findings. Our study is an attempt to assess whether high riskHPV detection by PCR-based assay have a role in the risk assessment of abnormal cytology among the Chinese younger women. Most previous related studies were mainly focused on general population or patients with cervical cancer in developed countries. Moreover, previous studies comparing HPV testing with conventional (Kuhn et al., 2000; Cuzick et al., 2003) and liquid-based cytology consistently found higher sensitivity with HPV testing (Clavel et al., 2001; Kulasingam et al., 2002). However, loss in specificity of HPV DNA is most relevant in younger women because of increased HPV prevalence. In this study, our results showed that HPV testing alone with cytology triage could be a feasible alternative for screening younger women with abnormal cytology in developed region of China. Our work supports that high risk HPV test provide enhanced sensitivity and specificity for triage of abnormal cytology in Chinese younger women.

With the introduction of cervical cytology in China, the more women are diagnosed as having a cervical cytological result of ASUS and LSIL, especially for younger non-pregnant women. The triage of abnormal cytology, especially ASC-US and LSIL, is still controversial causing increased anxiety to the gynecologist and the women involved. In the USA, HPV DNA testing is recommended as an adjuvant to cervical cytology screening (Wright et al., 2004). In China, women who live in urban regions have access to opportunistic screening for cervical cancer, which includes cytology or HPV DNA testing. Previous study has found the HPV DNA test to be a highly accurate screening test across various locations and age groups in China with optimum screening accuracy (Zhao et al., 2010). In general, the epidemiological research on human HPV has to date been performed using either the Digene's Hybrid Capture 2 (HC2) HPV DNA testing or PCR based methods. The HC2 test, the technology commonly used for HPV-DNA testing in high-resource countries, has been recommended by the U.S Food and Drug Administration (FDA) as a general screening test for the early detection of cervical cancer in the USA and it has been extensively validated in many large studies. Compared to the HC2 test, PCR based method seems more popular in china because of the simplicity, applicability, affordability and feasibility. A compared research have showed that both HC2 and real-time High Risk HPV test showed similar sensitivity and NPV for predicting HSIL/CIN2+ and triage of ASC-US (Wong et al., 2011). In our study, we apply the real time PCR to detect the 10 HPV high risk type, including HPV16, 18, 31, 33, 35, 45, 52, 53, 56, 58, which is prevalent in Chinese population (Wu et al., 2010). Our results showed that this test is feasibility, flexibility, and acceptability by women and providers for achieving the highest effect possible by optimizing coverage of the populations at risk.

In USA, HPV testing is not approved to guide

colposcopy triage of women with abnormalities of higher grade than ASC-US. Moreover, this test is not recommended to replace cytology or as an adjunct to cytology for women under the age of 30, in whom most HPV infection is transient and benign (Ho et al., 1998), although HPV testing as an adjunct to cytology was more commonly used for women under 30 years of age than for older women.(2005, 2003) Indeed, higher rate of transient infections was found in younger women. In women of Europe and North America, the peak of HPV infection happens in their 20s, with a steady decrease in prevalence as age increases (Cuzick et al., 2006). Although the HPV DNA test is not recommended as a primary screening test in young women in other countries, the high sensitivity and specificity of HPV DNA testing in young Chinese women suggest that a different strategy might be applicable in China (Zhao et al., 2010). The guideline by the Chinese government in 2004 recommended that the initial screening time should be between the ages of 20 and 30 years in economically developed areas and for high-risk groups appropriately early enough to examine. In this study, while the pooled real time High Risk HPV test was found to demonstrate a high sensitivity in predicting CIN2+ among ASC-US. For CIN2+, high risk HPV DNA test achieved a higher specificity and PPV. When analysis was restricted to all younger women with ASCUS+ cytology, high risk HPV test achieved a higher NPV for CIN2+ and CIN3+. These figures are same as those reported in similar studies on triage of ASC-US and LSIL conducted (Kim et al., 2002; Tsoumpou et al., 2011).

It has been reported that HPV testing for high-risk types was more sensitive than liquid-based cytology. Many of them, however, were based on self-referred women (Petry et al., 2003; Salmeron et al., 2003) . In addition, some were conducted in populations at high risk. Results of these previous studies are consistent with our findings in a Chinese younger population, which same as a study from China showed that HPV DNA test sensitivity did not differ greatly among women in different age groups (Li et al., 2010). Among younger women with abnormal cytology, a negative HPV DNA test for high-risk types is a good predictor of the absence of high-grade dysplasia. Moreover, among patients with the equivalent of HSIL cytology, a positive HPV DNA test indicated the presence of CIN3+. Findings from our analysis show that HPV DNA testing is a highly sensitive and moderately specific test for CIN grade 2 or worse and grade 3 or worse lesions in the Chinese shanghai population. We do acknowledge that there are some limitations of this study include: its sample size, the absence of long-term follow-up data, and used high risk HPV test does not identify the HPV subtypes and provide genotype information. Some study showed that HPV viral load is highly correlated with cytologic abnormality (Kovacic et al., 2006).

In conclusions, our results indicate that high risk HPV DNA testing is highly sensitive and moderately specific for CIN grade 2 or worse in Chinese women younger than 36 years. LBC primary testing followed by high risk HPV DNA triage improved sensitivity and increased false-positives for cervical cancer screening and are suitable for developed regions in China.

References

ACOG Practice Bulletin (2003): clinical management guidelines for obstetrician-gynecologists. Number 45, August 2003. Cervical cytology screening (replaces committee opinion 152, March 1995). *Obstet Gynecol*, **102**, 417-27.

ACOG Practice Bulletin (2005). Clinical Management Guidelines for Obstetrician-Gynecologists. Number 61, April 2005. Human papillomavirus. *Obstet Gynecol*, **105**, 905-18.

Cytological surveillance compared with immediate referral for colposcopy in management of women with low grade cervical abnormalities (2009): multicentre randomised controlled trial. *BMJ*, **339**, b2546.

Arbyn M, Buntinx F, Van Ranst M, et al (2004). Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst*, **96**, 280-93.

Arbyn M, Kyrgiou M, Simoens C, et al (2008). Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. *BMJ*, **337**, a1284.

Bao YP, Li N, Smith JS, et al (2008a). Human papillomavirus type-distribution in the cervix of Chinese women: a meta-analysis. *Int J STD AIDS*, **19**, 106-11.

Bao YP, Li N, Smith JS, et al (2008b). Human papillomavirus type distribution in women from Asia: a meta-analysis. *Int J Gynecol Cancer*, **18**, 71-9.

Clavel C, Masure M, Bory JP, et al (2001). Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Br J Cancer*, **84**, 1616-23.

Cox JT, Schiffman M, Solomon D (2003). Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. *Am J Obstet Gynecol*, **188**, 1406-12.

Cuzick J, Clavel C, Petry KU, et al (2006). Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*, **119**, 1095-101.

Cuzick J, Szarewski A, Cubie H, et al (2003). Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet*, **362**, 1871-6.

Ho GY, Bierman R, Beardsley L, et al (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med*, **338**, 423-8.

Khan MJ, Partridge EE, Wang SS, et al (2005). Socioeconomic status and the risk of cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. *Cancer*, **104**, 61-70.

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Kim JJ, Wright TC, Goldie SJ (2002). Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. *JAMA*, **287**, 2382-90.

Kim K, Zang R, Choi SC, et al (2009). Current status of gynecological cancer in China. *J Gynecol Oncol*, **20**, 72-6.

Kovacic MB, Castle PE, Herrero R, et al (2006). Relationships of human papillomavirus type, qualitative viral load, and age with cytologic abnormality. *Cancer Res*, **66**, 10112-9.

Kuhn L, Denny L, Pollack A, et al (2000). Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. *J Natl Cancer Inst*, **92**, 818-25.

Kulasingam SL, Hughes JP, Kiviat NB, et al (2002). Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. *JAMA*, **288**, 1749-57.

Kulkarni SS, Vastrad PP, Kulkarni BB, et al (2011). Prevalence and distribution of high risk human papillomavirus (HPV) types 16 and 18 in carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. *Asian Pac J Cancer Prev*, **12**, 645-8.

Kyrgiou M, Koliopoulos G, Martin-Hirsch P, et al (2006). Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. *Lancet*, **367**, 489-98.

Li C, Wu M, Wang J, et al (2010). A population-based study on the risks of cervical lesion and human papillomavirus infection among women in Beijing, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*, **19**, 2655-64.

Lorincz AT (1996). Hybrid capture method for detection of human papillomavirus DNA in clinical specimens: a tool for clinical management of equivocal Pap smears and for population screening. *J Obstet Gynaecol Res*, **22**, 629-36.

Munoz N, Bosch FX, de Sanjose S, et al (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*, **348**, 518-27.

Ogilvie GS, Patrick DM, Schulzer M, et al (2005). Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. *Sex Transm Infect*, **81**, 207-12.

Petry KU, Menton S, Menton M, et al (2003). Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. *Br J Cancer*, **88**, 1570-7.

Salmeron J, Lazcano-Ponce E, Lorincz A, et al (2003). Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos State, Mexico. *Cancer Causes Control*, **14**, 505-12.

Saslow D, Runowicz CD, Solomon D, et al (2002). American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin*, **52**, 342-62.

Silfverdal L, Kemetli L, Andrae B, et al (2009) Risk of invasive cervical cancer in relation to management of abnormal Pap smear results. *Am J Obstet Gynecol*, **201**, 188 e1-7.

Solomon D, Davey D, Kurman R, et al (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*, **287**, 2114-9.

Tsoumpou I, Valasoulis G, Founta C, et al (2011). High-risk human papillomavirus DNA test and p16INK4a in the triage of LSIL: A prospective diagnostic study. *Gynecol Oncol*, **121**, 49-53.

Wong OG, Lo CK, Szeto E, et al (2011). Efficacy of Abbott RealTime High Risk HPV test in evaluation of atypical squamous cells of undetermined significance from an Asian screening population. *J Clin Virol*, **51**, 136-8.

Wright TC, Jr., Schiffman M, Solomon D, et al (2004). Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol*, **103**, 304-9.

Wu D, Cai L, Huang M, et al (2010). Prevalence of genital human papillomavirus infection and genotypes among women from Fujian province, PR China. *Eur J Obstet Gynecol Reprod Biol*, **151**, 86-90.

Zhao FH, Lin MJ, Chen F, et al (2010). Performance of high-risk human papillomavirus DNA testing as a primary screen for cervical cancer: a pooled analysis of individual patient data from 17 population-based studies from China. *Lancet Oncol*, **11**, 1160-71.