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Baseline prevalence and type distribution of human papillomavirus in healthy Chinese women aged 18–25 years enrolled in a clinical trial

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Abbreviations: AGC: atypical glandular cells; ASC-H: atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions; ASC-US: atypical squamous cells of undetermined significance; CDC: Center for Disease Control and Prevention; CICAMS: Cancer Institute and Hospital, Chinese Academy of Medical Sciences; CI: confidence interval; CIN: cervical intraepithelial neoplasia; ELISA: enzyme-linked immunosorbent assay; EU: ELISA unit; HPV: human papillomavirus; hr: high-risk; HSIL: high-grade squamous intraepithelial lesions; LSIL: low-grade squamous intraepithelial lesions; MPL: 3-*O*-desacyl-4′-monophosphoryl lipid A; PCR: polymerase chain reaction; TVC: total vaccinated cohort

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Baseline human papillomavirus (HPV) prevalence and type distribution were evaluated in young Chinese women enrolled in a clinical trial of an HPV vaccine (ClinicalTrials.gov registration NCT00779766). Cervical specimens and blood samples were collected at baseline from women aged 18–25 years ($n = 6,051$) from four sites across Jiangsu province. Cervical specimens were tested for HPV DNA by SPF_{10} PCR-DEIA-$LiPA_{25}$ version 1, and HPV-16/18 type-specific polymerase chain reaction. Anti-HPV-16 and anti-HPV-18 antibody titres were quantified by enzyme-linked immunosorbent assay. At baseline, 15.3% of women were DNA positive for any of 14 HPV high-risk (hr) types (HPV-16/18/31/33/35/39/45/51/52/56/58/59/66/68). The most commonly detected hrHPV types in cervical specimens were HPV-52 (4.0%) and HPV-16 (3.7%). High-risk HPV DNA-positivity increased with severity of cytological abnormalities: 39.3% in atypical squamous cells of undetermined significance, 85.0% in low-grade squamous intraepithelial lesions and 97.8% in high-grade squamous intraepithelial lesions (HSIL). The hrHPV types most frequently detected in HSIL were HPV-16 (63.0%), HPV-18 (17.4%), HPV-52 (17.4%), HPV-58 (15.2%) and HPV-33 (15.2%). The hrHPV types most frequently detected in cervical intraepithelial neoplasia 2+ were HPV-16 (66.1%), HPV-33 (16.1%), HPV-52 (16.1%), HPV-58 (14.5%) and HPV-51 (11.3%). Multiple hrHPV infections were reported for 24.4% of hrHPV DNA positive women. Regardless of baseline HPV DNA status, 30.5% and 16.0% of subjects were initially seropositive for anti-HPV-16 and anti-HPV-18, respectively. In conclusion, the high baseline seropositivity rate and intermediate prevalence of cervical hrHPV types in Chinese women aged 18–25 years underlines the importance of early HPV vaccination in this population.

What's new?

In China, cervical cancer is the second most frequent cancer among women aged 15–44 years. The authors collected baseline data on prevalence and type distribution of human papillomavirus (HPV) from more than 6,000 healthy Chinese women aged 18–25 years participating in a large vaccine efficacy trial. Regardless of cytology, 15.3% of women were positive for high-risk HPV types, with HPV-52 (4.0%), HPV-16 (3.7%), HPV-51 (1.7%) and HPV-58 (1.5%) being the most frequently detected. This high baseline prevalence of high-risk HPV types underscores the importance of early vaccination among Chinese women.

In China, cervical cancer ranks as the eighth most frequent cancer among all women and the second most frequent cancer among women aged 15–44 years.[1] In 2008 an estimated 75,000 new cases of cervical cancer occurred in China and approximately 34,000 women died from the disease.[1] However, the burden of disease may be more substantial than this estimation, which was based on only a few available datasets, with a higher incidence than this observed in some rural areas.[2] Using available epidemiological evidence from both urban and rural areas in mainland China it was estimated that the annual number of new cases of cervical cancer nationally, in the absence of intervention, could increase by approximately 40–50% from the year 2010–2050.[2]

Persistent infection with high-risk (hr) human papillomavirus (HPV) is a necessary cause for the development of cervical cancer.[3,4] Thus, prophylactic HPV vaccination offers the potential to substantially reduce the burden of disease, when given to adolescent females prior to sexual debut and subsequent exposure to HPV. Vaccination against HPV may be particularly useful in China, where there has been limited success in the implementation of universal, effective and regular screening programs. Two HPV vaccines, a HPV-16/18 AS04-adjuvanted vaccine (*Cervarix*®, GlaxoSmithKline Vaccines) and a HPV-6/11/16/18 aluminium-adjuvanted vaccine (*Gardasil*®, Merck), are now licensed worldwide, including Asian regions or countries with ethnic Chinese majority populations such as Hong Kong, Macau, Taiwan and Singapore, but are not yet available in mainland China.

An understanding of HPV prevalence and type distribution in China, compared with other parts of the world, is relevant to assess the potential future impact of HPV vaccination. Herein we report baseline HPV data for healthy Chinese women aged 18–25 years who were enrolled in a large, randomised, controlled, Phase II/III clinical trial to evaluate the efficacy, immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine.[5] Objectives of this baseline analysis were to evaluate HPV prevalence and DNA genotype distribution in young Chinese women, by cytological and histological status, by age, and by region (urban or rural), and to evaluate HPV-16 and HPV-18 serological status at study entry.

Methods

Study design

Baseline HPV prevalence and type distribution data are reported from a phase II/III, double-blind, randomised, controlled study evaluating the efficacy, immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine.[5] This study is registered with ClinicalTrials.gov, number NCT00779766. Enrolment in the study started in October 2008 and follow-up is ongoing. The trial was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and the International

Conference on Harmonisation Good Clinical Practice guidelines. The study protocol and informed consent form were reviewed and approved by the ethics committees of the Center for Disease Control and Prevention (CDC) Jiangsu Province, and the Cancer Foundation of China. Written informed consent was obtained from each participant prior to the performance of any study-specific procedures.

Participants

Healthy Chinese women aged 18–25 years at the time of first vaccination, with a negative urine pregnancy test, were enrolled at four sites in Jiangsu Province, China (Binhai CDC, Jintan CDC, Lianshui CDC and Xuzhou CDC). Virgins were not enrolled in the study due to cultural and ethical considerations. Women of childbearing potential were to be abstinent or to have used adequate contraceptive precautions for 30 days prior to the first vaccination and agreed to have continued such precautions for 2 months after completion of the vaccination series. Women had to have a single intact cervix. Women who were pregnant or breastfeeding, had an immunosuppressive or immunodeficient condition, a history of colposcopy, an allergic disease likely to be exacerbated by any component of the vaccine or previously received HPV vaccination, 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) or AS04 adjuvant were excluded. The selection of participants was population-based. All women in the target age range living in the area covered by each of the selected CDC sites were invited to participate in the clinical trial if they met the selection criteria. The women were contacted and enrolled by the local CDC staff and recruitment was stopped when the target sample size was reached.

Cytology and histopathology

A cervical sample was collected from each subject pre-vaccination using the sampling device provided and rinsed into a collection vial containing *PreservCyt*® medium (Hologic Inc, Bedford, MA). Specimens were shipped to a central laboratory, the Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS), for processing and testing. The cervical sample was divided into two aliquots. One l mL aliquot was removed from the original *PreservCyt*® sample for HPV DNA PCR testing. The remaining sample was evaluated for cytology using the *ThinPrep*® Pap Test (Hologic Inc, Bedford, MA) and results were reported according to the Bethesda 2001 classification system.[6] If atypical squamous cells of undetermined significance (ASC-US) were identified, the residual *PreservCyt*® specimen was tested for hrHPV DNA using the *HC2 High-Risk HPV DNA Test*™ (Qiagen Inc, Gaithersburg, MD).

The pre-specified algorithm for the clinical management of abnormal cytological results and colposcopy referral has been described previously.[5] Briefly, colposcopy was recommended after reports of hrHPV DNA positive (by HC2 HPV DNA test) ASC-US, low-grade squamous cell intraepithelial lesion (LSIL) independent of HPV DNA results, atypical glandular cells (AGC), high-grade squamous intraepithelial lesion (HSIL) or atypical squamous cells in which HSIL could not be excluded (ASC-H). For any suspected cervical lesions at colposcopy, biopsy was recommended.

Histopathological analysis was performed by a panel of expert gynaecological pathologists at CICAMS. An independent endpoint committee reviewed data for women with cervical intraepithelial neoplasia (CIN), to make final case assignments.

HPV DNA testing

Samples were tested for the presence of 14 hrHPV DNA types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 11 low-risk HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70 and 74) by polymerase chain reaction (PCR) using the SPF_{10} PCR-DEIA-$LiPA_{25}$ version 1 detection system (manufactured by Labo Biomedial Product, Rijswijk, the Netherlands, based on licensed Innogenetics technology). All HPV positive samples were also tested using HPV-16 and HPV-18 type-specific PCR, as described previously.[7] A positive HPV-16 or HPV-18 result was defined as detection of either type by SPF_{10} PCR-DEIA-$LiPA_{25}$ or by type-specific PCR. A sample did not have to be positive for both tests.

Serology

Before vaccination, a blood sample was collected from each subject for the measurement of antibodies against HPV-16 and HPV-18 using enzyme-linked immunosorbent assay (ELISA).[8] Serological assays were performed by the National Institute for Food and Drug Control, China. The assay cut-off was 8 ELISA units per mL (EU/mL) for anti-HPV-16 and 7 EU/mL for anti-HPV-18. Seropositivity was defined as an antibody titre greater than or equal to the assay cut-off value.

Statistical methods

Sample size assumptions are described by Zhu *et al.*[5] The target enrolment was approximately 6,000 subjects. The primary analysis population was the total vaccinated cohort (TVC), which included all vaccinated subjects for whom data were available. Categorical data were summarised descriptively using frequencies, that is, number of subjects in given category (n) and corresponding percentage, calculated as number of subjects in given category divided by number of subjects with evaluable results (N) $\times$ 100. Descriptive summaries were produced using *SAS*® version 9.2.

The p-values were calculated using Chi square test to evaluate the statistical significance of differences in HPV prevalence between populations from urban and rural areas. No other statistical tests were done.

Results

A total of 6,081 Chinese women were enrolled at four sites in the Jiangsu Province of China and 6,051 subjects were included in the TVC (Table 1). Mean (standard deviation) age of participants was 23.0 (1.73) years at baseline. Twenty-two subjects were older than 25 years at baseline and therefore outside the protocol-defined age range of 18–25 years.

Table 1. Demographic and baseline characteristics (total vaccinated cohort)

	Total $N = 6,051$
Age at vaccination, years	
Mean (SD)	23.0 (1.73)
Median (min, max)	23.0 (18–34)
Region within Jiangsu Province, *n* (%)	
Xuzhou City	1,684 (27.8)
Jintan County	1,542 (25.5)
Binhai County	1,202 (19.9)
Lianshui County	1,623 (26.8)
Type of centre, *n* (%)	
Urban[1]	1,684 (27.8)
Rural[2]	4,367 (72.2)

[1]Subjects enrolled at Xuzhou CDC.
[2]Subjects enrolled at Binhai CDC, Jintan CDC and Lianshui CDC.

Approximately 28% of participants were enrolled at a site covering an urban area (Xuzhou CDC) and 72% of participants were enrolled at sites covering rural areas (Binhai CDC, Jintan CDC and Lianshui CDC) (Table 1).

The distributions of the tested HPV types in the TVC, irrespective of cytology, and by cytological status, are shown in Table 2. Baseline data were not available for 16 (0.3%) women due to unsatisfactory cervical samples. At study entry, 18.5% (95% confidence interval [CI]: 17.6, 19.6) of women were DNA positive for at least one of the 25 tested HPV types: 15.3% (95% CI: 14.4, 16.2) of women were positive for at least one of the 14 hrHPV types and 5.4% (95% CI: 4.8, 6.0) of women were positive for at least one of the 11 low-risk HPV types. The most commonly detected hrHPV types in cervical specimens from all women, regardless of cytology, were HPV-52 (4.0%, 95% CI: 3.6, 4.6) and HPV-16 (3.7%, 95% CI: 3.2, 4.2). Other hrHPV types detected in more than 1% of women were HPV-51 (1.7%), HPV-58 (1.5%), HPV-39 (1.3%), HPV-66 (1.3%), HPV-18 (1.2%), HPV-33 (1.1%) and HPV-56 (1.1%). Overall, multiple hrHPV infections were reported for 3.7% of women (Table 3). Out of those women who were DNA positive for at least one hrHPV type, 24.4% had multiple hrHPV types. The prevalence of hrHPV DNA, regardless of cytology, was 17.9% for women enrolled at one site in an urban location (Xuzhou) and 14.3% for women enrolled at the other three sites in rural locations (Binhai, Jintan and Lianshui) (Table 3). This difference in hrHPV prevalence was statistically significant ($p = 0.0006$). The prevalence of low-risk HPV DNA was 5.9% in subjects from the urban area and 5.2% in subjects from rural areas, with no statistically significant difference ($p = 0.2709$). Multiple hrHPV types were detected in 4.7% of women at the urban site and 3.4% of women at the rural sites.

The majority of women (5416/6035, 89.7%) had no cytological abnormalities at study entry; 10.3% had cytological abnormalities, including 5.8% (349/6035) with ASC-US, 3.4% (207/6035) with LSIL, 0.8% (46/6035) with HSIL, 0.2% (12/6035) with ASC-H and 0.1% (5/6035) with AGC (Table 2). The frequency of women who were positive for hrHPV DNA increased with increasing severity of cytological abnormalities: 10.3% in women with normal cytology, 39.3% in ASC-US, 85.0% in LSIL and 97.8% in HSIL. The most frequently detected hrHPV type in HSIL specimens was HPV-16 (63.0%, 95% CI: 47.2, 76.4). Other frequently detected (>10%) hrHPV types were HPV-18 (17.4%), HPV-52 (17.4%), HPV-33 (15.2%), HPV-58 (15.2%) and HPV-51 (10.9%).

Cervical intraepithelial neoplasia (CIN) 1+ was diagnosed in 1.4% (82/6035) of women and CIN2+ in 1.0% (62/6035) women (Table 2). All women diagnosed with a histological abnormality were HPV DNA positive for at least one hrHPV type. The most prevalent HPV type in women with a CIN2+ lesion was HPV-16 (66.1%, 95% CI: 52.7, 77.4). Other frequently detected (>10%) hrHPV types were HPV-33 (16.1%), HPV-52 (16.1%), HPV-58 (14.5%) and HPV-51 (11.3%). HPV-18 DNA was detected in 8.1% of women with a CIN2+ lesion.

Overall, 31.5% of participants had evidence of exposure to HPV-16 (*i.e.*, HPV DNA positive and/or anti-HPV-16 seropositive) and 16.7% had evidence of exposure to HPV-18 (Table 4). Regardless of baseline HPV DNA status, 30.5% of participants were initially seropositive for anti-HPV-16 antibodies and 16.0% were initially seropositive for anti-HPV-18 antibodies (Table 4).

When results were analysed by age, the rates of hrHPV DNA positivity, and anti-HPV-16 and anti-HPV-18 seropositivity, were similar across the age range of 18–25 years, and in the small number of subjects aged >25 years (Fig. 1).

Discussion

This prospective study in over 6,000 healthy mainland Chinese women aged 18–25 years is the largest HPV vaccine efficacy study conducted to date in China. The reported baseline data from this study provide information on HPV prevalence and type distribution in China, which will be useful for assessing the potential future impact of HPV vaccination and other control programmes (*e.g.*, cervical screening) in the country.

Overall baseline HPV prevalence (19%) in this study conducted in Jiangsu Province was similar to HPV prevalence estimated from population-based studies in Shenyang City (17%), Shenzhen City (18%) and Shanxi Province (15%),[9–11] but was much higher than that observed in Beijing (7%),[12] suggesting some heterogeneity in HPV prevalence across China. It is noteworthy that the Beijing study used a less sensitive PCR (MY09/11 consensus primers),[13] which could at least partially explain the difference observed. The observed HPV prevalence rate in our study was also similar to that found in the general population in areas with high incidence rates of cervical cancer in areas of Latin America and India,[14–17] although lower than in some high-risk areas in sub-Saharan Africa (26%),[18] and in the Guanacaste trial

Table 2. HPV DNA type distribution in cervical samples, and cytological and histological status at study entry (total vaccinated cohort)

			Cytological status												Histological status			
	Total $N = 6{,}035$[1]		Normal $N = 5{,}416$		ASC-US $N = 349$		LSIL $N = 207$		HSIL $N = 46$		ASC-H $N = 12$		AGC $N = 5$		CIN1+ $N = 82$		CIN2+ $N = 62$	
HPV type	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
High-risk HPV																		
HPV-16	222	3.7	112	2.1	33	9.5	43	20.8	29	63.0	2	16.7	3	60.0	48	58.5	41	66.1
HPV-18	74	1.2	38	0.7	11	3.2	15	7.2	8	17.4	0	0.0	2	40.0	7	8.5	5	8.1
HPV-31	59	1.0	36	0.7	13	3.7	5	2.4	4	8.7	1	8.3	0	0.0	3	3.7	3	4.8
HPV-33	65	1.1	27	0.5	9	2.6	21	10.1	7	15.2	1	8.3	0	0.0	15	18.3	10	16.1
HPV-35	31	0.5	16	0.3	4	1.1	8	3.9	3	6.5	0	0.0	0	0.0	6	7.3	3	4.8
HPV-39	81	1.3	48	0.9	16	4.6	15	7.2	2	4.3	0	0.0	0	0.0	7	8.5	5	8.1
HPV-45	33	0.5	24	0.4	1	0.3	7	3.4	1	2.2	0	0.0	0	0.0	2	2.4	0	0.0
HPV-51	102	1.7	65	1.2	11	3.2	21	10.1	5	10.9	0	0.0	0	0.0	8	9.8	7	11.3
HPV-52	243	4.0	166	3.1	31	8.9	35	16.9	8	17.4	0	0.0	3	60.0	18	22.0	10	16.1
HPV-56	69	1.1	28	0.5	9	2.6	28	13.5	4	8.7	0	0.0	0	0.0	6	7.3	3	4.8
HPV-58	90	1.5	40	0.7	14	4.0	28	13.5	7	15.2	1	8.3	0	0.0	13	15.9	9	14.5
HPV-59	22	0.4	14	0.3	1	0.3	7	3.4	0	0.0	0	0.0	0	0.0	3	3.7	1	1.6
HPV-66	78	1.3	45	0.8	10	2.9	20	9.7	3	6.5	0	0.0	0	0.0	4	4.9	2	3.2
HPV-68	56	0.9	39	0.7	8	2.3	7	3.4	2	4.3	0	0.0	0	0.0	4	4.9	4	6.5
Any high-risk HPV[2]	924	15.3	558	10.3	137	39.3	176	85.0	45	97.8	4	33.3	4	80.0	82	100	62	100
Low-risk HPV																		
HPV-6	31	0.5	18	0.3	6	1.7	6	2.9	1	2.2	0	0.0	0	0.0	2	2.4	2	3.2
HPV-11	32	0.5	26	0.5	2	0.6	4	1.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV-34	18	0.3	12	0.2	2	0.6	4	1.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV-40	15	0.2	8	0.1	3	0.9	4	1.9	0	0.0	0	0.0	0	0.0	1	1.2	0	0.0
HPV-42	7	0.1	5	0.1	2	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV-43	35	0.6	26	0.5	6	1.7	3	1.4	0	0.0	0	0.0	0	0.0	1	1.2	1	1.6
HPV-44	26	0.4	22	0.4	2	0.6	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV-53	88	1.5	63	1.2	13	3.7	11	5.3	1	2.2	0	0.0	0	0.0	3	3.7	2	3.2
HPV-54	65	1.1	59	1.1	6	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV-70	10	0.2	8	0.1	0	0.0	2	1.0	0	0.0	0	0.0	0	0.0	1	1.2	1	1.6
HPV-74	25	0.4	21	0.4	3	0.9	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Any low-risk HPV[2]	324	5.4	246	4.5	40	11.5	36	17.4	2	4.3	0	0.0	0	0.0	8	9.8	6	9.7
Any HPV[2]	**1119**	**18.5**	**723**	**13.3**	**156**	**44.7**	**187**	**90.3**	**45**	**97.8**	**4**	**33.3**	**4**	**80.0**	**82**	**100**	**62**	**100**

[1]Baseline data were not available for 16 participants due to unsatisfactory cervical samples.
[2]The sum of the percentages for each individual HPV type does not equal the overall total for "any HPV type," as some women had more than one HPV type detected.
AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; CIN1+, cervical intraepithelial neoplasia grade 1 or higher; CIN2+, cervical intraepithelial neoplasia grade 2 or higher; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; *N*, number of subjects with available results; *n*, number of subjects in given category; %, $n/N \times 100$.

conducted in Costa Rica (50%), which used the same HPV DNA detection methodology.[19] The overall rate of hrHPV DNA positivity appeared to be slightly higher for women in an urban setting than a rural setting, in accordance with a pooled analysis of population-based studies in China.[20]

The prevalence of hrHPV types in our study is similar to other studies conducted in China, in which HPV-16, HPV-52 and HPV-58 are generally among the most frequently identified genotypes, although previous studies have usually placed HPV-16 as the most common type in the general population.[9–12] In the large global trial PATRICIA, HPV-16, HPV-18 and HPV-31 were the most frequently identified hrHPV types.[21] The variation in ranking of HPV types across different studies could be due to differences in demographics of

the population studied, source of DNA samples, or laboratory methodologies. As reported previously, HPV-52 and HPV-58 appear to be more predominant in China[9–12,22,23] and other East Asian countries[24,25] than in non-Asian populations.[26] Approximately one-quarter of women with an hrHPV infection in the present study had more than one hrHPV type detected, consistent with other population-based studies conducted in China in which multiple infections accounted for 26–38% of all infections.[9–12]

Table 3. Number (%) of hrHPV types in cervical samples at study entry in the total vaccinated cohort and in hrHPV positive women

	n	Total vaccinated cohort %	hrHPV positive women %
All regions		***N* = 6,035**[1]	***N* = 924**
Any hrHPV	924	15.3	100
1 type	699	11.6	75.6
Multiple types	225	3.7	24.4
2 types	171	2.8	18.5
3 types	38	0.6	4.1
4 types	11	0.2	1.2
5 types	4	0.1	0.4
6 types	1	0	0.1
Urban[2]		***N* = 1,683**	***N* = 301**
Any hrHPV	301	17.9	100
1 type	222	13.2	73.8
Multiple types	79	4.7	26.2
Rural[3]		***N* = 4352**	***N* = 623**
Any hrHPV	623	14.3	100
1 type	477	11.0	76.6
Multiple types	146	3.4	23.4

[1]Baseline data were not available for 16 participants due to unsatisfactory cervical samples.
[2]Subjects enrolled at Xuzhou CDC.
[3]Subjects enrolled at Binhai CDC, Jintan CDC and Lianshui CDC.
hrHPV, high-risk human papillomavirus DNA positive; *N*, number of subjects with available results; *n*, number of subjects in given category; %, $n/N \times 100$.

The observed incidence of HSIL cytology (0.8%) was slightly lower than the range of 1–4% reported previously in studies conducted in China.[2] This might be explained by the fact that a younger population of women, aged 18–25 years, was evaluated in the present study compared with previous population-based studies in China, in which women ranged in age from 15 to 59 years.[2] However, the prevalence of HSIL appeared higher than that reported in the large global trial PATRICIA (0.3%) in women of a similar age range (15–25 years).[21] HPV DNA was detected in all but one of the women with HSIL cytology in the present study (98%). This rate is higher than previous reports, in which overall HPV prevalence in HSIL was estimated at 77% from a meta-analysis of Chinese studies[22] and 85% from a meta-analysis of worldwide studies.[27] We attribute the higher incidence in our study to the source of DNA samples (exfoliated cervical cells), the universal use of the highly sensitive SPF_{10} PCR-DEIA-$LiPA_{25}$ detection system, and the central cytohistological review procedure. In contrast, previous studies included in meta-analyses used various DNA sample sources (fresh or fixed tissue) and PCR testing protocols, and cytohistological diagnoses were not standardised.[27]

The distribution of HPV in the overall population does not represent the distribution in women with cervical lesions, due to the fact that some HPV types progress more easily than others. It is well documented that HPV-16 and HPV-18 have a propensity for persistence and progression to cervical lesions compared with some other hrHPV types,[28] and that the relative prevalence of these two genotypes increases

Table 4. Serological status by HPV DNA status at study entry (total vaccinated cohort)

HPV type	HPV DNA status (from cervical sample)	Antibody status	Total *N* = 6,035[1] *n*	Total *N* = 6,035[1] %
HPV-16	DNA negative	Seronegative	4,132	68.5
		Seropositive	1,681	27.9
	DNA positive	Seronegative	62	1.0
		Seropositive	160	2.7
HPV-18	DNA negative	Seronegative	5,027	83.3
		Seropositive	932	15.4
	DNA positive	Seronegative	39	0.6
		Seropositive	35	0.6
		Missing serology	2	–

[1]Baseline data were not available for 16 participants due to unsatisfactory cervical samples.
N, number of subjects with available results; *n*, number of subjects in given category; %, $n/N \times 100$.

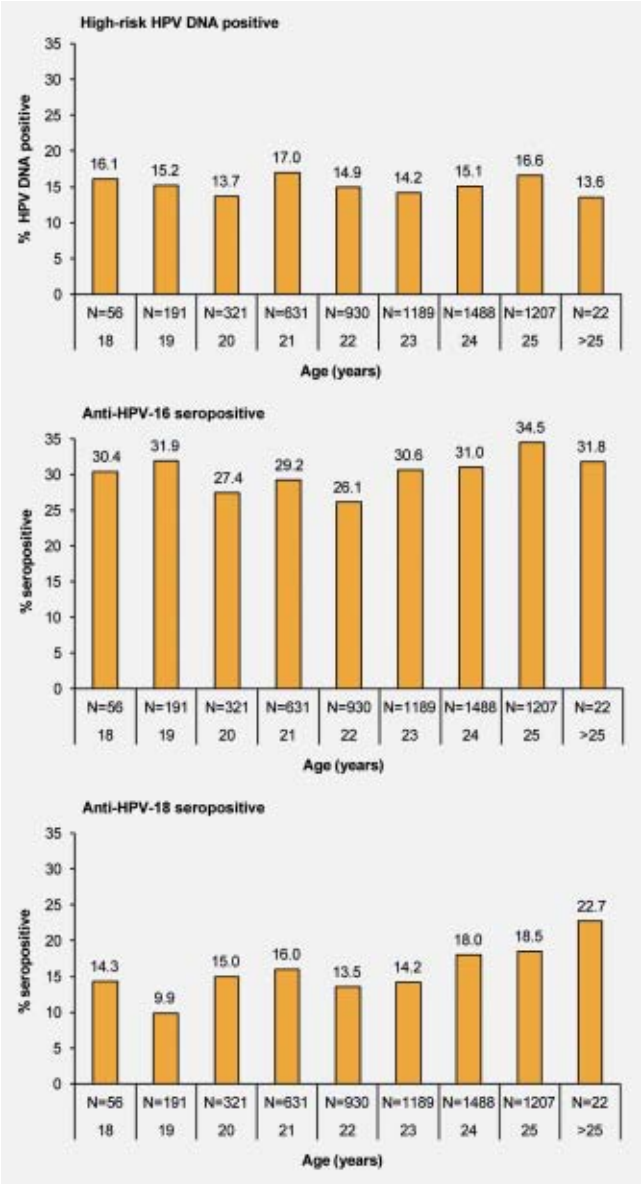

Figure 1. High-risk HPV DNA genotype status and serostatus at study entry by age (total vaccinated cohort). *N*, number of women in each age group. Numbers above each bar are the percentage of women in each category. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with increasing severity of cervical lesions.[27] We also observed this, with both HPV-16 and HPV-18 DNA positivity rates increasing more than five-fold from ASC-US through to HSIL.

We used abnormal cytology as the primary screen for colposcopy referral, with HC2 test positivity used as triage for referral of ASC-US women. This may, in part, explain the lower prevalence of CIN2+ in our study compared with a meta-analysis of other Chinese studies.[20] However, the younger age of our population compared with other studies is the most likely explanation for this lower incidence. The detection of hrHPV DNA in all women with CIN2+ in our study, and the predominance of HPV-16 in these women, is concordant with a large epidemiological study conducted in mainland China by Chen *et al.*,[29] which also used the SPF_{10} PCR-DEIA-$LiPA_{25}$ system. Other common HPV types in CIN2+ were also similar in the present study (HPV-52, -33, -58, -51, -18 and -39) and the study conducted by Chen *et al.* (HPV-58, -33, -52, -31 and -18), although relative rankings and proportions differed.[29]

The prevalence of hrHPV infection was similar across the age range of 18–25 years in our study. This might be explained by the fact that all participants were sexually active (virgins were not enrolled due to cultural and ethical considerations) and were, therefore, at risk of HPV infection. The official minimum age for marriage for women is 20 years in China, but social norms are changing and a substantial proportion of women commences sexual activity before this age. We did not evaluate risk factors for HPV infection, but in a previous epidemiological study conducted in Jiangsu Province, the number of lifetime sexual partners, husbands' ex-marital sexual relationships and multiple pregnancies were associated with an increased risk of HPV infection.[30] A recent cross-sectional epidemiologic survey showed a trend toward earlier sexual debut and riskier sexual behaviours in younger age groups of Chinese women, with the median age of sexual debut being 17 years.[31] These findings suggest that early implementation of HPV vaccination in young adolescent girls before completion of their 9-year compulsory education, will better contribute to the prevention of HPV infection and cervical cancer in China.

The strength of this study was the large sample size of over 6,000 subjects. Another asset was that all cytological and biopsy samples were evaluated at a central laboratory in China ensuring consistency in diagnoses. Additionally, standardised testing of cervical samples for HPV DNA using the SPF_{10} PCR-DEIA-$LiPA_{25}$ detection system allowed accurate genotyping of a broad spectrum of HPV types. Type-specific PCRs were also used for HPV-16 and HPV-18. This testing algorithm offered particularly high analytical sensitivity for HPV-16 and HPV-18 genotypes,[7] although it is known that broad-spectrum PCR detection systems, such as SPF_{10} PCR-DEIA-$LiPA_{25}$, can result in decreased sensitivity for some less prevalent HPV types due to competition in cases of multiple HPV infection.[7,32] A limitation was that the trial was conducted in one region of China (Jiangsu Province) and HPV prevalence and distribution data may not be representative of mainland China as a whole. Another potential limitation is that all subjects enrolled in this trial were sexually active and generally healthy at study entry, which might not fully represent the general population of this age range.

In conclusion, our study showed a high baseline seropositivity rate and intermediate prevalence of cervical hrHPV types in healthy Chinese women aged 18–25 years in Jiangsu Province. Early prophylactic vaccination, before sexual debut,

has the potential to substantially reduce the incidence of cervical cancer and precancer in China.

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