

**ASSOCIATION OF REPRODUCTIVE HISTORY WITH HUMAN PAPILLOMAVIRUS  
AND CERVICAL INTRAEPITHELIAL NEOPLASIA SEVERITY**

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Public Health (Epidemiology).

Chapel Hill  
2007

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## ABSTRACT

SUZANNE BELINSON: Association of Reproductive History with Human Papillomavirus and Cervical Intraepithelial Neoplasia Severity  
(Under the direction of Katherine Hartmann)

Our objective was to uncover potential links between Human Papillomavirus infection, common reproductive outcomes and high-grade cervical pre-cancer. We evaluated common reproductive risk factors, by varied stratifications of histologic grade, among 2,055 women positive and 6,657 women negative for Human Papillomavirus (HPV), who were enrolled in the Shanxi Province Cervical Cancer Screening Study II. Logistic regression was used to generate odds ratios and their corresponding 95% confidence intervals.

Risk-factor profiles diverged for Cervical Intraepithelial Neoplasia (CIN) II compared to CIN III, but were broadly similar for CIN II compared to CIN I. An increased risk of CIN III versus CIN II was seen for higher gravidity ( $\geq 3$  pregnancies) [odds ratio (OR)=1.6 (95% confidence interval [CI]: 1.0, 2.6)] and sexual intercourse within four months of childbirth [OR=2.0 (1.3, 3.2)]. Risks associated with reproductive factors appeared comparable for CIN II and CIN I, except an inverse association observed for sexual intercourse within four months of childbirth for CIN II versus CIN I [OR= 0.64 (0.42, 0.97)]. If CIN III and CIN II are biologically similar, risk-factor profiles would be expected to be more similar between CIN III and CIN II. Instead, risk factor profiles between CIN II and CIN I were more similar.

Utilizing these results, we investigated a broader spectrum of reproductive risk factors for CIN III versus  $\leq$  CIN II. Higher gravidity ( $\geq 3$  pregnancies) was associated with higher risk of CIN III versus  $\leq$  CIN II [OR=1.5 (1.0, 2.1)], as was intercourse within four months of childbirth [OR=1.7 (1.2, 2.3)], and age. It is biologically plausible that elevated levels of hormones during pregnancy or immediately postpartum may act as promoters in cervical carcinogenesis, aiding the

progression of cervical disease. These results add to the accumulating evidence that CIN II may be biologically more similar to CIN I than to CIN III, and that reproductive co-factors play an important role in the progression of HPV to high-grade pre-cancer. These results can provide impetus for investigators with prospective data to follow-up women with CIN II, and to analyze risk factors by histological grade.

## **ACKNOWLEDGEMENTS**

The five years of work needed to complete this dissertation have left me permanently changed and strengthened. The completion of the work would not have been possible without a network of support. I am most grateful for my parents, who taught me that anything is attainable, and who are always there to catch me when I fall. This project would not have come to completion without the dedication of my committee; they worked hard and fast to meet my deadlines. Jamie you are my heart; your support and encouragement was essential. You replenish me when I feel there is nothing left. To my daughter Madeline, you are why I do what I do. I hope that I make you all proud.

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## LIST OF ABBREVIATIONS

CI	confidence interval
CICAMS	Cancer Institute Hospital, Academy of Medical Sciences
CIN	cervical intraepithelial neoplasia
DNA	deoxyribonucleic acid
ECC	endocervical curettage
HPV	human papillomavirus
IARC	International Agency for Research on Cancer
IUD	intrauterine device
mRNA	messenger ribonucleic acid
NCI	National Cancer Institute
OC	oral contraceptive
OR	odds ratio
PCR	polymerase chain reaction
SPOCCS II	Shanxi Province Cervical Cancer Screening Study II
STD	sexually transmitted disease
TFR	total fertility rate

## **CHAPTER I**

### **REVIEW OF THE LITERATURE**

#### A. Conceptual Framework

Human Papillomavirus (HPV) is a common sexually-transmitted infection and the central cause of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer. Dysplastic changes, termed CIN, are classified into three grades, CIN I, CIN II and CIN III, based on increasing degrees of cellular change and disorganization. Currently, a two-tiered histological grouping has been adopted [1] (CIN I, CIN II/III) to reflect putative differences in the natural history between CIN I (lower probability of progressing) and CIN II/III (a dysplastic process with greater likelihood for progression to cancer) [1]. It has been hypothesized that a proportion of CIN II lesions may be more biologically similar to CIN I than to CIN III [2]. If confirmed, future treatment guidelines for women, especially young women, with CIN II diagnoses may need revision.

Infection with oncogenic (high-risk) HPV types alone is not sufficient for cervical carcinogenesis. Thus, other exogenous and endogenous factors act in conjunction with oncogenic HPV infection to influence the progression from infection to invasive cancer [3]. Identification of these HPV co-factors within a group of HPV-infected women can help identify those women at the highest risk for progression to CIN III and invasive cervical cancer.

Parity was one of the earliest risk factors associated with cervical cancer risk [4]. In recent studies, the relative risks of cervical cancer among women who have had five or more births have ranged from 3.8 (squamous cell carcinoma) [5] to 4.4 [6], compared with nulliparous women or 5.1 compared with nulliparous or primiparous women [7]. The means by which parity influences

progression to cervical cancer is not entirely clear. Hypotheses include: the maintenance of the transformation zone, [5] elevated levels of circulating hormones [8], immunosuppression caused by pregnancy [9] and cervical trauma related to vaginal delivery [10]. Further research is thus needed to investigate mechanisms by which parity may influence the risk of cervical carcinogenesis.

In China, invasive cervical cancer is the seventh leading cause of cancer-related deaths in women, with an estimated incidence rate of 5.2/100,000 women among those reporting. As a comparison, in 2002 the age-adjusted incidence rate for cervical cancer was 8.7/100,000 for all races in the United States [11, 12]. In some provinces of China, such as the Shanxi province in the Northeast of the country, the risk of invasive cervical cancer is estimated to be higher at 14.5/100,000 women. Due to the one child policy in China, on average women have fewer children than in other less-developed nations, particularly in Chinese urban centers. In the countryside there are more pregnancies and more births than in Chinese urban centers. In one study in Xiangyuan County, Shanxi Province, China where the estimated invasive cervical cancer incidence rate is estimated to be 40.7/100,000 women [13], the mean number of pregnancies for women enrolled in a cross sectional study of cervical cancer screening was 3.05 with a range of 0-13 births [14].

## B. Background and Significance

### *1. Human Papillomavirus, Invasive Cervical Cancer*

High-risk HPV types have been clearly established as the central cause of invasive cervical cancer and its related precursors [15]. A study conducted in 22 countries found that the presence of high-risk HPV types is nearly universal in invasive cervical cancer (99.6%) [16]. A case-control study in nine countries using highly-sensitive polymerase chain reaction (PCR)-based primers found that HPV types 16 and 18 alone are associated with 60-70% of invasive cervical cancer cases [17]. A recent review similarly found that the most common HPV types in 10,058

squamous invasive cervical cancer cases were, in order of decreasing prevalence, HPV 16 (46-63%), 18 (10-14%), 45 (2-8%), 31 (2-7%), 33 (3-5%), 58, 52, 35, 59, and 56 in all regions except Asia where HPV types 58 (6%) and 52 (4%) were relatively more common [18, 19]. In a large series of 1,518 women aged 15-72 years from a routine care setting in France, 44.0% (150/338) of young women (aged 15-30 years) were classified as HPV high-risk positive by Hybrid Capture II [20]. It is estimated that high-risk HPV infection is immunologically cleared in most women within 12 to 24 months [21-23], although some studies report clearance times that may be slightly shorter. Some women who do not clear their high-risk HPV infection progress to develop CIN III [24], and of those it is estimated that approximately one-third of women with CIN III will progress to develop invasive cervical cancer if untreated [25].

The major steps in cervical cancer carcinogenesis are infection with HPV, persistence of HPV infection over time, and progression from infection to pre-cancer and invasion [26]. The p53 protein, a tumor suppressor gene, prevents cell growth in the presence of cell damage. HPV oncogenic proteins E6 and E7 can both inhibit p53, thus leading to carcinogenesis. Some women, who do not clear their HPV infection, may develop persistent infections, and thus have an increased risk of progression to higher grades of CIN. Low-grade lesions (CIN I) may be caused by low or high-risk HPV infections [27]. In contrast, high-grade lesions (CIN III) are almost always associated with high-risk HPV types, are characteristic of E6 and E7 expression, and often show integration of the HPV viral genome into the host cell [28]. It is likely that uncontrolled E6/E7 expression is a phenomenon that distinguishes the process of cell transformation from a productive viral infection [2]. E6/E7 messenger ribonucleic acid (mRNA) has been found to be associated with high-grade cervical pre-cancer and cancer [29], although further prospective studies are indicated.

## *2. Progression from HPV infection to CIN III*

Although CIN was initially described as a continuum of histological changes (all of which were considered true precursors of invasive disease if untreated [27]), our understanding of the

natural history of cervical neoplasia has since been revised [27, 30] to acknowledge that CIN does not inevitably progress through each higher grade of CIN to cancer. When dysplasia progresses, it is thought to be orderly, i.e. not “skipping” from lower grades to cancer. However, progression between some levels, for instance from CIN I to CIN II, is the exception rather than the rule. Consequently, the classification of cervical cancer precursors by histology may need revision to more accurately reflect natural history of disease progression and to better inform clinical decision making [27]. Currently, a two-tiered histological grouping has been adopted [1] (CIN I, CIN II/III) to reflect putative differences in the natural history between CIN I (lower probability of progressing) and CIN II/III (a dysplastic process with a greater likelihood of progression to cancer) [1].

Recent research suggests CIN II may not be a discrete histological grade that can be reliably classified, but instead an overlap between CIN I (a non-neoplastic HPV infection) and CIN III (a neoplastic cervical cancer precursor) [2, 31]. It has been hypothesized that a proportion of CIN II lesions are biologically similar to CIN I and have greater probability of regressing over time, while other CIN II lesions are more characteristic of CIN III lesions [2]. Limited by study size, few studies have been able to complete the analyses required to compare risk factors by histological grade.

### *3. HPV Cofactors*

While HPV infection is necessary, it is not sufficient to cause invasive cervical cancer; other factors in conjunction with HPV infection lead to persistent HPV infection and progression to invasive cervical cancer. Cofactors can be divided into three categories: 1) environmental or exogenous factors, including cervical trauma, diet, oral contraceptive (OC) use, smoking, co-infection with HIV and other sexually transmitted agents; 2) HPV viral cofactors such as infection with specific types, viral load and viral integration; 3) host cofactors including genetic factors and other host factors related to the host immune response. The most well-established of

these cofactors are multiparity, smoking, young age at first intercourse and long term oral contraceptive use [3].

#### *4. Parity and Cervical Cancer*

Parity has consistently been identified as a risk factor for cervical cancer development. Since multiparity is still common in many less-developed countries, the contribution of parity in the aetiology of cervical cancer development has relevant public health implications [32, 33]. The magnitude of the effect of parity has been reported with relative risk estimates ranging from null or close to null values [6, 34-40] to 4.4 for five pregnancies compared with nulliparous women [6]. In the International Agency for Research on Cancer (IARC) multicenter study, an odds ratio (OR) of 3.8 was found for seven pregnancies when compared with nulliparous women or 2.3 when compared with women who had one or two full-term pregnancies [5]. In Latin America, a relative risk of 5.1 was found for women with 14 or more pregnancies compared with nulliparous or primiparous women [7]. Most recently, the International Collaboration of Epidemiological Studies of Cervical Cancer published a report stating that after controlling for age at first full-term birth, the relative risk for invasive cervical carcinoma among parous women was 1.76 (95% confidence interval [CI]: 1.53-2.02) for  $\geq 7$  full term pregnancies compared to women with 1-2 full term pregnancies [15]. This study reanalyzed the data of 16,563 women with cervical carcinoma and 33,542 controls from 25 epidemiologic studies. While certainly the largest analysis to date, the questions of what mechanism is responsible for the association between parity and cervical cancer and at which stage of carcinogenesis parity affects risk remain unanswered.

##### a. Potential Biologic Mechanisms for the Effect of Parity on Cervical Cancer Development

There are many possible explanations of the associations seen between parity and cervical cancer. Pregnancy may increase the risk of cervical cancer due to the fact that pregnancy maintains the transformation zone on the ectocervical region for the full period of the pregnancy, leaving the women at greater risk of HPV infection. The number of metaplastic cells in the

transformation zone have been shown to increase during pregnancy [5]. These cells are most susceptible to HPV infection in this immature phase of development [35]. In a woman who has experienced multiple pregnancies, the metaplastic transformation zone will have repeatedly been exposed to carcinogenetic agents (i.e. HPV infection). It is this repeated exposure experienced over multiple pregnancies that may intensify the actions of carcinogenic infectious agents causing an increased risk for cervical cancer [41]. During pregnancy, there are high concentrations of circulating oestrogen and progesterone hormones. There is some epidemiologic evidence for a role of endogenous hormones in cervical carcinogenesis [8], and several laboratory studies of cervical cell lines and HPV-16 transgenic mice [42-46]; however, a case-control study of endogenous hormones and cervical cancer found no evidence that plasma levels of sex hormones have an important role on the risk of cervical cancer in HPV infected women [40]. An additional explanation for the role of hormones during pregnancy may be that they could favor, or accelerate cervical carcinogenesis with a mechanism similar to that put forward to explain the increased risk of cervical cancer among oral contraceptive users [47, 48]. The data consistently points to oral contraceptives promoting some step in the process of cervical carcinogenesis rather than having a role in facilitation of infection or persistence [49]. Pregnancy induced immunosuppression however may favor the infection or aid in the oncogenic properties of HPV [9].

The mechanism may not be related to pregnancy per se but cervical inflammation and trauma during delivery. In the IARC multicenter study women who reported cesarean but not vaginal deliveries had an odds ratio of 0.98 (95% CI: 0.36, 2.7) when compared to nulliparous women, and parous women who reported only cesarean deliveries showed a decreased risk compared with women who reported vaginal deliveries only, but the 95% CI was broad (0.1, 1.1) due to the rarity of cesarean delivery in the study [5].

##### *5. China*

China has the largest population in the world (1.2 billion people; approximately 300 million women of reproductive age) and the largest number of invasive cervical cancer cases in the world

[50]. Although a one-child policy was introduced in China in 1979, the total fertility rate (TFR) never fell below a 2.5 child-per-woman average in rural areas, although it dropped to about 1.2 in urban areas. By the mid-1980s, less than one-fifth of all eligible married couples had signed the one-child certificate -- a contract which granted couples and their child economic and educational advantages in return for promising not to have more than one child. Throughout the 1980s, nearly half of all reported births were second, third, or higher order births. Various surveys suggested that the desire to have at least two children remains strong among Chinese couples. In the Shanxi Province Cervical Cancer Screening Study II (SPOCCS II), a study of 8,079 women in the Shanxi province of China, this trend continued with women reporting a mean of 3.05 pregnancies (0-13) and a mean of 2.26 live births (0-8) [14].

Invasive cervical cancer is the second most common cancer in women worldwide and the leading cause of cancer mortality in women from the developing world [32]. Of the estimated 600 women that die each day of cervical cancer, 80% are from the developing world where access to cervical cancer screening and therapeutic interventions is limited [32, 51]. In China, over 100,000 cases of invasive cervical cancer are diagnosed each year, representing over one quarter of the cervical cancer cases worldwide [13]. Each year approximately 20,000 women in China die of invasive cervical disease [13].

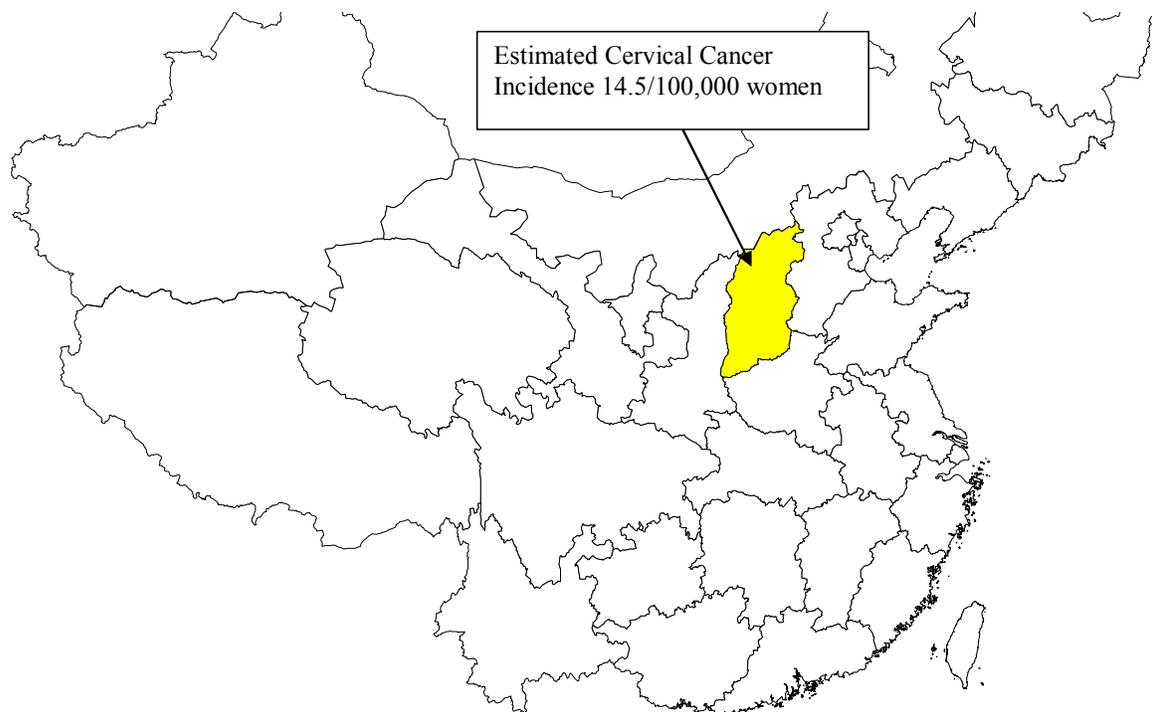
#### a. Cervical Cancer Incidence, Mortality, and Prevention in China

Cervical cancer mortality rates in Chinese women declined from 10.7/100,000 women in the 1970s to 3.9/100,000 women in the 1990s due to limited screening in large cities [13]. However, with more than 300 million women of reproductive age (15-49 years) in China, there are wide regional variations in the documented mortality rates of invasive cervical cancer. Two provinces in China (Shanxi and Gansu) have some of the highest reported cervical cancer mortality rates in the world (See Figure 1). These invasive cervical cancer mortality rates, standardized to the world population, are 40.7/100,000 (Yangcheng, Shanxi Province) and 42.0/100,000 women (Wudu, Gansu Province) [13].

Table 1. Estimated cervical cancer incidence and mortality in Yangcheng County, Shanxi Province (1/100,000 women) [52]

Age (years)	30-	35-	40-	45-	50-	55-	60-	65-	70-	Total
	34	39	44	49	54	59	64	69	74	
Incidence per 100,000 women	32.6	136.1	234.2	484.5	249.4	298	232.4	174	94.9	132.1
Mortality per 100,000 women	3.1	24.4	52.3	141.9	153.2	176.1	146	87	113.9	52.1

Figure 1. Map of China with Shanxi Province Highlighted



### C. Summary

Infection with oncogenic HPV is common. HPV may cause histologic changes which will regress over time or progress to cervical cancer if untreated. Dysplastic changes are categorized into a two-tiered system CIN I (lesions with a low probability of progression) and CIN II/CIN III

(lesions with a higher likelihood for progression). Recently, this classification has come under reconsideration as studies show CIN II may behave more like CIN I than CIN III.

Determining whose HPV infection will progress to cervical cancer is one of the unanswered questions in the field. Part of this answer may lie in the study of risk-factor profiles. The identification of risk factors associated with HPV infection and each subsequent stage of cervical abnormality may offer clues.

Over 100,000 cases of cervical cancer are diagnosed each year in China, representing over one quarter of the worldwide cervical cancer incidence. Data from a large study of Chinese women offers an opportunity to look at reproductive risk factors for HPV infection and CIN.

## **CHAPTER II**

### **SPECIFIC AIMS**

#### A. Specific Aims and Hypotheses

The aims of this study were:

**Specific Aim 1:** To determine if CIN II lesions are epidemiologically more similar to CIN I or CIN III lesions in terms of risk predictors, among women enrolled in the SPOCCS II study.

Hypothesis: CIN II may be differentially misclassified when grouped with CIN III. By investigating the risk factor profiles of CIN II compared to CIN I and CIN III, we can determine where they are similar and where they diverge. We believed CIN II would show more similarities with CIN I than with CIN III.

**Specific Aim 2:** To describe established reproductive cofactors on the risk of CIN III compared to those for CIN I/HPV, among women in the SPOCCS II study.

Hypothesis: Histological diagnosis can act as a marker for disease progression. By investigating the cofactor profiles between women at various stages of cervical disease in a cross-sectional study, it may be possible to determine why some women progress and others do not. We believed that parity would be a significant predictor of who progresses and who does not.

## CHAPTER III

### METHODS

#### A. Study Overview

We conducted a research study to:

- 1) Determine if CIN II lesions are epidemiologically more similar to CIN I or CIN III lesions in terms of risk predictors, among women enrolled in the SPOCCS II study.
- 2) Describe established reproductive cofactors on the risk of CIN III compared to those for CIN I/HPV, among women in the SPOCCS II study.

Many studies have highlighted the role of possible cofactors, like parity, in cervical carcinogenesis [49, 53-63]. Most of these studies accounted for HPV infection through statistical adjustments.

Using a cross-sectional study design, we identified risk-factor profiles for HPV infection and CIN. Models were restricted to HPV positive women to reduce confounding by HPV infection. As a sensitivity analysis, HPV negative women were added and HPV infection status was adjusted for in the models. Odds ratios and 95% confidence intervals were generated.

#### B. Design

##### *1. Study Population*

The current analyses used data collected in the Shanxi Province Cervical Cancer Screening Study II. These analyses add to the body of information already gained from this study.

Between May 2001 and June 2002, the SPOCCS II cervical cancer screening study was conducted in Yangcheng and Xiangyuan counties, Central China, as previously described [14]. In brief, women aged 35 to 50 years were invited to participate in a cross-sectional, cervical cancer

collaborative project between the Cancer Institute of the Chinese Academy of Medical Sciences in Beijing, China and the Cleveland Clinic Foundation in Cleveland, Ohio. A cluster-sampling frame was used, where communes within each county were the units for the clusters. Eligibility criteria included being a resident of one of 15 county communes, non-pregnant, having an intact uterus, no history of pelvic irradiation or cervical cancer, and no screening within the last five years. Of a total of 9,034 women attending the Xiangyuan Women’s and Children’s Clinic and the Yangcheng Cancer Hospital, 8,798 women (97%) met study eligibility criteria. SPOCCS II enrolled 8,497 women aged 27-56 years; these women had a self-collected test for HPV detection, a physician-collected test of cervical exfoliated cells for HPV, and provided cervical samples for liquid-based cytology [14]. Sensitivities and specificities of these screening tests are presented in Table 2 [14]. The study aimed to increase the sensitivity of the self-test to detect high-grade disease by obtaining the specimen with a conical shaped brush (HC Cervical Sampler®, Digene, Inc. Gaithersburg, Maryland) versus a Dacron swab (used in SPOCCS I), and instructing the women to insert the brush high (6 to 7 cm) into the vagina [64].

Table 2. Sensitivity and Specificity of Screening Tests in SPOCCS II.

Screening Test	Biopsy Result		Sensitivity % (95% CI)	Specificity % (95% CI)
	≥CIN II	<CIN II		
Liquid based Cytology ≥ LSIL				
Abnormal	294	555	78.4	93.2
Normal	81	7,567	(74.3, 82.5)	(92.6, 93.8)
Liquid based Cytology ≥ HSIL				
Abnormal	331	1,523	88.3	81.2
Normal	44	6,599	(85.0, 91.6)	(80.4, 82.0)

Self-collected HPV				
Abnormal	328	1,850	87.5	77.2
Normal	47	6,272	(84.2, 90.8)	(76.2, 78.2)
Physician-collected HPV				
Abnormal	363	1,652	96.8	79.7
Normal	12	6,470	(95.0, 98.6)	(78.9, 80.5)

CIN=cervical intraepithelial neoplasia, LSIL=low grade squamous intraepithelial lesion, HSIL=high grade squamous intraepithelial lesion, HPV=high-risk human papillomavirus detected by Hybrid Capture II®, CI=Confidence Interval. Positive HPV test is defined at 1.0 picograms (pg).

Of 8,497 women, there were 7,781 women with negative biopsies, 341 with the most abnormal biopsy showing CIN I, 173 with CIN II, 181 with CIN III, and 21 with invasive cancer. Therefore, 375 of 8,497 (4.4%) had  $\geq$ CIN II. Table 3 contains data on reproductive factors from SPOCCS II.

Table 3. Demographic and Reproductive Factors for 2,055 HPV Positive and 6,657 HPV Negative Women in the SPOCCS II Study, Stratified by Final Histology.

Characteristic	Normal		Normal		
	HPV	HPV	CIN I	CIN II	CIN III
	Negative	Positive			
	N=6,657	N=1,405	N=299	N=167	N=184
Married (%)	99	98	97	96	99
Median Age at First Intercourse (Range), years	20 (15-31)	20 (15-33)	(15-28)	(16-26)	(16-24)
Intercourse within Four Months of Childbirth (%)	34	31	37	28	44

Median Number Lifetime					
Sexual Partners (range)	1 (1-27)	1 (1-10)	1 (1-8)	1 (1-10)	1 (1-12)
Median Gravidity (range)	3 (0-13)	3 (0-9)	3 (0-8)	3 (0-10)	3 (0-8)
Median Parity (range)	2 (0-8)	2 (0-7)	2 (0-5)	2 (0-5)	2 (0-5)
Median Number of Abortions					
(range)	0 (0-9)	0 (0-9)	0 (0-4)	0 (0-4)	0 (0-4)

## 2. Methods for the Study

Statistical analysis for this study was two-fold. First, through risk-factor profiles we determined if CIN II should be grouped with CIN III as is current practice or if it is more similar to CIN I (see specific aim 1). Second, we attempted to answer the question of why some women in the SPOSSC II trial had cervical disease of a higher grade than others, with a focus on common reproductive risk factors (see specific aim 2).

Data from the SPOCCS II trial was analyzed generating odds ratios for the association between multiple covariates including number of pregnancies, number of live births, age, and HPV infection/cervical intraepithelial neoplasia CIN I, CIN II and CIN III.

### a. Classification of the Exposure

Exposure to oncogenic HPV infection was gathered by both self-collected cervical vaginal specimens and physician-collected samples from the cervix. These samples were evaluated for thirteen high-risk oncogenic HPV deoxyribonucleic acid (DNA) types (HPV 16,18,31,33,35,39,45,51,52,56,58,59, and 68) using the Digene second-generation hybrid-capture assay [65]. Per manufacturers' instructions, a value of  $\geq 1.0$  pg/ml HPV DNA was used as the cut-off for positivity.

A standardized questionnaire was administered in a confidential setting by trained health workers to elicit information on risk factors potentially associated with cervical cancer and its

precursors, including sexual, diet, and reproductive history (please see the appendix for a copy of the questionnaire).

#### b. Classification of the Outcome

Outcome data in this study was based on cervical biopsy specimens from SPOCCS II. The biopsy protocol is as follows: Women with abnormal cytology results and/or HPV positive test results were examined by a colposcope and biopsies were collected with a two-mm bronchoscopy biopsy instrument. The cervix was examined by quadrant and all colposcopically detected cervical abnormalities were biopsied. In quadrants that appeared normal, biopsies were still obtained at the squamo-columnar junction at 2, 4, 8, and 10 o'clock depending on the quadrant. Endocervical curettage (ECC) was also performed. Therefore all participants colposcoped had a minimum of five biopsies including the ECC.

#### c. Comparison Group Selection

In general the comparison group should be selected from the population that also gave rise to the cases. For cervical cancer cases this population is women who are infected with HPV.

It has been noted that a comparison group defined this way likely attenuates the risks associated with cofactors (i.e. parity) that are associated with the acquisition and potential persistence of HPV infection, so it avoids potential residual confounding by HPV [40, 66]. Munoz and colleagues suggest that studies including HPV negative women among the controls in case-control studies might have underestimated the influence of parity [5]. Researchers have highlighted the fact that while this may not be the best comparison group it is the achievable one. The most accurate comparison group would be one containing women who were infected with HPV at the same age as the cases but who did not go on to develop cervical cancer [40, 66]. Women without HPV infection were included in identical analyses to estimate the change in odds ratio associated with their inclusion.

### *3. Data Management*

A strict system of data management was utilized in the SPOCCS II trial. At the first visit, all women were assigned a confidential code. All identifiers were removed from the data and kept in a locked separate file. For the purpose of this research women are identified by their code only.

All data forms in SPOCCS II were collected and transported to the Cancer Institute Hospital, Academy of Medical Sciences (CICAMS) in Beijing where the data was entered, managed and cleaned. The “cleaning” procedures for the SPOCCS II data included quality control procedures employing range checks and assessment of completeness and consistency across variables. Further data management was performed at the National Cancer Institute (NCI) when the data was being prepared for additional analyses. To ensure uniformity across epidemiologic analyses performed using the SPOCCS II data, the dataset from the NCI was utilized for these analyses.

### *4. Data Analysis*

#### a. Specific Aim 1

Estimates of median values and proportions of women with suspected risk factors for CIN were calculated, stratified by histological status. Prevalence odds ratios and corresponding 95% confidence intervals for grades of CIN were calculated by unconditional multiple logistic regression, adjusted for age (in 3 groups: 35-39, 40-44, 45-50 years). Models were restricted to HPV positive women to reduce residual confounding by HPV infection [66].

Modeling strategies have been tested to ensure the correct adjustment for HPV is made. Using the IARC series of case-control studies, Castellsague and colleagues estimated the impact of different HPV adjustment strategies on the association of environmental cofactors and invasive cervical cancer risk. Three models were fitted for each cofactor. Of the three strategies tested, models that were restricted to HPV positive subjects yielded higher associations (1.1- 2.0 fold higher) than those derived from HPV adjusted models [3]. If HPV restriction is seen as the strictest approach to HPV adjustment, models that adjust for HPV rather than restrict the analysis could be underestimating the magnitude of the association. The same study concluded that

regardless of the strategy that they used the same conclusions were reached regarding the direction and statistical significance of the association between the cofactor and invasive cervical cancer [3]. In the present analyses, HPV-restricted models were utilized to analyze the potential associations between the cofactors and invasive cervical cancer.

Parity was defined as a woman's number of live births and gravidity as the number of pregnancies. As few women reported having never been pregnant (72 women, 0.8%) and few were nulliparous (i.e. no previous live births, 1.2%), the parity variable was collapsed into a binary variable (0-2 and  $\geq 3$ ).

Models were reduced using backward elimination [67, 68] until only those variables producing a change  $\geq 10\%$  remained. Pregnancy and live births were highly correlated and thus not included in the same model. The final multivariate model included terms for age, number of pregnancies, intercourse within four months of childbirth, and number of induced abortions.

Results of models restricted to HPV positive women and to those that adjusted for HPV status were completed. All analyses were performed using Stata 8.0 and Stata 9.0 analytic software (Stata Corp. LP, College Station, Texas).

#### b. Specific Aim 2

Two sets of cross-sectional analyses were designed. The first was restricted to only HPV positive women [66]. A strict assessment of cofactors requires a study group that is known to be exposed to HPV. Based on results from the analyses performed for specific aim 1 we altered the stratification of the outcome in these analyses. Odds ratios and 95% confidence intervals are presented for each cofactor tested for comparisons of CIN III vs.  $\leq$  CIN II and for CIN I and/or HPV positive women vs. HPV negative women.

Variable categories were examined and recoded if necessary. Univariate distributions of the variables of interest were then examined. Please see the appendix for a list of the variables of interest. These variables (i.e. number of pregnancies, number of live births, smoking history, self-report of sexually transmitted disease [STD] history) are important since there are hypotheses

stating that cervical trauma, smoking status, co-infection with other STDs are important cofactors for the progression of HPV infection to invasive cervical cancer [48, 69-72].

A second analysis was completed where HPV was adjusted for in the model. Women negative for HPV infection were included in the analyses as there may be cofactors that act to make women more susceptible to HPV infection. In these models other potential confounders of the HPV and CIN relationship were evaluated by using the same backward elimination method [67, 68]. First, all potential confounders were included in the full model. Second, using the *epiconf* procedure in STATA the difference in the crude and adjusted estimates when the potential confounder was removed from the model was calculated. Confounders were eliminated until only those variables producing a change  $\geq 10\%$  in the point estimate for the main exposure remained in the model. Those remaining variables were considered confounders of the relationship between HPV and CIN.

### c. Power Calculation

The crude odds ratio of the association between number of pregnancies and CIN III vs.  $\leq$  CIN II is 1.6 [95% CI (1.1, 2.2)]. Table 4 shows the levels of power to detect odds ratios from 1.3 to  $\geq 4$  for the analyses. Power calculations were completed using Episheet [73, 74].

Table 4. Power to detect associations between number of pregnancies and CIN III vs.  $\leq$  CIN II with 184 cases of cervical cancer.

Odds Ratio	Power for HPV+ control group (n=1,871)	Power for HPV+ and HPV- controls (n=8,580)
1.3	35%	37%
1.5	68%	71%
2.0	98%	98%
3.0	99%	99%

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4.0 or greater	100%	100%
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## CHAPTER IV

### RESULTS

#### **A. Manuscript 1. Risk factors for cervical intra-epithelial neoplasia II seem more similar to grade I than grade III**

##### *1. Introduction*

Infection of the cervix with Human Papillomavirus (HPV) does not uniformly lead to histologic changes. Viral replication within cells and integration into the DNA of the cervical cells causes the range of cellular changes seen microscopically in biopsy specimens. Dysplastic changes, termed cervical intraepithelial neoplasia (CIN), are classified in three grades based on increasing degrees of cellular change and disorganization. Although CIN was initially described as a continuum of histological changes (all of which were considered true precursors of invasive disease if untreated) [27], our understanding of the natural history of cervical neoplasia has since been revised [27, 30] to acknowledge that CIN does not inevitably progress through each higher grade of CIN to cancer. When dysplasia progresses, it is thought to be orderly, i.e. not “skipping” from lower grades to cancer. However, progression between some levels, for instance CIN I to CIN II, is the exception rather than the rule. Consequently, the classification of cervical cancer precursors by histology may need revision to more accurately reflect natural history of disease progression and to better inform clinical decision making [27]. Currently, a two-tiered histological grouping has been adopted [1] (CIN I, CIN II/III) to reflect putative differences in the natural history between CIN I (lower probability of progressing) and CIN II/III (a dysplastic process with a greater likelihood of progression to cancer) [1].

Recent research suggests CIN II may not be a discrete histological grade that can be reliably classified, but instead an overlap between CIN I (a non-neoplastic HPV infection) and CIN III (a

neoplastic cervical cancer precursor) [2, 31]. It has been hypothesized that a proportion of CIN II lesions are biologically similar to CIN I and have greater probability of regressing over time, while other CIN II lesions are more characteristic of CIN III lesions [2]. If the current construct of CIN II is confirmed to behave more like CIN I or CIN III lesions, future clinical treatment guidelines for women, especially young women with CIN II diagnoses, may need to be reevaluated. Under consideration would be differential re-screening and treatment options, as well as incorporation of more sophisticated markers of risk of progression.

In order to describe whether CIN II lesions are epidemiologically more like CIN I or CIN III lesions in terms of the risk predictors among women with these diagnoses, we used data from a cross-sectional study in China: the Shanxi Province Cervical Cancer Screening Study II (SPOCCS II) [14]. In this population-based study, women were screened for cervical cancer. Women with cytological abnormalities and/or those who tested positive for cervical HPV DNA were triaged to colposcopy for universal histological confirmation of cervical disease status. Our primary aim was to determine if histologically confirmed CIN II lesions had risk factor profiles that more closely resembled CIN I or CIN III cases. We present results examining differences in the risk factor profiles among HPV DNA positive women with histological diagnoses of CIN I, CIN II and CIN III.

## *2. Materials and Methods*

*Subject recruitment, study design.* Between May 2001 and June 2002, the SPOCCS II cervical cancer screening study was conducted in Yangcheng and Xiangyuan counties, Central China, as previously described [14]. In brief, women aged 35 to 50 years were invited to participate in a cross-sectional, cervical cancer collaborative project between the Cancer Institute of the Chinese Academy of Medical Sciences in Beijing, China and the Cleveland Clinic Foundation in Cleveland, Ohio. A cluster-sampling frame was used, where communes within each county were the unit for the cluster. Eligibility criteria included being a resident of one of 15

county communes, non-pregnant, having an intact uterus, no history of pelvic irradiation or cervical cancer, and no screening within the last five years. Of a total of 9,034 women attending the Xiangyuan Women's and Children's Clinic and the Yangcheng Cancer Hospital, 8,798 women (97%) met study eligibility criteria.

A standardized questionnaire was administered in a confidential setting by trained health workers to elicit information on risk factors potentially associated with cervical cancer and its precursors, including sexual, diet, and reproductive history. All participating women performed a self-collected HPV test. Approximately 10 months later (range 3-16 months), women underwent a pelvic examination by a study staff physician to collect cervical cell samples for cytology and HPV testing. For women with abnormal cytology results and/or HPV positive test results, they were examined by a colposcope and biopsies collected with a two-mm bronchoscopy biopsy instrument. The cervix was examined by quadrant and all colposcopically detected cervical abnormalities were biopsied. In quadrants that appeared normal, biopsies were still obtained at the squamo-columnar junction at 2, 4, 8, and 10 o'clock depending on the quadrant. Endocervical curettage (ECC) was also performed. Therefore all participants colposcoped had a minimum of five biopsies including the ECC.

Study protocol and questionnaires were approved by human subjects review boards of the Cancer Institute/Hospital of the Chinese Academy of Medical Sciences, and the Cleveland Clinic Foundation.

*HPV DNA Detection.* Both self-collected cervical vaginal specimens and physician collected samples from the cervix were evaluated for thirteen high-risk oncogenic HPV DNA types (HPV 16,18,31,33,35,39,45,51,52,56,58,59, and 68) using the Digene second-generation hybrid-capture assay [65]. Per manufacturers' instructions, a value of  $\geq 1.0$  pg/ml HPV DNA was used as the cut-off for positivity. In this paper oncogenic HPV DNA will be referred to as HPV.

*Statistical Analyses.* Estimates of median values and proportions of women with suspected risk factors for CIN were calculated, stratified by histological status. Prevalence odds ratios

(ORs) and corresponding 95% confidence intervals (CIs) for grades of CIN were calculated by unconditional multiple logistic regression, adjusted for age (in 3 groups: 35-39, 40-44, 45-50 years). Models were restricted to HPV positive women to reduce residual confounding by HPV infection [66]. Models were reduced using backward elimination [67, 68] until only those variables producing a change  $\geq 10\%$  remained. Pregnancy and live births were highly correlated and thus not included in the same model. The final multivariate model included terms for age, number of pregnancies, intercourse within four months of childbirth, and number of induced abortions.

Parity was defined as a woman's number of live births and gravidity as the number of pregnancies. As few women reported having never been pregnant (72 women, 0.8%) and few were nulliparous (i.e. no previous live births, 1.2%), the parity variable was collapsed into a binary variable (0-2 and  $\geq 3$ ).

An analysis was conducted to compare results of models restricted to HPV positive women to those that adjusted for HPV status. Analyses were performed using Stata 8.0 and Stata 9.0 analytic software (Stata Corp. LP, College Station, TX).

### *3. Results*

Overall, a total of 8,798 women participated, of whom 4% had histologically-confirmed CIN I, 2% had CIN II and 2.1% had CIN III. Prevalence of HPV increased from 85.2% among women with CIN-1 to 97.4% for CIN-3. Of the 8,062 women with normal histology, 1,405 (17.4%) were HPV positive. Infection with HPV was associated with an increased risk for CIN II versus CIN I [OR= 5.0 (2.1, 11.9)], however, no difference in HPV prevalence was found when comparing CIN III versus CIN II cases: [OR =1.3 (0.39, 4.4)].

Among 2,055 women who tested positive for HPV, the prevalence of histologically confirmed CIN was as follows: 14.4% had CIN I, 8.0% had CIN II, and 8.9% had CIN III (Table 1). The majority with HPV infection (67.7%) were histologically normal (negative for CIN).

Participants' median age ranged from 40 years for women who were HPV positive and less than or equal to CIN I, to 43 years for HPV positive and CIN III.

More than 96% of women were married; most reported having one lifetime sexual partner. Independent of the grade of cervical disease, women had a median number of two live births and three pregnancies, with a median age at first intercourse of 20 years. Most women use female sterilization as their contraceptive method and this also did not vary by grade of CIN. The proportion of women reporting intercourse within four months of childbirth differed by histological grade, and ranged from 28 to 44%.

*Age-adjusted and multivariate models for CIN II versus CIN III and CIN I.* Age did not appear to be associated with risk of higher histological grade of cervical neoplasia (Table 2). An increased risk of CIN III versus CIN II was seen for higher gravidity ( $\geq 3$  pregnancies) [OR=1.6 (1.0, 2.6)] and sexual intercourse within four months of childbirth [OR=2.0 (1.3, 3.2)]. Risks associated with reproductive factors appeared comparable for CIN II and CIN I, except an inverse association was observed for sexual intercourse within four months of childbirth for CIN II versus CIN I [OR= 0.64 (0.42, 0.97)]. All observed associations were of the same magnitude in the multivariate models (Figure 1). For comparisons of CIN II separately to CIN III and to CIN I, no associations were found for educational attainment, history of vaginal discharge, numbers of home deliveries, number of live births, number of induced abortions or current method of contraception. Results from HPV restricted models and HPV adjusted models for CIN II versus CIN III and CIN I were nearly identical (data not shown).

*Multivariate models for CIN III versus  $\leq$  CIN II and CIN II/III versus  $\leq$  CIN I.* Women over age 45 years showed an increased risk of having a diagnosis of CIN III versus  $\leq$  CIN II [OR=1.9 (1.3, 2.8) versus women 35-40 years] (Table 3). Higher number of births ( $\geq 3$  live births) was associated with a 70% greater risk of CIN III (versus  $\leq$  CIN II) compared to women with fewer than three births. Intercourse within four months of childbirth was also associated with CIN III (versus  $\leq$  CIN II) [OR = 1.8 (1.3, 2.5)]. No associations for CIN III (versus  $\leq$  CIN II) were found

for lifetime number of sexual partners, number of live births, or number of abortions. When multivariate models were re-run for CIN II/III (versus  $\leq$  CIN I), no associations remained statistically significant, with the exception of age over 45 years [OR=1.8 (1.3, 2.4 versus women 35-40 years)].

#### *4. Discussion*

This study of 2,055 HPV positive women, all with a minimum of five biopsies, in Shanxi Province, China, provides evidence that risk factors for histologically-confirmed CIN II are more similar to those for CIN I than for CIN III. Information on risk factors was obtained through private interviews, conducted in the local dialect by trained interviewers, using a previously piloted questionnaire. When comparing CIN risk-factor profiles, they diverged for CIN II compared to CIN III, but were broadly similar for CIN II compared to CIN I. If CIN III and CIN II are biologically similar, we would expect the risk factor profiles to be more similar for CIN III and CIN II. Instead, risk factor profiles between CIN II and CIN I cases were more similar. Based on these results, CIN II is more similar to CIN I and may represent non-neoplastic HPV infections rather than a direct cervical cancer precursor such as CIN III. Multivariate models comparing CIN III versus  $\leq$  CIN II were similar to CIN III versus CIN II comparisons. In contrast, no risk factors, other than age, were identified as meaningfully different in comparisons of the combined category CIN II/III to  $\leq$  CIN I, reinforcing our assumption that CIN II and CIN I are more similar than CIN II and CIN III.

Our study is one of the few of sufficient size and uniform use of histology that is able to directly compare CIN III to CIN II. It builds on previous findings that CIN II lesions may not be more biologically similar to CIN III, at least as reflected in risk factor profiles [31, 75, 76]. For example, among 2,366 women with oncogenic HPV enrolled in the U.S. ALTS trial, smoking was strongly associated with a diagnosis of  $\geq$  CIN III, but was not associated with a diagnosis of CIN II, except among women with HPV-16 [31]. No significant differences in reproductive

factors were found between women diagnosed with CIN II or CIN III, when compared to  $\leq$ CIN I in ALTS [77] (as found in our Chinese study), although the number of ALTS participants reporting three or more pregnancies or live births was limited. Too few women in our study from China smoked in order to examine this risk factor with sufficient power. Further evidence that the characteristics and behaviors of CIN II may be distinct from CIN III includes data from a large population-based prospective study among women with HPV infection that found that time from last normal cytological smear was a risk factor for prevalent CIN III, but not for  $\leq$ CIN II [76].

Although population-based, our findings from two rural counties in Shanxi province cannot be considered representative of the entire Chinese population. Reliance on self-reported data may have led to under or over reporting of reproductive variables. Self-reported data are always difficult to verify, but due to the one child policy in China there has been strong incentive to have reliable reproductive information and to report pregnancies, abortions, live and still births to the government [78]. Although there was a large range in the numbers of pregnancies (0-10) and live births (0-10), these numbers were compared to means for the province (typical for a rural environment) and they fell within normal limits.

We realize there may be concern with our histological diagnoses due to inherent difficulties in obtaining standardized pathological diagnoses. Many studies report on the poor reproducibility of CIN II versus CIN III, and CIN I versus HPV changes. Interobserver variation for diagnosing CIN II is greater than for CIN III [75]. A potentially large proportion of CIN II cases could be misclassified and actually be true CIN I cases. In order to describe potential changes in estimates related to misclassification, estimates were recalculated using data from a review of study biopsies. Biopsies from SPOCCS I (a SPOCCS II precursor) were recently reviewed for agreement and the proportion of biopsies said to be misclassified were applied to the results from this study: on review, 92% remained CIN 2, 2.4% CIN 1, 4.8% normal histology and 1.2% CIN 3). Odds ratios and 95% CI, from the present study, were recalculated for higher gravidity ( $\geq 3$  pregnancies), where no change in the magnitude of the effect was observed. Given the result of

the quality control study including double-reading of pathology slides, the change in the magnitude of the effect was small, suggesting that misclassification of CIN was negligible in this study. The fact that 92% of CIN II remained classified as CIN II would suggest it can in fact be sorted into a histological classification distinguishable from CIN I and CIN III. Epidemiologic data from this study would suggest that CIN II has biologic characteristics more similar to CIN I rather than the conventional grouping with CIN III. It further suggests that studies who choose to group CIN II with CIN III are differentially misclassifying CIN II and diluting the effect of covariates as they relate to CIN III. Data from prospective studies is needed that follows women with untreated CIN II to document the natural history see if it behaves more like CIN III or CIN I. These preliminary data would lead us to hypothesize the latter.

Our study results are consistent with others that suggest that CIN II may represent a more heterogeneous group of lesions than originally believed. If confirmed to be true by future prospective studies, different treatment options may need to be considered for a proportion of women with CIN II diagnoses in order to avoid potential over-treatment. Current recommendations are to treat women with histologically confirmed CIN II or CIN III with either ablative (e.g. cryotherapy) or excisional treatment (e.g. loop electrocautery excision procedure, conization) [79]. While clinical trials have generally failed to show differences in treatment modalities [80-82], excisional treatment allows for pathologic diagnosis of the excised tissue. Excision is often the treatment of choice and is always recommended for women with unsatisfactory colposcopy, or recurrent disease [79]. Treatment of CIN is not without complications, especially for women who are still in their childbearing years [83]. While uncommon, significant pregnancy complications have been associated with larger loop electrosurgical excision procedure [84-86]. Treatment of CIN II may require review; more research is needed to see if our findings are confirmed in other studies.

In conclusion, our data add to the accumulating evidence that a substantial proportion of CIN II may be biologically different from CIN III, sharing more similarities to CIN I. We encourage

those with prospective data to perform case-case analyses to identify risk factor differences among the histologic cervical grades.

Table 5. Selected Characteristics of Study Participants among 2,055 HPV DNA Positive Women in the SPOCCS II Study, Stratified by Histology.

Characteristic	HPV Positive	CIN I	CIN II	CIN III
	Normal Cytology N=1,405	N=299	N=167	N=184
Proportion of all HPV Detected Lesions (%)	67.7	14.4	8.0	8.9
Median Age (range), years	40 (35-50)	40 (35-50)	41 (35-50)	42 (35-50)
Percent Married (%)	98	97	96	99
Median Age at First Intercourse (range), years	20 (15-33)	20 (15-28)	20 (16-26)	20 (16-24)
Percent Having Intercourse Within Four Months of Childbirth (%)	31	37	28	44
Median Number of Lifetime Sexual Partners (range)	1 (1-10)	1 (1-8)	1 (1-10)	1 (1-12)
Median Number of Pregnancies (range)	3(0-9)	3 (0-8)	3 (0-10)	3 (0-8)
Median Number of Live Births (range)	2 (0-7)	2 (0-5)	2 (0-5)	2 (0-5)

Table 6. Age-adjusted<sup>1</sup> Odds Ratios for Selected Characteristics among HPV DNA positive Women in the SPOCCS II Study, Comparisons by Histology

Characteristic	CIN III vs. CIN II		CIN II vs. CIN I	
	Cases/Controls	OR (95%CI)	Cases/Controls	OR (95%CI)
<b>Age (years)</b>				
35-39	62/62	1.0	62/142	1.0
40-44	52/55	0.95 (0.56, 1.6)	55/81	1.6 (0.99, 2.5)
45-50	70/50	1.4 (0.84, 2.3)	50/76	1.5 (0.95, 2.4)
<b>Educational Attainment</b>				
None	12/12	1.0	12/16	1.0
Primary	62/49	1.2 (0.51, 3.0)	49/90	0.74(0.32, 1.7)
Middle	89/75	1.2 (0.52, 3.0)	75/140	0.79 (0.35, 1.8)
Over Senior high	21/31	0.73 (0.27, 2.0)	31/53	0.89 (0.37, 2.2)
<b>Age at First Intercourse (years)</b>				
≥ 21	67/71	1.0	71/114	1.0
19-20	41/31	1.2 (0.76, 2.0)	31/64	0.86 (0.56, 1.3)

12-18	76/65	1.3 (0.70, 2.3)	31/64	0.71 (0.41, 1.2)
<b>Lifetime Sexual Partners</b>				
1	122/107	1.0	107/174	1.0
2	26/37	0.63 (0.36, 1.1)	37/74	0.82 (0.51, 1.3)
≥ 3	36/23	1.4 (0.75, 2.5)	23/51	0.75 (0.43, 1.3)
<b>Reported # Pregnancies</b>				
0-2	50/64	1.0	64/104	1.0
≥ 3	134/103	1.6 (1.0, 2.6)	103/195	0.84 (0.56, 1.3)
<b>Reported # Live Births</b>				
0-2	126/108	1.0	108/202	1.0
≥ 3	57/58	0.78 (0.49, 1.2)	58/96	1.1 (0.71, 1.6)
<b>Intercourse Within Four Months of</b>				
<b>Childbirth</b>				
No	103/120	1.0	120/187	1.0
Yes	80/46	2.0 (1.3, 3.2)	46/111	0.64 (0.42, 0.97)
<b>Reported # Abortions</b>				
None	113/111	1.0	111/199	1.0

1	41/37	1.1 (.66, 1.9)	37/57	1.1 (.71, 1.8)
$\geq 2$	29/18	1.7 (.89, 3.3)	18/42	0.79 (.43, 1.5)
<b>Current Contraception Use</b>				
Female Sterilization	158/132	1.0	132/237	1.0
Intrauterine Device	15/19	0.72 (.35, 1.5)	19/44	0.80 (.45, 1.4)
Oral Contraceptives	2/4	0.45 (.08, 2.5)	4/3	2.8 (.60, 12.7)
Condoms	0/1	-	1/1	2.4 (.14, 38.4)
None	9/11	0.71 (.28, 1.8)	11/14	1.5 (.64, 3.3)

<sup>1</sup>Age adjustment was by categorical years as follows; 35-39, 40-44, 45-50.

Note: Numbers rounded to 1.

Table 7. Odds Ratios for Selected Characteristics Among all Women in the SPOCCS II Study, Comparisons by Histology

Characteristic	CIN III vs. ≤ CINII		CIN II/III vs. ≤ CIN I	
	Cases/Controls	ORs (95%CI) <sup>1</sup>	Cases/Controls	ORs (95%CI) <sup>1</sup>
<b>Age (years)</b>				
35-39	62/824	1.0	124/762	1.0
40-44	52/598	1.1 (0.78, 1.7)	107/543	1.2 (0.90, 1.6)
45-50	70/449	1.9 (1.3, 2.8)	120/399	1.8 (1.3, 2.4)
<b>Reported # of Pregnancies</b>				
0-2	50/688	1.0	114/624	1.0
≥3	134/1183	1.7 (1.1, 2.5)	237/1080	1.2 (0.92, 1.6)
<b>Intercourse Within Four Months of</b>				
<b>Childbirth</b>				
No	103/1274	1.0	223/1154	1.0
Yes	80/584	1.8 (1.3, 2.5)	126/538	1.2 (0.96, 1.6)
<b>Reported # of Abortions</b>				
None	113/1177	1.0	224/1066	1.0
1	41/452	0.76 (0.50, 1.1)	78/415	0.83 (0.61, 1.1)

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$\geq 2$

29/229

1.0 (0.62, 1.6)

47/211

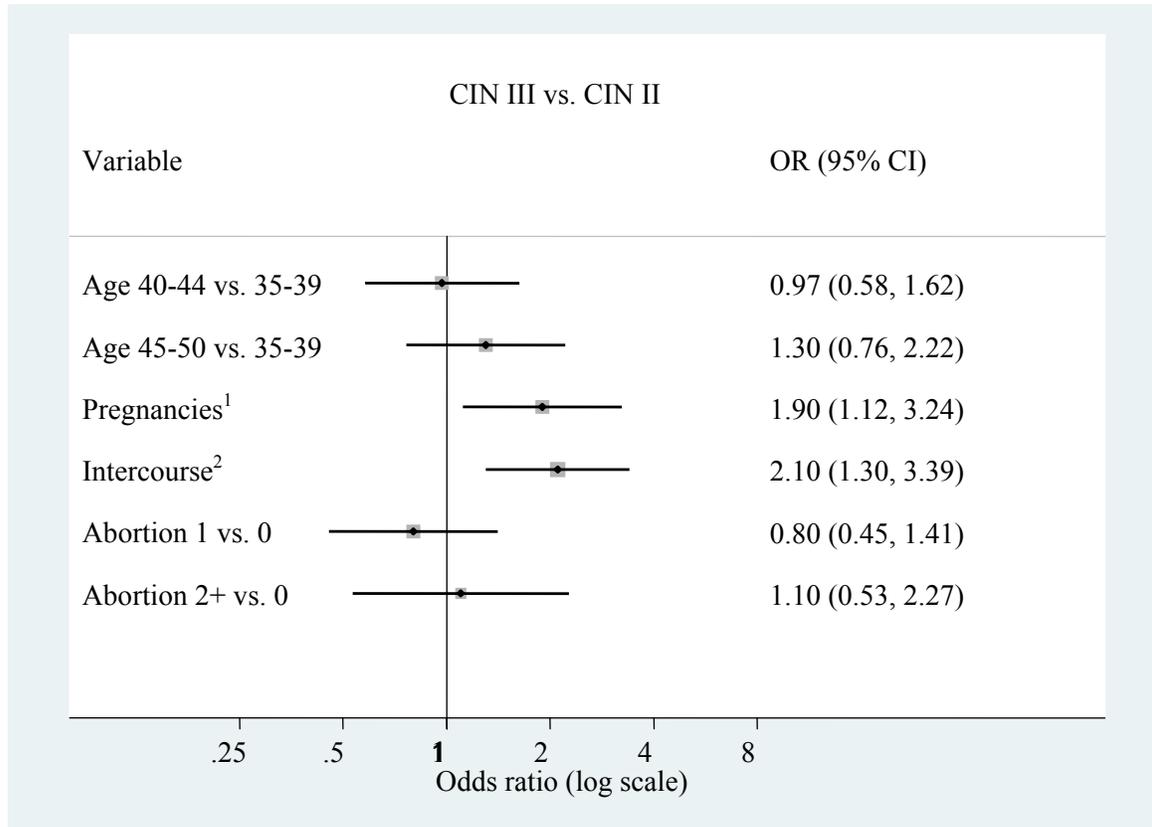
0.99(0.67, 1.5)

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<sup>†</sup>Models adjusted for all variables in the table.

Note: Age adjustment was by categorical years as follows; 35-39, 40-44, 45-50.

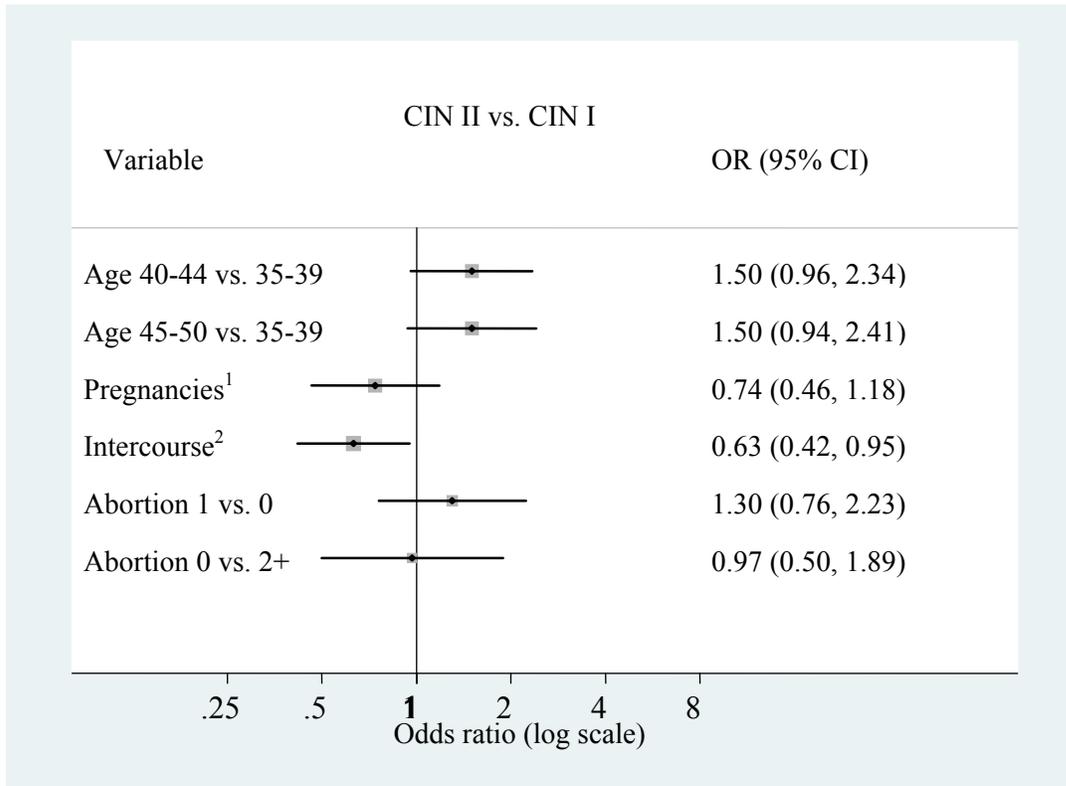
Figure 2. Multivariate Odds Ratios and 95% Confidence Intervals for Selected Factors for CIN III vs. CIN II among Women in the SPOCCS II Trial.



<sup>1</sup> Variable in the model was pregnancies 0-2 vs. 3+

<sup>2</sup> Variable in the model was intercourse within four months of childbirth (yes/no).

Figure 3. Multivariate Odds Ratios and 95% Confidence Intervals for Selected Factors for CIN II vs. CIN I Among Women in the SPOCCS II Trial.



<sup>1</sup> Variable in the model was pregnancies 0-2 vs. 3+

<sup>2</sup> Variable in the model was intercourse within 4 months of childbirth yes/ no

## **B. Manuscript 2. Reproductive risk factors for Human Papillomavirus infection and cervical intraepithelial neoplasia III in the Chinese SPOCCS II study.**

### *1. Introduction*

Worldwide, invasive cervical cancer (ICC) is the second most common cancer in women with approximately 470,000 new cases [11]. Oncogenic types of Human Papillomavirus (HPV) infection have clearly been established as a necessary cause of cervical cancer. The contrast between the high lifetime cumulative incidence of cervical HPV infection and the relatively low lifetime risk of cervical cancer suggests the influence of other etiologic cofactors that act in conjunction with HPV to increase a woman's risk of cervical carcinogenesis. Thus, further understanding of HPV co-factors that increase a woman's risk of progression from cervical HPV infection to ICC is warranted. Candidate cofactors may be split into three groups [3]: 1) environmental cofactors, such as use of oral contraceptives, smoking, diet, and cervical trauma; 2) viral cofactors, such as infection with multiple HPV types or infection with specific oncogenic types; and 3) host factors, such as factors related to the host's immune response and endogenous hormones.

Factors related to reproduction have been associated with both the risk of HPV infection (e.g. early onset intercourse, multiple partners) [26] and the risk of developing cervical cancer among HPV DNA positive women (e.g. parity) [5, 10]. Specific reproductive factors could act as HPV cofactors within any of the three aforementioned categories: 1) Environment: cervical trauma, temporary exogenous hormonal exposure [3]; 2) Viral type: HPV viral types acquired and timing of acquisition during pregnancy or postpartum [3] ; 3) Host: modification of immune responses during pregnancy or change of estrogenization of tissues during lactation [3]. It is not clear however in which part of the carcinogenic process reproductive factors may play a role [87]. Some may increase a woman's susceptibility to HPV infection while others affect the probability of an infection becoming persistent, while still others may affect progression from one

histological state to another more serious grade of cervical disease [87]. Figure 4 is a proposed causal diagram for the relationship between oncogenic HPV infection, reproductive risk factors and CIN III. Further, little is known concerning whether host factors related to the successful progression of a pregnancy to a live birth might also be related to the risk of progression of HPV infection to CIN III.

In a previous publication, we determined that risk factors for CIN II are more similar to CIN I than CIN III. In order to investigate reproductive risk factors, we have utilized this information and present here reproductive risk factors for CIN III versus  $\leq$  CIN II from a large cross-sectional study in rural China. Differences in risk factor profiles are also presented by comparing women with either CIN I or HPV DNA, to HPV-negative women with normal cytology from a study of population-based samples of women screened for cervical cancer. All women with cytological abnormalities and/or HPV DNA positive results were referred to colposcopy for histological confirmation of cervical disease status. Our aim was to determine whether reproductive risk factors may increase a woman's likelihood of current HPV infection (association with CIN I/HPV) or the likelihood that an HPV infection will progress to high-grade dysplasia (association with CIN III).

## *2. Materials and Methods*

*Subject recruitment, study design.* Between May 2001 and June 2002, the SPOCCS-II cervical cancer screening study was conducted in Yangcheng and Xiangyuan counties, Central China, as previously described [14]. In brief, women aged 35 to 50 years were invited to participate in a cross-sectional, collaborative cervical cancer project between the Cancer Institute of the Chinese Academy of Medical Sciences in Beijing, China and the Cleveland Clinic Foundation in Cleveland, Ohio. A cluster-sampling frame was used, where communes within each county were the units for the clusters. Eligibility criteria included being a resident of one of 15 county communes, non-pregnant, having an intact uterus, no history of pelvic irradiation or

cervical cancer, and no screening within the last five years. Of a total of 9,034 women attending the Xiangyuan Women's and Children's Clinic, 8,798 women (97%) met study eligibility criteria.

A standardized questionnaire was administered in a confidential setting by trained health workers to elicit information on risk factors potentially associated with cervical cancer and its precursors, including sexual, diet, and reproductive history. All participating women performed a self-collected HPV test [14, 88]. After approximately 10 months (range 3-16), women underwent a pelvic examination by a study staff physician to collect cervical cell samples for cytology and HPV DNA testing. All women with abnormal cytology results and/or HPV-positive test results on either sample, were examined with a colposcope and biopsies were collected with a 2-mm bronchoscopy biopsy instrument. The cervix was examined by quadrant and all colposcopically detected cervical abnormalities were biopsied. In quadrants that appeared normal, biopsies were still obtained at the squamo-columnar junction at 2, 4, 8, and 10 o'clock depending on quadrant. Endocervical curettage (ECC) was also performed. Therefore all participants had a minimum of five biopsies including the ECC.

Study protocol and questionnaires were approved by human subjects review boards of the Cancer Institute/Hospital of the Chinese Academy of Medical Sciences, and the Cleveland Clinic Foundation.

*HPV DNA Detection.* Both self-collected cervico-vaginal specimens and physician collected-samples were evaluated for thirteen high-risk oncogenic HPV types (HPV 16,18,31,33,35,39,45,51,52,56,58,59, and 68) using the Digene second generation hybrid capture assay (HC II) [65]. Per manufacturers' instructions, a value of  $\geq 1.0$  pg/ml HPV DNA was used as the cut-off for positivity. HPV DNA testing was performed blinded to other test results. HPV DNA will be hence referred to as HPV.

*Statistical Analyses.* Estimates of medians and proportions of suspected risk factors for CIN were calculated, stratified by histological status. Prevalence odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for grades of CIN were calculated by unconditional multiple

logistic regression, adjusted for age (categorized into 3 groups: 35-39, 40-44, 45-50 years). Models were restricted to HPV-positive women to reduce residual confounding by HPV infection [66], with the exception of the model comparing women with either CIN I or HPV DNA (CIN I/HPV positive cytology normals) versus HPV-negative women with normal cytology. Potential confounders examined included age, educational attainment, age at first intercourse, number of sexual partners, current contraceptive use, intercourse within four months of childbirth, and number of previous pregnancies, live births, or induced abortions. Model variables were eliminated using backward elimination [67, 68] until only those variables producing a change of  $\geq 10\%$  remained. Pregnancy and live births were highly correlated and thus not included in the same model. Final multivariate models included terms for age, number of pregnancies, intercourse within four months of childbirth, number of induced abortions, number of sexual partners and the use of an intrauterine device for contraception.

Parity was defined as woman's number of live births and gravidity as the number of pregnancies. As few women reported having never been pregnant (72 women, 0.82%) or were nulliparous (i.e. no previous live births, 1.2%), the parity variable was collapsed into a binary variable (0-2 and  $\geq 3$ ).

An analysis was conducted comparing results of models restricted to HPV positive women to those that adjusted for HPV status. Analyses were performed using Stata 8.0 and Stata 9.0 analytic software (Stata Corp LP, College Station, TX).

### *3. Results*

Among 8,798 women who participated in the SPOCC II study, 4% had histologically-confirmed CIN I, 2% had CIN II and 2.1% CIN III (Table 1). HPV positivity was 85.2% among those with CIN I, 96.5% for CIN II, and 97.3% for CIN III. 17.4% of the 8,062 women with normal histology were HPV positive.

Of the 8,712 women for whom data was available on all variables, 76.4% were histological normal (CIN negative) without any detectable HPV DNA infection (n=1,405), 16.1% had HPV infection and normal histology (n=1,405), 3.4% had CIN I (n=299), 2% had CIN II (n=167), and 2% had CIN III (n=184). Participants' median age increased with higher histological grade, ranging from 40 years for women who were cytology-negative and HPV-positive to 43 years for HPV-positive CIN III.

More than 96% of women were married; most reported having one lifetime sexual partner. Independent of the grade of cervical disease, women had a median number of two live births and three pregnancies, with a median age at first intercourse of 20 years. Most women use female sterilization as their contraceptive method and this also did not vary by grade of CIN. The proportion of women reporting intercourse within four months of childbirth differed by histological grade, and ranged from 28 to 44%.

The risk of CIN III versus  $\leq$  CIN II was associated with older age: [OR= 2.1 (1.4, 3.0) for 45-50 versus 35-44 years], higher gravidity ( $\geq 3$  pregnancies) [OR=1.5 (1.0, 2.1) versus 0-2 pregnancies] and sexual intercourse within four months of childbirth [OR=1.7 (1.2, 2.3)]. Women who reported using an intrauterine device (IUD) appeared to be at a lower risk of CIN III versus  $\leq$  CIN II [OR= 0.52 (.30, .91) versus female sterilization]. No association was observed for a woman's reported age at initiation of sexual intercourse [OR=1.3 (0.89, 1.8) 12-18 versus  $\geq 21$  years], or reported number of sexual partners [OR=1.2 (.80, 1.8)  $\geq 3$  versus 1 partner] Results of the multivariate analyses (Figure 5) were similar to those in the age-adjusted analyses.

When comparing women who had either CIN I or were HPV positive versus those that were dually negative for HPV and cytology, age was not associated with risk. Conversely, there appeared to be an increased risk for women who initiated sexual intercourse at ages 19-20 [OR=1.3 (1.1, 1.5) versus  $\geq 21$  years]) and with a greater number of reported sexual partners (2 versus 1) [OR=1.5 (1.3, 1.7)]. A nearly identical OR and CI was observed for women with  $\geq 3$  sexual partners versus those reporting only one lifetime sexual partner. A woman's number of

reported pregnancies was not associated with CIN I/HPV DNA positivity. Results of multivariate analyses were generally the same, with the exception of age at first intercourse which after adjusting for number of sexual partners and number of abortions did not remain significant: [OR= 1.10 (.93, 1.3) for 19-20 versus  $\geq$  21 years] and [OR=1.0 (.88, 1.14) for 12-18 versus  $\geq$  21 years] (Figure 6).

No associations were seen for number of live births or any of the three constructed pregnancy success variables for both comparisons of cervical histological grades, in either the age- or multivariate-adjusted analyses.

Risk factors for engaging in intercourse within four months of childbirth were examined among women with normal cytology, and included older age, fewer reported sexual partners and fewer reported pregnancies. Women who reported to have engaged in intercourse within four months of childbirth were also more likely to have  $\leq$  50% of pregnancies resulting in live birth. Women with normal cytology who used an IUD [OR=2.9 (2.5, 3.4)], oral contraceptives [OR=2.8 (1.8, 4.3)], or no contraceptives [OR=1.3 (1.0, 1.7)] were also more likely to report having intercourse within four months of child birth versus those who were sterilized. Reported number of abortions and expected reproductive success were not associated with risk of CIN in any of the analyses. When abortions were excluded from the pregnancy success variable, the association increased rather than decreased risk [OR= 2.0 (1.2, 3.5).]

#### *4. Discussion*

This study of 8,712 women, all with a minimum of five biopsies, in Shanxi province, China provides evidence that selected reproductive risk factors, including having more than three pregnancies and a history of sexual intercourse within four months of childbirth may have a role in the progression to CIN III from lower grades of cervical disease. In contrast, susceptibility to CIN I/HPV infection did not appear to be clearly associated with the reproductive factors investigated in this survey, and was more clearly associated with a woman's reported history of

lifetime sexual partners. Host factors related to the successful progression of pregnancy to live births were not shown to be related to either histological grade comparison. Information on risk factors was obtained through private interviews, conducted in the local dialect by trained interviewers, using a previously piloted questionnaire.

A prior publication on the SPOCCS II study analyzed risk factors for HPV infection and CIN III or greater among the same women included in this present report [89]. However, relatively few reproductive variables were analyzed (parity and number of sexual partners) in this previous report. The present analysis explored comparisons of different histological categorizations, using alternate stratifications in order to investigate a broader range of reproductive factors affecting the risk of HPV infection, and progression to high-grade pre-cancer (CIN III). Our prior analyses indicated that risk factor profiles for CIN II are more similar to CIN I than CIN III [90], and thus the case group for high-grade disease was restricted to CIN III, rather than to the combined outcome of CIN II/CIN III.

The major steps in cervical cancer carcinogenesis are infection with HPV, persistence of HPV infection over time, and progression from infection to pre-cancer and invasion [26]. The p53 protein, a tumor suppressor gene, prevents cell growth in the presence of cell damage. HPV oncogenic proteins E6 and E7 can both inhibit p53, thus leading to carcinogenesis. Initial HPV infections are common, with most women developing immunity against HPV within two years with cessation of viral replication [27]. Some women however develop persistent infections, and thus have a higher risk of progression to higher grades of CIN. Currently, a two-tiered system describes the morphologic classification of non-invasive HPV-associated cervical lesions: CIN I (low-grade lesion) a non-neoplastic lesion with a low likelihood for progression and CIN II/CIN III high-grade lesions with a greater likelihood for progression to invasive cervical cancer [27]. Low-grade lesions may be caused by low or high-risk HPV infections [27]. In contrast, high-grade lesions are almost always associated with high-risk HPV types, are characteristic of E6 and E7 expression, and often show integration of the HPV viral genome into the host cell [28]. It is

likely that the uncontrolled E6/E7 expression is a phenomenon that distinguishes the process of cell transformation from a productive viral infection [2]. E6/E7 mRNA has been found to be associated with high-grade cervical pre-cancer and cancer [29], although further prospective studies are indicated.

Our analyses suggest that reproductive risk factors, notably pregnancy history, likely play a more important role in the progression to CIN III than in susceptibility to HPV infection, which appear largely dependent upon sexual behavioral risk factors. Our results that a woman's number of pregnancies may modulate the risk of progression of CIN II or less to CIN III are consistent with previous reports [3, 87, 91]. Our findings add evidence to the existing body of evidence that pregnancies may affect a women's risk of high-grade pre-cancer. A recent pooled analysis of 16,563 women with cervical cancer and 33,542 controls from 25 epidemiologic studies found a direct relationship between number of full term pregnancies and CIN III or cancer. Relative risks were found to increase with each additional pregnancy [10].

Observed associations between CIN I/HPV positives and number of sexual partners are also consistent with other studies [89, 92-94]. In a pooled analysis of the IARC HPV prevalence studies, a significant trend of decreasing HPV positivity was seen with increasing age at first intercourse. HPV infection risk, however, was not significantly increased for women initiating intercourse at age 15 compared to women initiating at age 24 [95]. Our multivariate results suggested that abortion history was not a risk factor for HPV, in contrast with two other studies that found a lower risk of HPV among women with a reported history of abortions from Thailand [19] and Vietnam [92]. Based on our data we found no evidence that women with more pregnancies were are a greater risk for HPV infection.

Pregnancy may increase the risk of cervical cancer due to the maintainance of the transformation zone on the ectocervical region during the full period of the pregnancy, thus increasing a woman's risk of HPV infection. The maintaince of the transformation zone is due to circulating estrogen and progesterin levels that become elevated during pregnancy. The number of

metaplastic cells in the transformation zone have been shown to increase during pregnancy [5]; it is during this immature phase of development that cells are most susceptible to HPV infection [35]. In a woman who has experienced multiple pregnancies, the metaplastic transformation zone will have repeatedly been exposed to carcinogenetic agents including HPV infection. Repeated exposure experienced over multiple pregnancies may intensify the actions of carcinogenic infectious agents causing increased risk for cervical cancer [41].

Research data suggest a role of endogenous hormones in cervical carcinogenesis in both epidemiological [8] and laboratory studies [42-46]. Hormones during pregnancy may also favor, or accelerate cervical carcinogenesis, with a mechanism similar to that for oral contraceptives [47, 48]. The data consistently points to oral contraceptives promoting process of cervical carcinogenesis rather than having a role in facilitation of HPV acquisition or persistence [49]. However, a case-control study found no evidence that plasma levels of sex hormones (estrogen and progesterone) are associated with an increased risk of cervical cancer in HPV infected women [40].

Pregnancy could also act as a promoter, rather than an initiator in the process of cervical carcinogenesis, a hypothesis supported by our data. Papillomavirus replication depends on elevated levels of transcription viral genes, which in turn can be activated in response to progesterone [96-98]. Alternatively, the mechanism may not be related to pregnancy per se, but to cervical inflammation and trauma during delivery. In the IARC multicenter study, women who reported cesarean but not vaginal deliveries had a lower risk of cervical cancer [OR=0.98 (0.36, 2.7)] when compared to nulliparous women. Further, parous women who reported only cesarean deliveries had a lower risk of cervical cancer compared with women who reported vaginal deliveries only, but the 95% CI of study effect estimates were broad (0.1-1.1) due to the rarity of cesarean delivery in the study [5]. Pregnancy-induced immunosuppression may also favor the infection or aid in the oncogenic properties of HPV [9]. Our data would suggest that pregnancies play a role in the progression to CIN III, and do not increase a woman's risk of HPV infection.

Our observed association between CIN III and intercourse within four months of birth is novel. A similar variable was tested among 759 women with invasive cancer and 1,430 controls in a case-control study in Latin America, where no association was found [7]. To gain understanding of this association, we conducted analyses to identify predictors of intercourse within four months of birth. Older age was highly associated with having intercourse within four months of childbirth and having more than one lifetime sexual partner was protective. However the association of earlier return to intercourse remained even after adjustment for age and number of sexual partners. Women who had a high proportion of pregnancies resulting in live births (excluding abortions) were more likely to engage in intercourse soon after childbirth, but for the variable that included abortions in its calculation, having medium to high success made these women less likely to engage in intercourse soon after childbirth. Although the possible association between intercourse within four months of delivery and CIN III is intriguing, additional work is needed to clarify both the actual timing of intercourse and the potential biological mechanisms. Intercourse within 6-8 weeks of delivery would occur in a time of significant inflammatory response and tissue remodeling; exposure to semen or additional microtrauma from intercourse could exacerbate processes which facilitate progression [99]. Conversely, assuming that the majority of women in Shanxi province breast-feed, hormonal changes related to lactation may affect immune response. In this study approximately 30% of women had resumed intercourse within four months of childbirth. This is substantially lower than was reported for women in an urban population in Zibo, China where 93% of women had resumed sexual intercourse at four months postpartum [100].

Similarly, it is unclear to what extent the association between number of pregnancies and CIN III is due to some factor related to the pregnancy (such as effects of pregnancy hormones on immune function or physical properties of the cervix affecting progression risk), or some independent host factor associated with both risk of HPV progression and the ability to achieve

pregnancy. If the former, we would have expected both the number of pregnancies and number of live births to be associated with CIN III risk.

While population-based, our findings from two rural counties in Shanxi province cannot be considered representative of the very diverse Chinese population. The cross-sectional nature of the data limits our ability to draw causal links and estimate the timing of each risk factor's contribution to cervical carcinogenesis. Cohort studies allow for observation of the timing of oncogenic HPV infection, and progression from oncogenic HPV infection to CIN III. Cross-sectional data only provide measurements at a single time point and therefore, we utilized data within histologic grades as a proxy for progression. Reliance on self-reported data may have led to under- or over-reporting of reproductive variables. The quality of self reported data are often difficult to verify, but due to the one child policy in China there has been strong incentive to have reliable reproductive information and to report pregnancies, abortions, live and still births to the government [78]. While initially surprised to see the range in numbers of pregnancies (0-10) and live births (0-10), these numbers were validated against means for the province (typical for a rural environment) and fall within normal limits.

In conclusion, our findings add to the accumulating evidence that reproductive co-factors play an important role in the progression of persistent high-risk HPV infection to CIN III, whereas sexual behavior may be a more important risk factor for HPV acquisition. Prospective data is needed to further untangle the effects of these variables. Determining the stage or stages of carcinogenesis affected is an important next step in understanding the natural history of the HPV and cervical cancer relationship.

Table 8. Selected Characteristics of Study Participants among 2,055 HPV DNA Positive and 6,657 HPV Negative Women in the SPOCCS II Study, Stratified by Histological Diagnosis

Characteristic	HPV Negative,		HPV Positive		
	Normal	Normal			
	Cytology	Cytology	CIN I	CIN II	CIN III
	N=6,657	N=1,405	N=299	N=167	N=184
Median Age (range), years	41 (35-50)	40 (35-50)	40 (35-50)	41 (35-50)	42 (35-50)
Percent Married (%)	99	98	97	96	99
Median Age at First Intercourse (Range), years	20 (15-31)	20 (15-33)	20 (15-28)	20 (16-26)	20 (16-24)
Percent Having Intercourse Within 4 Months of Childbirth (%)	34	31	37	28	44
Median Number Lifetime Sexual Partners (range)	1 (1-27)	1 (1-10)	1 (1-8)	1 (1-10)	1 (1-12)
Median Number of Pregnancies (range)	3 (0-13)	3 (0-9)	3 (0-8)	3 (0-10)	3 (0-8)
Median Number of Live Births (range)	2 (0-8)	2 (0-7)	2 (0-5)	2 (0-5)	2 (0-5)

Table 9. Crude and Age-Adjusted Odds Ratios for Selected Reproductive Characteristics among 2,055 HPV DNA Positive Women and 6,657 HPV Negative Women in the SPOCCS II Study.

Characteristic	CIN III vs ≤CIN II			CIN I or HPV positive Normal Cytology vs. CIN negative/HPV negative		
	Cases/Control	OR (95% CI) <sup>1</sup>	OR (95% CI) <sup>2</sup>	Cases/Control	OR (95% CI) <sup>1</sup>	OR (95% CI) <sup>2</sup>
	<hr/>					
Age (years)						
35-39	62/824	1.0	1.0	762/2940	1.0	1.0
40-44	52/598	1.2 (0.79, 1.7)	1.2 (0.79, 1.7)	543/2055	1.0 (0.90, 1.2)	1.0 (0.90, 1.2)
45-50	70/449	2.1 (1.4, 3.0)	2.1 (1.4, 3.0)	339/1662	0.93 (0.81, 1.1)	0.93 (0.81, 1.1)
<hr/>						
Age at First Intercourse (years)						
≥ 21	67/827	1.0	1.0	756/3108	1.0	1.0
19-20	41/343	1.5 (0.98, 2.2)	1.1 (0.74, 1.8)	312/1086	1.2 (1.0, 1.4)	1.3 (1.1, 1.5)
12-18	76/701	1.3 (0.95, 1.9)	1.3 (0.89, 1.8)	636/2462	1.1 (0.94, 1.2)	1.1 (0.96, 1.2)
<hr/>						
Lifetime Sexual Partners						
1	122/1217	1.0	1.0	1110/4875	1.0	1.0
2	26/365	0.71 (0.46, 1.1)	0.70 (0.45, 1.1)	328/4875	1.4 (1.3, 1.7)	1.5 (1.3, 1.7)
≥ 3	36/289	1.2 (0.84, 1.8)	1.2 (0.80, 1.8)	266/787	1.5 (1.3, 1.7)	1.5 (1.3, 1.8)

Reported # Pregnancies						
0-2	50/688	1.0	1.0	624/2536	1.0	1.0
≥ 3	134/1183	1.6 (1.1, 2.2)	1.5 (1.0, 2.1)	1080/4121	1.1 (0.95, 1.2)	1.1 (0.96, 1.2)
Reported # Live Births						
0-2	126/1265	1.0	1.0	1157/4626	1.0	1.0
≥ 3	57/593	0.97 (0.70, 1.3)	0.81 (0.58, 1.1)	535/1973	1.1 (0.97, 1.2)	1.1 (0.99, 1.2)
Intercourse Within Four Months of Childbirth						
No	103/1274	1.0	1.0	1154/4404	1.0	1.0
Yes	80/584	1.7 (1.2, 2.3)	1.7 (1.2, 2.3)	538/2195	0.94 (0.83, 1.0)	0.94 (0.83, 1.0)
Reported # Abortions						
None	113/1177	1.0	1.0	1066/4293	1.0	1.0
1	41/452	0.94 (0.65, 1.4)	0.99 (0.68, 1.4)	415/1431	1.2 (1.0, 1.3)	1.2 (1.0, 1.3)
≥ 2	29/229	1.3 (0.86, 2.0)	1.5 (0.94, 2.3)	211/875	0.97 (0.82, 1.1)	0.96 (0.82, 1.3)

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Current Contraception Use						
Female Sterilization	158/1448	1.0	1.0	1316/5295	1.0	1.0
Intrauterine Device	15/293	0.47 (0.27, 0.81)	0.52 (0.30, 0.91)	274/947	1.2 (1.0, 1.3)	1.2 (1.0, 1.3)
Oral Contraceptives	2/21	0.87 (0.20, 3.8)	1.1 (0.25, 4.7)	17/92	0.74 (0.44, 1.3)	0.74 (0.44, 1.2)
Condoms	0/8	-	-	7/17	1.1 (0.69, 4.0)	1.6 (0.68, 3.9)
None	9/99	0.83 (0.41, 1.7)	0.86 (0.42, 1.7)	88/305	1.2 (0.91, 1.5)	1.2 (0.91, 1.5)

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<sup>1</sup> Crude OR and 95% CI.

<sup>2</sup> Age-Adjusted OR and 95% CI; Numbers rounded to 1.0.

Notes: Controls for the comparison with CIN 1 are HPV negative women with normal cytology. Age adjustment was by categorical years as follows: 35-39, 40-44, 45-50.

Table 10. Multivariate Odds Ratios for Intercourse within Four Months of Childbirth among 8,062 Control Women in the SPOCCS II Study.

Characteristic	Negative Cytology OR (95% CI)
<b>Age (years)</b>	
35-39	1.0
40-44	1.2 (1.0, 1.3)
45-50	1.6 (1.4, 1.8)
<b>Lifetime Sexual Partners</b>	
1	1.0
2	0.5 (.41, .55)
≥ 3	0.3 (.26, .38)
<b>Reported # of Pregnancies</b>	
0-2	1.0
≥3	0.5 (.41, .53)
<b>Reported # of Abortions</b>	
None	1.0
1	2.1(1.9, 2.5)
≥ 2	3.5 (3.0, 4.2)
<b>Current Form of Contraception</b>	
Female Sterilization	1.0
IUD	3.3 (2.9, 3.8)
Oral Contraceptive	3.4 (2.2, 5.1)
Condoms	0.8 (.31, 2.2)
None	2.7 (2.1, 3.3)

Note: Age adjustment was by categorical years as follows: 35-39, 40-44, 45-50.

Figure 4: Causal Diagram for the Relationship between HPV infection, Reproductive Factors and the risk of CIN III.

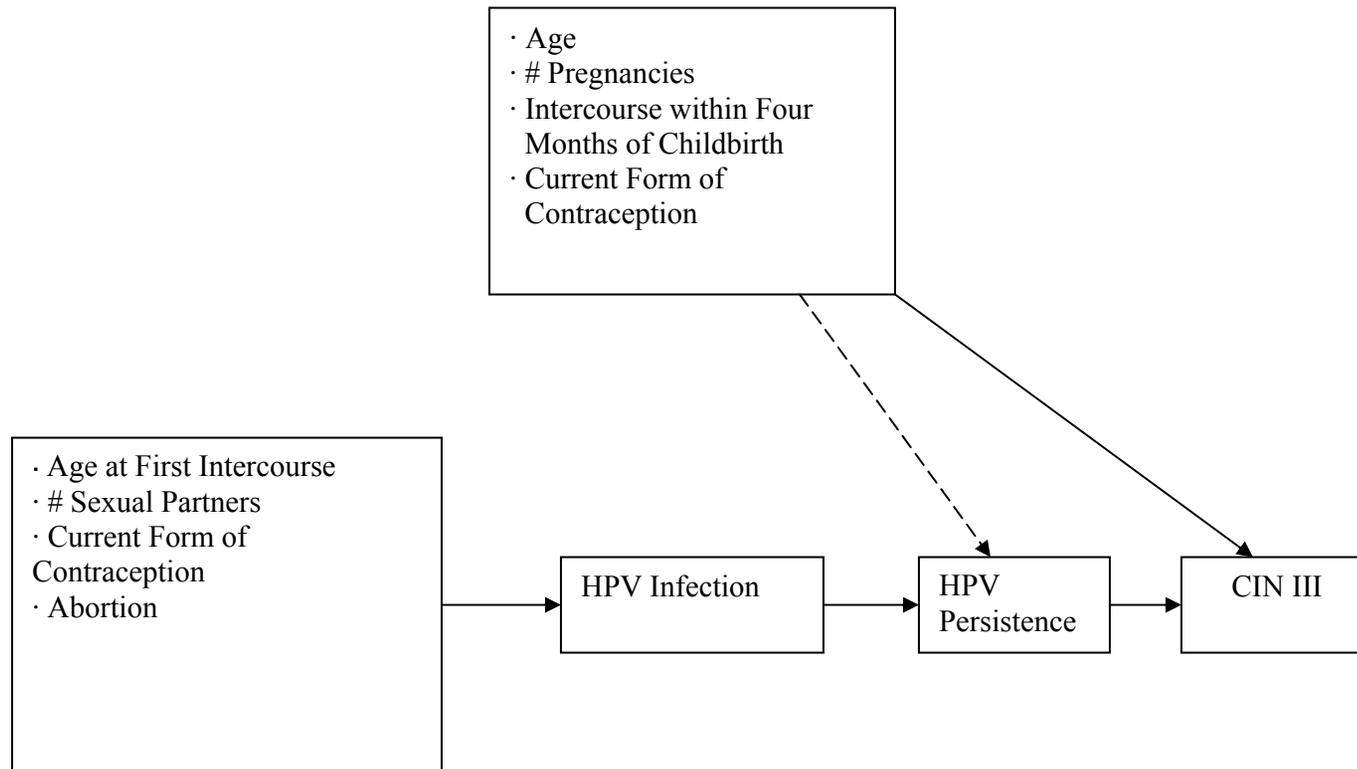
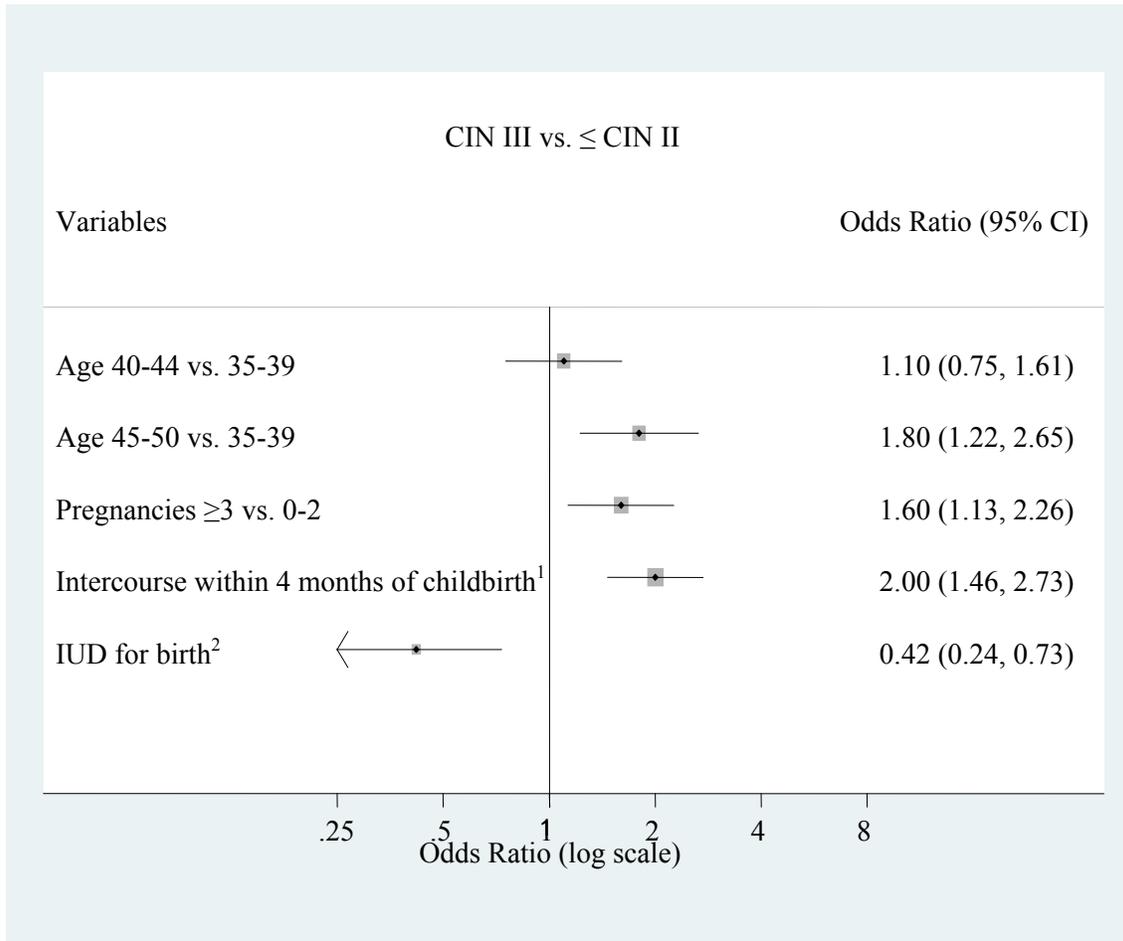


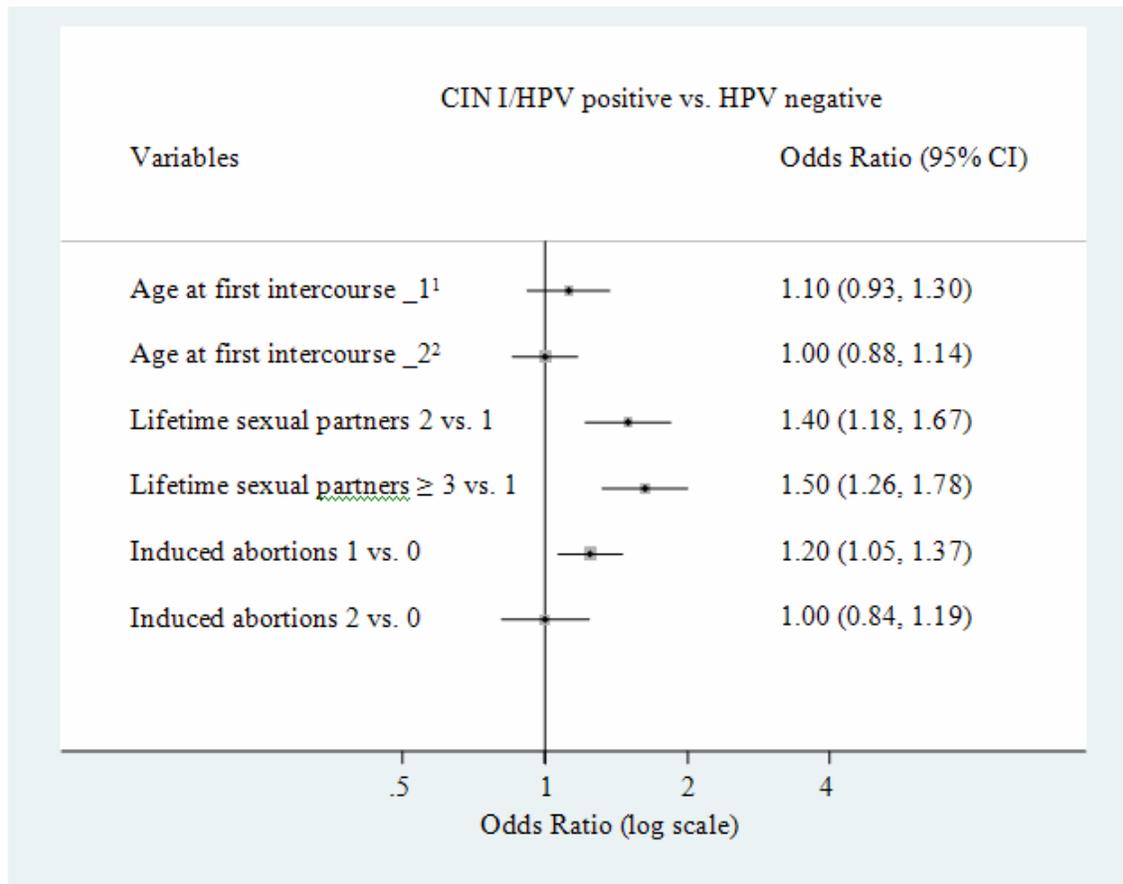
Figure 5. Multivariate OR and 95% CI for Selected Reproductive Risk Factors for CIN III vs.  $\leq$  CIN II among women in the SPOCCS II trial.



<sup>1</sup> Variable in the model was intercourse within four months of childbirth (yes/no)

<sup>2</sup> Variable in the model was IUD vs. female sterilization

Figure 6. Multivariate OR and 95% CI for Selected Reproductive Risk Factors for CIN I/HPV positive vs. HPV negative among women in the SPOCCS II trial.



<sup>1</sup>Variable in the model was age at first intercourse of 19-20 versus  $\geq 21$

<sup>2</sup>Variable in the model was age at first intercourse of 12-18 versus  $\geq 21$

## **CHAPTER V**

### **CONCLUSIONS**

#### A. Summary

Results from this study add to the evidence that CIN II may be more similar to CIN I than to CIN III, as shown through risk factor profiles. Reproductive risk factors may play an important role in the progression from an HPV lesion to CIN III, while sexual behavior is important for the acquisition of the initial HPV infection. Potential misclassification of CIN II lesions may be leading to over-treatment of CIN lesions that would regress over time if left alone. Reproductive risk factors may have a role in the progression of HPV to high-grade cervical pre-cancer. It is biologically plausible that elevated levels of hormones during pregnancy and immediately postpartum may act as promoters in cervical carcinogenesis, aiding in the progression of cervical disease. Results from this study provide impetus to further investigate the role that some reproductive cofactors may have as promoters.

#### B. Study Limitations

The reliability of the self-reported data on current and previous sexual behavior, as in any study, may be compromised if women were reluctant to disclose sexual behavioral information. Due to the one child policy in China there has been strong incentive to have reliable reproductive information and to report pregnancies, abortions, live and still births to the government. Ranges of pregnancies and live births were compared to means for the province (typical for a rural environment). Female interviewers were used who received training in approaching study participants in a culturally acceptable way and assuring them that all responses would remain confidential.

Women who test positive for HPV today may have been infected in the past and past infection may have played a role in their current health state. Although persistent HPV infections are pointed to as the cause of cervical cancer, the role of past infections is unclear.

In general, it is a limitation to use cross-sectional data when looking at an infection that is transient, as is HPV. In this specific situation the majority of women are married (98%), the level of reported extra-marital affairs is low and all women are over the age of 35, leading us to believe that the incidence of new infections is low and that the majority of these infections in fact represent the persistent infections that occasionally progress to cervical cancer.

### C. Study Strengths

Data from the Shanxi Province Cervical Cancer Screening study II (SPOCCSII) was utilized for several reasons. The dataset contains 8,497 women, with histologic confirmation of disease for women who experienced a positive HPV test and/or positive cytology. The extensive histologic confirmation of disease eliminated some issues of misclassification and verification bias. These data provided a fairly homogeneous population with respect to risk factors like age, smoking status and marital status while at the same time being diverse enough to provide variation on other important cofactors (i.e. pregnancies). This offered an opportunity to look at women who do not differ in some of the major areas that affect cervical status, such as smoking.

Due to the concern regarding the heterogeneity of CIN II lesions, biopsies from SPOCC I (a SPOCCS II precursor ) were reviewed for agreement, the proportion of biopsies said to be misclassified were applied to the results of this study. On review 92% of CIN II remained CIN II, 2.4% was reclassified as CIN I, 4.8% as normal histology and 1.2% as CIN III. When odds ratios and 95% confidence intervals were recalculated for higher gravidity, no change in the magnitude of the effect was observed, suggesting that misclassification in this study was negligible.

#### D. Future Directions

The majority if not all cervical cancers are attributable to HPV infection [101-103]. Histological classifications along the path from HPV infection to CIN III or cancer are under review. The current challenges are: to determine if CIN II has low progression potential, as is the case with CIN I, or if it is more similar to CIN III and to unravel the potential roles of cofactors in the acquisition of HPV infection, development of persistent infection, and the rare progression from HPV infection to cervical cancer.

While the results from this study cannot answer the questions of cofactor roles in HPV infection, persistence, and progression, we encourage those with prospective data to complete case-case analysis to identify risk factor differences among histological grades, and to follow up women with CIN II to see if they progress more similarly to CIN III or to CIN I.

## **APPENDICES**

- A. Variables Available for Analysis with their Original Coding in the SPOCCS II Dataset
- B. Questionnaire from the SPOCCS II study

**Appendix A. Variables Available for Analysis with their Original Coding  
in the SPOCCS II Dataset**

<b>Variables</b>	<b>Code</b>
Subject age	Numeric Age
Marriage status	1=Married; 2= Divorced; 3= Widow; 4= Single
Education	1=No formal schooling; 2=Primary school; 3=Middle school; 4=Over senior high school
Bathing location	1=Bathing in public house; 0=Bathing at home
Current smoke	1=Yes; 2=No
Drink alcoholic beverage	1=Yes; 2=No
Age of first menstrual period	Numeric Age
Days between menstrual periods	0=Missing
Days for each period	0=Missing
Menopausal status	1=Menopause;2=No menopause; 3=Premenopause; 4=Missing
Age at menopause	Numeric Age 0=Missing
Husband multiple sexual partners	1=Yes; 0=No
Pregnancies number	Numeric

Live birth number	Numeric
Stillbirth number	Numeric
Miscarriage number	Numeric
Abortion number	Numeric
Births delivered in hospital	Numeric
Births delivered at home	Numeric
At what month had sexual intercourse during the pregnancy	Numeric
At what month had sexual intercourse after delivery	Numeric
	Numeric Age
Age at first sexual intercourse	0=Missing
Subject multiple sexual partners	1=Yes; 2=No
How many sexual partners in your life	Numeric
Sexual frequency during the youth period	Numeric
Sexual frequency during the mid-age period	Numeric
	1 = Always; 2 = Sometimes;
Genital wash before sexual intercourse	3 = Rarely or Never
	1 = Always; 2 = Sometimes;
Genital wash after sexual intercourse	3 = Rarely or Never
	1= IUD; 2= Birth control pill;
	3=Condom or rubber;
	4=Operation-female sterilization;
Birth control methods	5=No; 9=Missing
Ever diagnosis of Hepatitis	1=Yes; 2=No; 9=Missing

	Numeric Age
Age at first diagnosis for hepatitis	0=Missing
Ever diagnosis of Cancer	1=Yes; 2=No; 9=Missing
Age at first diagnosis for cancer	Numeric Age
	0=Missing
Ever diagnosis of Cervical inflammation	1=Yes; 2=No; 9=Missing
Diagnosis times for cervical inflammation	Numeric
Ever diagnosis of Urinary tract infection	1=Yes; 2=No; 9=Missing
Diagnosis times for urinary tract infection	Numeric
Ever diagnosis of Gonorrhea	1=Yes; 2=No; 9=Missing
Diagnosis times for gonorrhea	Numeric
Ever diagnosis of Syphilis	1=Yes; 2=No; 9=Missing
Diagnosis times for syphilis	Numeric
Ever diagnosis of Condyloma	1=Yes; 2=No; 9=Missing
Diagnosis times for condyloma	Numeric
Ever diagnosis of Genital herpes	1=Yes; 2=No; 9=Missing
Diagnosis times for genital herpes	Numeric
Ever diagnosis of Genital ulcers	1=Yes; 2=No; 9=Missing
Diagnosis times for genital ulcers	Numeric
Ever diagnosis of Vagina Trichomoniasis	1=Yes; 2=No; 9=Missing
Diagnosis times for vagina trichomoniasis	Numeric
Ever diagnosis of Mycosis	1=Yes; 2=No; 9=Missing
Diagnosis times for mycosis	Numeric
Ever diagnosis of Other Sexual Transferred	
Disease	1=Yes; 2=No; 9=Missing

Diagnosis times for other sexual transferred disease	Numeric
Vaginal discharge	1=Yes; 2=No; 9=Missing
Cancer family	1=Yes; 2=No; 9=Missing
Pathology grade for cervical intraepithelial lesion	1=SCC; 2=CIN III; 3=CIN II; 4=CIN I; 5=Normal
HPV self test result	1=POS; 2=NEG; 9=Missing
HPV direct test result	1=POS; 2=NEG
County code	1=Xiangyuan; 2=Yangcheng
Cervical cancer family history	1=Yes; 0=No
Screening season	1=Spring; 2=Summer; 3=Winter



A. Section I General Information

- 1.1. How old are you now?
- 1.2. Marriage condition:  
0 = Unmarried; 1 = Married; 2 = Divorce; 3 = Widow
- 1.3. Do you consider yourself to be han, hui or another ethnic group?  
1 = Han; 2 = Hui; 3 = Other, specify \_\_\_\_\_
- 1.4. What is the highest grade or level of schooling you completed?  
0 = No formal schooling; 1 = Primary school;  
2 = Junior high school 3 = Over senior high school
- 1.5. What is your birthplace? \_\_\_\_\_ Province \_\_\_\_\_ county/City
- 1.6. Do you have sisters aged 30-50?  
1 = Yes (continue) 2 = No (skip to 1.8 )
- 1.7 Have they taken part in the study?  
1 = Yes (Names \_\_\_\_\_ )  
2 = No
- 1.8. When you bathe, do you use?  
1 = A shower; 2 = A bath tub in your home;  
3 = Public bathhouse; 4 = Other, specify \_\_\_\_\_
- 1.9. What is your chief source of drinking water?  
1 = Well, Pond / lake; 2 = Spring, River; 3 = Tap water
- 1.10. Have you been smoking for six months or more?  
1 = Yes (continue); 2 = No (skip to 1.17 )
- 1.11. At what age did you start smoking cigarettes regularly for six or more months?
- 1.12. When smoking, do you inhale into the chest?  
1 = Yes; 2 = No
- 1.13. ( During periods when you smoked ) What do/did you usually smoke ?  
1 = Cigarette (continue); 2 = Tobacco(skip to 1.15); 3 = Both

1.14. ( During periods when you smoked ) do/did you usually smoke filtered or unfiltered cigarettes?

1 = Filtered cigarette 2 = Unfiltered cigarette; 3 = Both

1.15. ( During periods when you smoked ), how many tobacco product ( per day / month ) do/did you smoke?

a. Tobacco  liang / month

b. Cigarettes  cigarettes/day

1.16. Do you smoke now?

1 = Yes;

2 = No

1.17. At what age did you stop smoking regularly? Age

1.18. How many years have you smoked? YR

1.19. Do you drink alcoholic beverages?

1 = Never

2 = Sometimes

3=Often

1.20. Tell me the kinds of alcoholic beverages that you drink?

1 = Beer; 2 = Wine; 3 = Grain liquor;

4 = Both beer and wine;

5 = Both wine and grain liquor;

6 = Both beer and grain liquor

7 = All three types of beverages;

8 = Another type of alcoholic drink, specify

## **B. Section II Menstrual History / Marriage and Birth**

2.1. At what age did you have your first menstrual period Age

2.2. At what age did your menstrual periods become established at regular intervals, that is, periods occurring at predictable intervals?

Age

2.3. On the average, over most of your adult life, how many days were there between menstrual periods?

DY

2.4. How many days of menstrual flow did you usually have each period? (Note that any days of spotting should not be included. Only days with a full flow should be counted.)

|\_|\_|DY

2.5. Have you had any changes in your menstrual cycle over the years?

1 = Yes(continue); 2 = No(skip to 2.7 )

|\_|

2.6. If yes, at what age did your cycle change?

Age |\_|\_|

2.7. Have you had menopause?

1 = Yes(continue); 2 = No(skip to 2.9 )

|\_|

2.8. In which month and year did you have your last menstrual period?

|\_|\_| - |\_|\_| YR - MO

2.9. When you (are/were) having your menstrual period, what (do/did) you usually use to collect the blood?

1 = Sanitary napkin; 2 = Paper; 3 = Other, specify\_\_\_\_\_

|\_|

2.10. How many times have you been married? ( Never married =0 ) |\_|\_| times

2.11. Do you have a partner now?

1 = Yes; 2 = No

|\_|

2.12. To your knowledge, did your husband / any of your husbands ever have sexual intercourse with another woman before or during your marriage?

1 = Yes; 2 = No

|\_|

2.13. Including livebirths, stillbirths and abortions, how many times have you been pregnant?

|\_|\_|

2.14. Did you ever receive any medical treatment prescribed by a doctor to become pregnant?

1 = Yes ( explain why \_\_\_\_\_ ); 2 = No

|\_|

2.15. Was the outcome of your pregnancy?

a. Livebirth |\_|\_| times

b. Stillbirth |\_|\_| times

c. Miscarriage |\_|\_| times

d. Abortion |\_|\_| times

2.16. Do you have a child with a congenital defect such as a cleft lip or a heart defect and so on?

- a.. Livebirth      1 = Yes ( specify \_\_\_\_\_ );      2 = No
- b.. Stillbirth      1 = Yes ( specify \_\_\_\_\_ );      2 = No

2.17. How many births have you delivered in following places?

- a. Hospital / Clinic       birth
- b. Home       birth
- c. Some other place, (specify \_\_\_\_\_)       birth

2.18. Have you ever had a cesarean section?

- 1 = Yes;      2 = No

2.19. (Besides the cesarean section) have you ever had any other problems during childbirth such as a rupture or tear of any part of the genital area?

- 1 = Yes;(continue)      2 = No(skip to 3.1);      3 = Don't know (skip to 3.1)

2.20. During your most recent pregnancy, until what month did you have sexual intercourse?

MO

2.21. How long after the end of the most recent pregnancy did you begin having sexual intercourse again?

MO

### C. Section III History/Hygiene Habit

The next questions are about your sexual history and hygiene habits. We realize this is a personal topic, but it is very important to study. Please take the time to recall this information as accurately as possible. We will maintain secrecy for you.

3.1. How old were you when you first had sexual intercourse with a man?

Age

00 = Never had intercourse (skip to section IV)

3.2 Did you have sexual intercourse with man besides your husband (sexual intercourse before marriage will be included)

- 1 = Yes;      2 = No

3.3. Through your life, how many men have you had sexual intercourse with?

3.4. On the average, how long did you have a sexual intercourse? Answer in the easiest

way. If the frequency is not constant in a period, mark the largest frequency. ( Never happened, please fill 0 )

1. Youth Period (~29)

|\_|\_| times / month

2. Middle-Age Period (30~49)

|\_|\_| times / month

3.5. Do you usually wash genital area before sexual intercourse?

1 = Always;      2 = Sometimes;      3 = Rarely or Never

|\_|

3.6. Do you usually wash genital area after sexual intercourse?

1 = Always;      2 = Sometimes;      3 = Rarely or Never

|\_|

#### **D. Section IV Contraceptive History**

I am interested in all the methods of birth control or family planning you have used since you had sexual active.

4.1. Now, I'd like to read to you a list of birth control methods. Please tell me if you and your partner have ever used any of these methods?

1 = IUD;      2 = Birth control pill;      3 = Condom or rubber  
4 = Operation-female sterilization;      5 = No (skip to section V after 4.2)

|\_|

4.2. What is the major reason you have never used a method of birth control or family planning?

1 = Never know about it;      2 = Partners is not fertile  
3 = Subjects is not fertile or has trouble becoming pregnant;      4 = Other reasons

|\_|

4.3. How old were you when you last used a method of birth control?

|\_|\_|

4.4. I'd now like to find out which pills you were using. Please tell me the name of the pill you often used?

1st pill: Name of pill \_\_\_\_\_ |\_|\_|YR

2nd pill: Name of pill \_\_\_\_\_ |\_|\_|YR

4.5. If you have used an IUD, how long have you used it?

\_\_\_\_\_

**E. Section V Medical History**

5.1. Have you been ever diagnosed with following disease (condition) before?

	1 = Yes / 2 = No	How old were you when you first had?
a: High blood pressure	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
b: Diabetes	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
c: Gall bladder disease	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
d: Thyroid disease	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
e: Tuberculosis	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
f: Hepatitis	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age
g: cancer	<input type="checkbox"/>	<input type="text"/> <input type="text"/> age

5.2. Have you been ever diagnosed with following disease (condition) before?

	1 = Yes / 2 = No	How many times have you suffered it?
a. Cervix erosion or chronic inflammation, polyp	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
b. Urinary tract infection	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
c. Gonorrhea	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
d. Syphilis	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
e. Condyloma	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
f. Genital herpes	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
g. Genital ulcers	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
h. Vagina Trichomoniasis	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
i. Mycosis	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times
j. Other Sexual Transferred Disease	<input type="checkbox"/>	<input type="text"/> <input type="text"/> times

5.3. Did you have a vaginal discharge or vaginal irritation?

1 = Yes (What color was the discharge? \_\_\_\_\_); 2 = No

5.4. Did you ever see a doctor or other health professional about genital condition such as a vaginal discharge or vaginal irritation?

1 = Yes(continue); 2 = No(skip to section 5.8)

5.5. Have you been received some treatment?

1 = Yes(continue); 2 = No(skip to section 5.8)

5.6. What type of treatment have you received?

1 = electrotherapy                      2 = refrigeration

3 = laser                                      4 = other treatment

5.7. Are there someone in your family have suffered some cancer?

1 = Yes(continue); 2 = No(skip to section VI)

5.8. Who are they suffered with some cancer?

Relative to you	Name	Alive	Cancer Name / ICD-10 code	Address
(1) _____	_____	<input type="checkbox"/>	_____/ _ _ _ _ _	_____
(2) _____	_____	<input type="checkbox"/>	_____/ _ _ _ _ _	_____
(3) _____	_____	<input type="checkbox"/>	_____/ _ _ _ _ _	_____

( **Note** : Alive:1=Yes 2=No

If there are more than two person in her family, including subject herself, have suffered with following cancer, it is necessary to fill in the Questionnaire for Family Study. These cancer types are cervix cancer, malignant neoplasm of body of uterus, malignant neoplasm of ovary and other uterine adnexa, malignant neoplasm of placenta, esophageal cancer, breast cancer and carcinoma in situ of breast and cervix. )

1. Section VI Records for interviewer

6.1 If you got support when you ask the questions?

1 = best; 2 = good; 3 = not bad; 4 = bad

6.2 Can the interview subject answer your questions accurately?

1 = Very Accurate; 2 = Accurate; 3 = not accurate for some parts;  
4 = Not accurate; 5 = Uncertain

6.3 Assessment for whole interview quality

1 = good; 2 = general; 3 = doubt; 4 = not satisfy

6.4 Which sections are not reliable? Please circle the section number you selected.

1, 2, 3, 4, 5

6.5 name of interview subject

1 = subject herself 2 = other person

6.6 Time of Interview Ended: :

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