Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Prevalence, Incidence, Clearance, and Associated Factors of Genital Human Papillomavirus Infection among Men: A Population-Based Cohort Study in Rural China

Mengfei Liu, Zhonghu He, Chanyuan Zhang, Fangfang Liu, Ying Liu, Jingjing Li, Zhongyao Xu, Qiyan Wang, Dong Hang, Na Shen, Yaqi Pan, Chuanhai Guo, Hong Cai, and Yang Ke

Abstract

Background: The natural history of human papillomavirus (HPV) infection in men on a population base has rarely been studied in general, particularly among Chinese men.

Methods: A total of 1,286 men ages 25 to 65 years from rural China were enrolled during 2009–2010 and their genital HPV infection status was assessed biannually for up to seven visits using PCR and sequencing methods. Prevalence analysis was performed among men with at least one valid HPV result ($N = 1,279$) and men with at least two consecutive HPV results ($N = 1,059$) were included in incidence and clearance analyses (median follow-up time, 31.8 months; interquartile range, 15.4–37.9 months).

Results: The prevalence and incidence of any HPV type, oncogenic, and nononcogenic HPV were 17.8%, 6.4%, 12.4%, and 14.6, 4.9, 10.8 per 1,000 person months, respectively. The median duration of infection with any HPV type, oncogenic, and nononcogenic HPV was 11.5, 6.8, and 11.5 months, respectively. The number of lifetime sexual partners was consistently associated with increased risk of prevalent and incident infection of HPV. Men ages 25 to 50 years had a higher incidence and longer duration of HPV infection than older men (51–65 years).

Conclusions and Impact: This epidemiologic investigation provides basic information of genital HPV infection among the Chinese male population; these data are crucial for the consideration of primary strategies against HPV-related carcinoma in the Chinese male and female population. *Cancer Epidemiol Biomarkers Prev; 23(12); 2857–65. ©2014 AACR.*

Introduction

Human papillomavirus (HPV) infection has been demonstrated to be the necessary cause of cervical cancer in women, and this virus also plays an important role in anogenital and oropharyngeal cancers (1). In the past decade, genital HPV infection in men has also brought about growing concern, not only because it may lead to benign or malignant pathologic changes in the external male genitalia, but also because of the fact that men may act as a viral reservoir and significantly affect HPV infection and HPV disease risk in women (2–5).

To rationally implement screening and immunologic intervention for HPV-related diseases, the natural history of HPV infection must be carefully investigated. Natural history studies in women have played a crucial role in developing vaccine-based HPV intervention programs for women (6–12). However, our knowledge about HPV infection in men is relatively inadequate at this time. To date, most of published data are based on volunteers, university students or soldiers, sexually transmitted disease clinic patients, men who have sex with men, HIV-infected men, or men from a relatively narrow age range (13–19). Thus, such studies have limited representation of the general population. Moreover, most available data are from North America and Europe and little data are available for Asian male populations, particularly for China.

In the current study, we established a cohort of rural Chinese men on the basis of a large-scale esophageal cancer cohort study (20), and followed up their genital HPV status for seven biannual visits. The aim of this study is to investigate the type-specific prevalence, incidence, and clearance of HPV infection in men from rural China, and to evaluate potential risk factors.

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Laboratory of Genetics, Peking University Cancer Hospital & Institute, Beijing, P.R. China.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

M. Liu and Z. He contributed equally to this article.

Corresponding Authors: Yang Ke, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Laboratory of Genetics, Peking University Cancer Hospital & Institute, No. 52 Fucheng Road, Haidian District, Beijing 100142, P.R. China. Phone: 86-10-88196762; Fax: 86-10-88196735; E-mail: keyang@bjmu.edu.cn; and Hong Cai, E-mail: drhcai@gmail.com

doi: 10.1158/1055-9965.EPI-14-0365

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

Materials and Methods

Study subjects

From 2007 to 2009, a population-based esophageal cancer cohort study was initiated in rural Anyang, China (Anyang Esophageal Cancer Cohort Study, AECCS; ref. 20). The current study uses a subcohort of the original study consisting of three target villages and the baseline investigation was started concurrently with the second section of the AECCS. The eligibility criteria for this study were as follows: permanent residency in the target villages; age, 25 to 65 years; no history of cancer, cardiovascular disease or mental disorder; no history of infection with Hepatitis B Virus, Hepatitis C Virus, or Human Immunodeficiency Virus; and willingness to cooperate with follow-up, and ability to provide informed consent. All eligible male cohort members were enrolled during 2009–2010 and were assessed biannually for up to seven visits.

Research protocols and materials were approved by the Institutional Review Board of the Peking University School of Oncology, China. All participants in this study provided written informed consent.

Specimen and data collection

At each evaluation, exfoliated cells from the penile shaft, glans penis, coronal sulcus, and scrotum of all subjects were collected by an experienced doctor using saline-soaked swabs as described previously (21). Cells adherent to the swab were then rinsed in 0.9% saline solution, and centrifuged at 5,000 rpm for 5 minutes. Supernatants were then discarded. All specimens were stored at −20°C and subsequently transported to our laboratory in Beijing and stored in ultralow temperature freezers (−70°C) pending DNA extraction and HPV detection. A computer-aided questionnaire was completed by all cohort members during the baseline investigation to obtain the demographic data, personal information, and potential risk factors for HPV infection.

Laboratory procedure

As described elsewhere (21), DNA was extracted using the Biomek 3000 Automated Workstation (Beckman Coulter) and then tested with Polymerase Chain Reaction (PCR) for the β-globin gene for quality evaluation. β-Globin–positive specimens were subsequently tested for HPV using PCR-based direct sequencing with a pair of SPF1/GP6^{+} primers that amplified an approximately 184-bp fragment of the L1 gene (22). The ABI 3730XL with BigDye 3.1 reagent (Applied Biosystems) was used for the Sanger sequencing–based genotyping procedure to evaluate the HPV types in this population, which was also used in previous studies (21, 23, 24). Samples with ambiguous HPV typing signals were subjected to further cloning and sequencing for multiple infections. The HPV types that were classified as oncogenic in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (25, 26). The nononcogenic types were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 67, 66, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

Statistical analysis

HPV prevalence was defined as the percentage of HPV-positive visits out of all the counted visits during the study period in participants with adequate HPV test results. Positive for any HPV type, oncogenic HPV or nononcogenic HPV was defined as being positive for at least one of any type HPV, 13 oncogenic HPV and 38 nononcogenic HPV, respectively. Prevalence estimates along with 95% confidence intervals (CI) were estimated using a null linear regression model implemented with the Generalized Estimating Equation (GEE) with a robust sandwich estimator of covariance to adjust for repeat measurements (27).

Participants were treated as the calculating unit in the incidence analysis and only the participants who were negative for a specific type of HPV at enrollment were included. Incident infections of any HPV type, oncogenic and nononcogenic HPV were defined as the first incident event of any type of HPV, 13 oncogenic HPV and 38 nononcogenic HPV, respectively. Incident infection was assumed to occur at the midpoint of the interval before the first HPV-positive visit. Person-time was calculated from enrollment to the first incident event and participants without HPV infection were censored at their last visits. Incidence rates were then calculated by dividing the number of transmission events by the total number of person-months and the 95% CIs were estimated on the basis of Poisson distribution (28). Kaplan–Meier method was used to estimate cumulative risk of incident infection of any HPV type, oncogenic HPV or nononcogenic HPV, and log-rank test was used to compare incidence between groups.

Type-specific infections were treated as the calculation unit in the clearance analysis. HPV clearance was defined as two sequential negative results and the clearance event was assumed to occur at the midinterval following the last HPV-positive visit. Clearance analysis was restricted in incident cases while newly acquired infections identified at a participant's last visit were not included. Median time to clearance and 95% CIs were estimated for groups of HPV using the clustered Kaplan–Meier method (29), and the Wei Lin Weissfeld (WLW) marginal model approach was used to assess the statistical significance between HPV groups (30).

For the risk factor assessment, GEE logistic regression models, Cox regression models, and WLW Cox regression models were used for HPV prevalence, incidence, and clearance analysis, respectively. Backward-selection method with a significance threshold of 0.1 was used to identify the variables included in the final multivariate models. Candidate variables included age at enrollment, marital status, type of job, education level, current smoking status, lifetime number of female sexual partners, bathing frequency in the winter, and washing external

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

genitalia before sex. Age, education level, and type of job were included in all the multivariate models as design factors.

Single missing visits (12% of total visits) were imputed using "prior observation carried backward" approach.

Statistical analyses were performed using STATA version 11.2 (STATA Corporation) and SAS version 9.2 (SAS Institute Inc.). Tests were two sided, and had a significance level of 0.05.

Results

A total of 1,286 participants participated in at least one visit of the study (Supplementary Table S1). Human β-globin gene was positive in 94.4% (5,439/5,761 visits) of specimens from 1,279 (99.5%) participants who were therefore considered as adequate for HPV DNA evaluation.

As shown in Table 1, the median age of the 1,279 β-globin–positive participants was 43 years (interquartile

Table 1. Selected demographic and behavior variables for genital HPV infection among men from rural China included in the prevalence analysis, included in the incidence analysis, and not included in the incidence analysis, 2009–2013

Variable	Participants included in the prevalence analysis[a] *n* (%)	Participants included in the incidence analysis[b] *n* (%)	Participants not included in the incidence analysis *n* (%)	*P*[c]
	N = 1,279	*N* = 1,059	*N* = 220	
Follow-up time (mo)				
Median (IQR)	25.3 (7.6–37.2)	31.8 (15.4–37.9)		
Mean (SD)	22.4 (14.9)	27.0 (11.8)		
Age at enrollment (y)				
Median (IQR)	43 (36–54)	44 (37–55)	36 (29.5–43)	
25–50	888 (69.4)	697 (65.8)	191 (86.8)	
51–65	391 (30.6)	362 (34.2)	29 (13.2)	<0.001
Marital status				
Not married	66 (5.2)	56 (5.3)	10 (4.6)	
Married	1,213 (94.8)	1,003 (94.7)	210 (95.4)	0.651
Type of job				
Farming	486 (38.0)	441 (41.6)	45 (20.5)	
Work in local area	374 (29.2)	301 (28.4)	73 (33.2)	
Work outside	419 (32.8)	317 (30.0)	138 (46.3)	<0.001
Education level				
Primary school or below	384 (30.0)	335 (31.6)	49 (22.3)	
Junior middle School	690 (53.9)	570 (53.8)	120 (54.5)	
Senior middle school or above	157 (12.3)	121 (11.4)	36 (16.3)	
Unknown[d]	48 (3.8)	33 (3.1)	15 (6.8)	0.001
No. of lifetime sexual partners				
0–1	1,120 (87.6)	934 (88.2)	186 (84.6)	
≥2	159 (12.4)	125 (11.8)	34 (15.4)	0.135
Current smoker				
No	561 (43.9)	463 (43.7)	98 (44.6)	
Yes	718 (56.1)	596 (56.3)	122 (55.4)	0.823
Bath frequency in winter (d)				
1–7	459 (35.9)	354 (33.4)	105 (47.7)	
8–30	630 (49.3)	531 (50.1)	99 (45.0)	
>30	190 (14.8)	174 (16.5)	16 (7.3)	<0.001
Wash genitalia before sex				
No	990 (77.4)	839 (79.2)	151 (68.6)	
Yes	289 (22.6)	220 (20.8)	69 (31.4)	0.001

Abbreviations: IQR, interquartile range; SD, standard deviation.

[a]Participants having valid HPV results for at least one visit were included in the prevalence analysis.

[b]Participants having valid HPV results for at least two consecutive visits were included in the incidence analysis.

[c]χ^2 test was used to compare between participants included in the incidence analysis and not included.

[d]The unknown category was not included in the χ^2 test.

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

range, 36–54 years). Most of these individuals were married and had an education level of junior middle school or below. Participant job categories were distributed so that one-third of the participants held each of three types of jobs. About behavior factors, 1,120 of 1,279 (87.6%) participants reported having 0 or 1 lifetime female sexual partners, 718 (56.1%) were current smokers, 630 (49.3%) reported having bathed every 8 to 30 days in winter, and 990 (77.4%) reported not washing genitalia before sex. Of these 1,279 men, 1,059 (82.8%) completed at least two consecutive visits in the study and were included in incidence analysis with a median follow-up time of 31.8 months (interquartile range, 15.4–37.9). The 220 men excluded from the incidence analysis were younger and more likely to work outside the local area, had received a higher level of education, and had better personal hygienic habits than the subjects included in incidence analysis (Table 1).

The overall prevalence of any HPV infection was 17.8% (1,099/6,182 visits), and 114 out of 1,099 positive visits (10.4%) had multiple infections (Supplementary Table S1). Prevalence of oncogenic and nononcogenic HPV infection was 6.4% (393/6,182 visits) and 12.4% (765/6,182 visits). Oncogenic HPV types with the highest prevalence were HPV-16, -18, and -58. Nononcogenic HPV types with the highest prevalence were HPV-3, -57, and -54 (Table 2). As compared with older men (51–65 years), younger men (25–50 years) had a significantly higher prevalence of any type, oncogenic and nononcogenic HPV infection (data not shown).

The incidence of any HPV type, oncogenic, and nononcogenic HPV infection was 14.6, 4.9, and 10.8 per 1,000 person-months, respectively (Table 2). Oncogenic HPV types with the highest incidence were HPV-16, -18, and -58. Nononcogenic HPV types with the highest incidence were HPV-3, -57, and -54. Incidence of nononcogenic HPV was significantly higher than oncogenic type HPV (Fig. 1). Younger men were at significantly higher risk for incident HPV infection for both any and nononcogenic HPV types (Fig. 1).

Median time to clearance of any, oncogenic, or nononcogenic HPV infection was 11.5, 6.8, and 11.5 months, respectively (Table 2). Of note, 55.5%, 57.3%, and 54.8% of any, oncogenic, and nononcogenic HPV infections were cleared 12 months after new acquisition of infection, and 78.5%, 84.0%, and 74.8% were cleared at 24 months, respectively. Half of HPV-16 and HPV-18 infections were cleared in approximately 6 months. Nononcogenic HPV types seemed to persist longer than oncogenic HPV types, but this difference did not reach a significant level ($P = 0.106$; Fig. 1). Younger men cleared infections significantly slower than older men in any and oncogenic HPV types (Fig. 1).

In the multivariate analyses, the number of lifetime female sexual partners was consistently associated with an increased risk of HPV infection. The risk of acquisition of both any and nononcogenic HPV infection was significantly increased in current smokers. Men who bathed infrequently in winter had a reduced risk of HPV infections in prevalent infection analysis, and also had a reduced risk of incident oncogenic HPV infection. Older age (51–65 years) was associated with a significantly lower risk of incident nononcogenic HPV infection and shorter duration of oncogenic HPV infection (Table 3).

Discussion

In this population-based study, we investigated the natural history of genital HPV infection in more than 1,000 men from rural China. Results of the study expanded our knowledge of the natural history of HPV infection in men. To our knowledge, this is the first report from China on this topic. As compared with previous studies, the population-based sampling frame and high response proportion in AECCS would maximally decrease the selection bias (20), and the conclusions of this study could thus be generalized to a broader rural population in China.

HPV prevalence and incidence for the male genitalia have been reported in some prospective cohort studies adopted relatively long (>12 months) and fixed follow-up design (4–6 months) in Western countries (17–19). For the oncogenic types, the prevalence estimates ranged from 16.6% to 30.0% (17, 18), and incidence estimates ranged from 15.5 to 24.7 per 1,000 person-months (18, 19). In this study, we found a much lower prevalence (6.4%) and also a lower incidence (4.9 per 1,000 person-months) for oncogenic HPV infection in Chinese men. Our previous cross-sectional investigation in the very same population found a similar but slightly lower prevalence estimate (6.1%) and the most common oncogenic types were HPV-16, HPV-18, and HPV-58 in both the studies (21). An international study also reported a lower prevalence of oncogenic HPV infection among men from the Asia-Pacific region (31). Differences among races, sampling methods, and the considerable more conservative sexual behavior in the population we studied may account for the low HPV prevalence and incidence in oncogenic types. For nononcogenic types, exploratory study had shown that a broad distribution of nononcogenic HPV types at the male genital region could not be genotyped by linear array, which was used in almost all prior studies (32). And this had largely limited the comparability between our results and the others'. On the basis of our result, we could infer that nononcogenic HPVs existed extensively at the male external genital site in this population.

We found that the median time to clearance of oncogenic HPV was 6.8 months. The U.S. men study reported that median time for clearance of oncogenic HPV infections was 5.8 months (18). In the HPV in Men (HIM) study, the corresponding median time was 7.2 months (17). These findings were consistent with our current recognition that most HPV infections in males are transient and would be cleared quickly. For type-specific clearance, HPV-16 cleared more rapidly in our population as compared with the HIM study (6.6 months vs. 12.2 months). However, the U.S. men study reported a median duration for HPV-16 (6.0 months), which is much closer to our study. Difference in clearance duration may be the result

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

Table 2. Prevalence, incidence, and clearance of HPV infection among men from rural China, 2009–2013[a]

HPV type	Prevalent cases $N = 1,279$	Prevalence, 95% CI[b] (%) $N = 6,182$	Incident cases[c] $N = 1,059$	Incidence rate, 95% CI[d] (per 1,000 person-months)	Newly acquired infections[e]	Cleared infections[f]	Median time to clearance, 95% CI[g] (mo)
Any	526	17.8 (16.2–19.3)	291	14.6 (13.1–16.4)	359	207	11.5 (6.5–12.4)[h]
Oncogenic[i]	218	6.4 (5.4–7.3)	123	4.9 (4.1–5.8)	115	76	6.8 (6.4–12.6)[h]
HPV-16	105	2.7 (2.1–3.3)	59	2.2 (1.7–2.9)	43	31	6.6 (6.2–12.4)
HPV-18	43	1.0 (0.7–1.3)	26	0.9 (0.6–1.4)	22	20	6.6 (6.0–6.8)
HPV-58	34	1.0 (0.6–1.3)	23	0.8 (0.6–1.2)	16	14	6.4 (5.9–12.6)
HPV-45	19	0.5 (0.2–0.6)	11	0.4 (0.2–0.7)	9	3	NE
HPV-35	13	0.5 (0.2–0.7)	7	0.3 (0.1–0.5)	6	2	NE
HPV-33	7	0.2 (0.0–0.4)	6	0.2 (0.1–0.5)	6	1	NE
HPV-52	8	0.2 (0.0–0.3)	6	0.2 (0.1–0.5)	4	2	18.9 (7.3–NE)
HPV-68	9	0.4 (0.1–0.7)	5	0.2 (0.1–0.4)	4	1	NE
HPV-56	6	0.1 (0.0–0.2)	3	0.1 (0.0–0.3)	2	1	11.7 (NE–NE)
Nononcogenic[j]	404	12.4 (11.1–13.6)	239	10.8 (9.6–12.3)	244	131	11.5 (6.5–12.6)[h]
HPV-3	136	3.3 (2.7–4.0)	88	3.3 (2.7–4.1)	61	33	11.6 (6.6–12.8)
HPV-57	76	1.9 (1.4–2.3)	58	2.1 (1.6–2.7)	41	23	11.9 (6.6–18.5)
HPV-54	46	1.3 (0.8–1.7)	29	1.0 (0.7–1.5)	20	11	6.6 (6.5–NE)
HPV-90	33	0.9 (0.5–1.3)	21	0.8 (0.5–1.2)	17	10	12.0 (6.6–12.4)
HPV-87	30	0.8 (0.5–1.2)	20	0.7 (0.5–1.1)	16	13	6.5 (5.8–11.7)
HPV-67	24	0.6 (0.3–0.8)	16	0.6 (0.4–0.9)	14	11	6.6 (5.9–11.5)
HPV-81	26	0.7 (0.4–1.0)	13	0.5 (0.3–0.8)	7	4	6.6 (5.8-NE)
HPV-91	14	0.4 (0.2–0.6)	8	0.3 (0.1–0.6)	7	3	12.8 (6.5–NE)
HPV-94	11	0.2 (0.1–0.4)	8	0.3 (0.1–0.6)	7	2	NE
HPV-43	15	0.4 (0.2–0.6)	8	0.3 (0.1–0.6)	7	3	NE
HPV-11	11	0.2 (0.1–0.4)	8	0.3 (0.1–0.6)	5	2	11.7 (6.6–NE)
HPV-30	10	0.3 (0.1–0.6)	6	0.2 (0.1–0.5)	5	3	13.2 (6.6-NE)
HPV-10	13	0.4 (0.2–0.6)	6	0.2 (0.1–0.5)	4	0	NE
HPV-6	7	0.2 (0.0–0.3)	5	0.2 (0.1–0.4)	4	2	6.6 (5.8–NE)
HPV-27	11	0.2 (0.1–0.4)	5	0.2 (0.1–0.4)	0	0	NE
HPV-84	6	0.1 (0.0–0.2)	4	0.1 (0.1–0.4)	4	1	6.7 (6.7–NE)
HPV-75	7	0.2 (0.0–0.3)	4	0.1 (0.1–0.4)	4	1	NE
HPV-70	7	0.3 (0.0–0.5)	4	0.1 (0.1–0.4)	3	1	6.6 (6.6–NE)

Abbreviation: NE, not estimable because too few cases.

[a]Only HPV types with >5 prevalent cases were listed.

[b]Prevalence was defined as the percentage of HPV-positive visits out of all counted visits during the study period in participants with adequate HPV test results ($n = 6,182$). 95% CIs were adjusted for correlations of repeat measurements.

[c]Incident cases were defined as the first HPV infection for a specific HPV type in one participant ($n = 1,059$).

[d]Incidence rate and 95% CIs were calculated on the basis of the number of events modeled as a Poisson variable for the total person-months.

[e]Only incident infections were included. Multi-type incident infections in one participant were judged as different newly acquired infections. Incident infections detected at a participant's last visit were not included for clearance analysis.

[f]Clearance was defined as two consecutive negative results after testing HPV positive for a specific type.

[g]Median time to clearance and 95% CIs were estimated using the Kaplan–Meier method.

[h]95% CIs were adjusted for possible within subject correlations.

[i]Oncogenic types in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

[j]Nononcogenic types in this study were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 66, 67, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

of both demographic and behavioral factors across populations and this requires further evaluation with large studies. However, this discrepancy should be interpreted with caution as there would be a lack of variation in the estimates of median duration and they would be very close to the integer multiples of the follow-up intervals

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

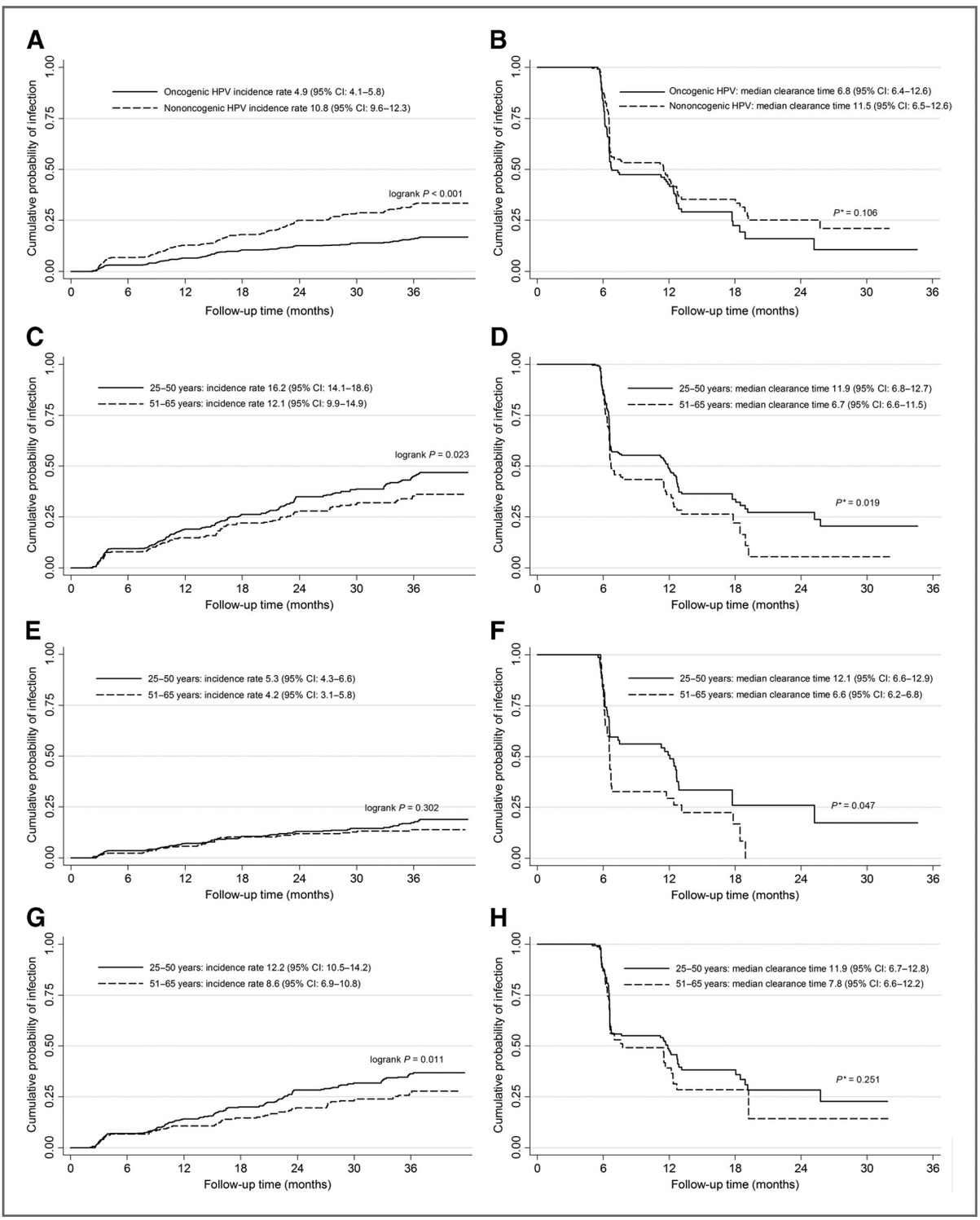

Figure 1. Kaplan–Meier estimates of the incidence (per 1,000 person-months) and time to clearance (mo) of HPV infection among 1,059 men from rural China, 2009–2013, stratified by carcinogenicity of HPV and age group. Asterisk indicates *P* values were calculated by Cox models using the WLW method to account for within-subject correlation. Confidence intervals in HPV clearance analysis were adjusted for within-subject correlations using the clustered Kaplan–Meier method. WLW, Wei Lin Weissfeld marginal model approach. A, incidence of oncogenic and nononcogenic HPV infection. B, clearance of oncogenic and nononcogenic HPV infection. C, incidence of any HPV infection, stratified by age group. D, clearance of any HPV infection, stratified by age group. E, incidence of oncogenic HPV infection, stratified by age group. F, clearance of oncogenic HPV infection, stratified by age group. G, incidence of nononcogenic HPV infection, stratified by age group. H, clearance of nononcogenic HPV infection, stratified by age group.

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

Table 3. Multivariate analyses of factors associated with prevalence, incidence, and clearance of any HPV, oncogenic[a], and nononcogenic[b] HPV infection in rural Chinese men, 2009–2013[c]

	Prevalence of HPV infection			Incidence of HPV infection			Clearance of HPV infection		
	Any HPV	Oncogenic HPV	Nononcogenic HPV	Any HPV	Oncogenic HPV	Nononcogenic HPV	Any HPV	Oncogenic HPV	Nononcogenic HPV
Variable	Adjusted OR[d] (95% CI)	Adjusted OR[d] (95% CI)	Adjusted OR[d] (95% CI)	Adjusted HR[d] (95% CI)	Adjusted HR[e] (95% CI)	Adjusted HR[d] (95% CI)	Adjusted HR[f] (95% CI)	Adjusted HR[f] (95% CI)	Adjusted HR[f] (95% CI)
Age (y)									
≤50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
>50	0.91 (0.68–1.22)	0.96 (0.61–1.53)	0.90 (0.66–1.23)	0.76 (0.56–1.05)	1.24 (0.77–2.01)	**0.65 (0.47–0.91)**	1.23 (0.88–1.72)	1.64 (0.99–2.72)	1.04 (0.67–1.60)
Education level									
Primary school or below	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Junior middle school or above	0.90 (0.71–1.14)	0.81 (0.56–1.18)	0.95 (0.73–1.23)	0.85 (0.65–1.11)	1.00 (0.66–1.53)	0.88 (0.66–1.17)	0.82 (0.59–1.12)	0.89 (0.55–1.46)	0.74 (0.49–1.12)
Type of job									
Farmer	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Worker in local area	1.01 (0.77–1.34)	1.05 (0.70–1.58)	1.01 (0.74–1.37)	0.94 (0.69–1.28)	1.10 (0.69–1.75)	1.07 (0.77–1.48)	1.09 (0.79–1.51)	0.87 (0.51–1.49)	1.26 (0.82–1.93)
Worker outside	0.99 (0.74–1.31)	0.72 (0.47–1.12)	1.13 (0.82–1.55)	0.94 (0.69–1.29)	1.11 (0.68–1.80)	0.89 (0.62–1.26)	0.82 (0.55–1.22)	1.40 (0.77–2.53)	0.66 (0.39–1.10)
Current smoker									
No	1.0	NA	1.0	1.0	NA	1.0	NA	NA	NA
Yes	**1.29 (1.04–1.60)**	NA	**1.26 (1.00–1.59)**	**1.28 (1.01–1.63)**	NA	**1.32 (1.01–1.73)**	NA	NA	NA
No. of lifetime sexual partners									
0–1	1.0	1.0	1.0	1.0	1.0	1.0	NA	NA	NA
≥2	**1.86 (1.40–2.46)**	**2.00 (1.35–2.96)**	**1.69 (1.24–2.30)**	**1.57 (1.12–2.20)**	**1.73 (1.06–2.81)**	**1.50 (1.05–2.15)**	NA	NA	NA
Bath frequency in winter (d)									
1–7	1.0	1.0	1.0	1.0	1.0	NA	NA	NA	NA
8–30	**0.70 (0.55–0.87)**	**0.53 (0.36–0.77)**	0.84 (0.65–1.08)	0.80 (0.61–1.05)	**0.48 (0.32–0.73)**	NA	NA	NA	NA
>30	**0.50 (0.35–0.71)**	**0.43 (0.24–0.76)**	**0.55 (0.37–0.83)**	0.80 (0.53–1.20)	**0.50 (0.26–0.94)**	NA	NA	NA	NA

NOTE: Variables with a significant level of 0.05 are shown in bold.

[a]Oncogenic types in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

[b]Nononcogenic types in this study were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 66, 67, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

[c]Backward selection with a threshold of 0.1 was used to generate the final multivariate model. Age, education level, and type of job were included in all multivariate models as design factors.

[d]All the listed variables remained in the final multivariate model.

[e]All the listed variables and wash genitalia before sex remained in the final multivariate model.

[f]Only age, education level, and type of job remained in the model.

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

due to the period sampling. This was particularly obvious in our study, as the biannual centralized follow-up was adopted and all study subjects were investigated within 2 to 3 weeks in each section.

Significantly higher prevalence, incidence, and longer duration of HPV infection was observed in the younger group (25–50 years) as compared with the older group (51–65 years), and in our previous descriptive study, we also observed a similar age pattern of HPV infection ($P = 0.039$; ref. 21). In other studies, however, this age trend was rarely detected, although faster clearance of HPV infections with increasing age was also found in the HIM study (17). We found that younger age is positively associated with more sexual partners in this population (Supplementary Table S2), which was not observed in the HIM cohort (17). As such, more sexual activity in younger men and higher exposure levels to HPV may have led to the age-related patterns observed for HPV acquisition and clearance in this study. Despite all this, further longitudinal studies in the Chinese population are required to verify our results.

Similar to previous studies (17–19, 33–36), the number of lifetime sexual partners was consistently associated with an increased risk of HPV infection. We also found that current smokers had a significantly elevated risk of nononcogenic HPV infection, which was in keeping with the HIM study (17). A potential mechanism is that smoking may increase the HPV viral load by weakening the cellular immune response (37). To investigate the possibility that the lifetime number of sexual partners acted as a confounder within the association between smoking and nononcogenic HPV incidence, stratification analysis by the lifetime sexual partner group was conducted. And similar effects (OR and HR) were observed in both groups, which suggested that the association between smoking and nononcogenic HPV was independent of sexual behavior. In addition, we also unexpectedly found that better personal hygiene is associated with a significantly elevated risk of HPV infection, and this has not been evaluated in previous studies of men. In this population, levels of personal hygiene (i.e., frequency of bathing) may reflect overall socioeconomic status (SES) due to the relatively poor economic and cultural conditions in rural China (38). Men with higher SES were more likely to work outside the local area for most of the year, which suggests that these individuals may engage in more unprotected sexual behavior and higher risk for HPV exposure. Finally, we found that age group was associated with incident infection of nononcogenic HPV and clearance of oncogenic HPV. This age trend should be interpreted with caution due to the potential pitfall of multiple testing in these borderline significant findings.

Two limitations of this study should be noted. First, 220 (17.2%) men without follow-up data were excluded from the incidence and clearance analyses, which might lead to a certain degree of underestimation of overall incidence and duration of HPV. But it would not significantly affect our main conclusions about prevalence, age-stratified analyses of natural history, and risk factor analysis. Second, although we have investigated more than 1,000 men for up to seven evaluations, we were still limited by statistical power when analyzing minor types. Large studies with more evaluation cycles are needed to validate our conclusions.

Basic information about the natural history of HPV infection across a broad age range is a prerequisite for the development of a cost-effective prophylactic strategy for HPV-related cancer. This study was the first prospective HPV natural history study among men from China. Results from this study will be important both in establishing primary prevention project of HPV-related carcinoma in Chinese women and in evaluating male HPV vaccination in China.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Ke, H. Cai, Z. He
Development of methodology: Y. Ke, Z. He, F. Liu, Y. Liu, J. Li
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Liu, C. Zhang, F. Liu, Y. Liu, Z. Xu, Q. Wang, D. Hang, N. Shen, Y. Pan, C. Guo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Liu, Z. He, Q. Wang, H. Cai
Writing, review, and/or revision of the manuscript: M. Liu, Z. He, Q. Wang, H. Cai
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Liu, F. Liu, Y. Liu, D. Hang
Study supervision: Y. Ke, H. Cai, Z. He

Acknowledgments

The authors thank Dr. Michael A. McNutt for editing and correction of this article.

Grant Support

This work was supported by Natural Science Foundation of China (grant number 30930102); "973" Project of National Ministry of Science and Technology Grant (grant numbers 2011CB504300, 2012CB910800); "863" Key Projects of National Ministry of Science and Technology Grant (grant numbers 2012AA02A209, 2012AA022502); Charity Project of National Ministry of Health (grant numbers 201202014, 200902002); and Natural Science Foundation of Beijing (grant number 7100001) to Y. Ke. This work was also supported by Natural Science Foundation of China (grant number 81473033) to Z. He.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 4, 2014; revised July 24, 2014; accepted September 5, 2014; published OnlineFirst September 12, 2014.

References

1. Zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009;384:260–5.
2. Buckley JD, Harris RW, Doll R, Vessey MP, Williams PT. Case-control study of the husbands of women with dysplasia or carcinoma of the cervix uteri. Lancet 1981;2:1010–5.
3. Zunzunegui MV, King MC, Coria CF, Charlet J. Male influences on cervical cancer risk. Am J Epidemiol 1986;123:302–7.
4. Agarwal SS, Sehgal A, Sardana S, Kumar A, Luthra UK. Role of male behavior in cervical carcinogenesis among women with one lifetime sexual partner. Cancer 1993;72:1666–9.

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

5. Bosch FX, Castellsague X, Munoz N, de Sanjose S, Ghaffari AM, Gonzalez LC, et al. Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst 1996;88:1060–7.
6. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005;32 Suppl 1:S16–24.
7. Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001;286:3106–14.
8. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370: 890–907.
9. Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357:1831–6.
10. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423–8.
11. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest 2011;121:4593–9.
12. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010;202:1789–99.
13. Canadas MP, Darwich L, Videla S, Sirera G, Coll J, Rafael ML, et al. Circumcision and penile human papillomavirus prevalence in human immunodeficiency virus-infected men: heterosexual and men who have sex with men. Clin Microbiol Infec 2013;19:611–6.
14. Moreira ED, Giuliano AR, Palefsky J, Flores CA, Goldstone S, Ferris D, et al. Incidence, clearance and progression to disease of genital human papillomavirus infection in heterosexual men. J Infect Dis 2014;210: 192–9.
15. Grabowski MK, Gray RH, Serwadda D, Kigozi G, Gravitt PE, Nalugoda F, et al. High-risk human papillomavirus viral load and persistence among heterosexual HIV-negative and HIV-positive men. Sex Transm Infect 2014;0:1–7.
16. Kjaer SK, Munk C, Winther JF, Jorgensen HO, Meijer CJ, van den Brule AJ. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev 2005;14:1528–33.
17. Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet 2011;377:932–40.
18. Giuliano AR, Lu B, Nielson CM, Flores R, Papenfuss MR, Lee JH, et al. Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. J Infect Dis 2008; 198:827–35.
19. Partridge JM, Hughes JP, Feng Q, Winer RL, Weaver BA, Xi LF, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. J Infect Dis 2007;196:1128–36.
20. Liu F, Guo F, Zhou Y, He Z, Tian X, Guo C, et al. The anyang esophageal cancer cohort study: study design, implementation of fieldwork, and use of computer-aided survey system. PLoS ONE 2012;7:e31602.
21. He Z, Liu Y, Sun Y, Xi LF, Chen K, Zhao Y, et al. Human papillomavirus genital infections among men, China, 2007–2009. Emerg Infect Dis 2013;19:992–5.
22. Huang SL, Chao A, Hsueh S, Chao FY, Huang CC, Yang JE, et al. Comparison between the Hybrid Capture II Test and an SPF1/GP6+ PCR-based assay for detection of human papillomavirus DNA in cervical swab samples. J Clin Microbiol 2006;44:1733–9.
23. Wang X, Tian X, Liu F, Zhao Y, Sun M, Chen D, et al. Detection of HPV DNA in esophageal cancer specimens from different regions and ethnic groups: a descriptive study. BMC Cancer 2010;10:19.
24. Guo F, Liu Y, Wang X, He Z, Weiss NS, Madeleine MM, et al. Human papillomavirus infection and esophageal squamous cell carcinoma: a case-control study. Cancer Epidemiol Biomarkers Prev 2012;21: 780–5.
25. Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6:204.
26. Castle PE. The evolving definition of carcinogenic human papillomavirus. Infect Agent Cancer 2009;4:7.
27. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121–30.
28. Ulm K. A simple method to calculate the confidence interval of a standardized mortality ratio (SMR). Am J Epidemiol 1990;131:373–5.
29. Ying Z WL. The Kaplan-Meier estimate for dependent failure time observations. J Multivar Anal 1994;50:17–29.
30. Wei LJ LD, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modelling marginal distributions. J Am Stat Assoc 1989;84:1065–73.
31. Vardas E, Giuliano AR, Goldstone S, Palefsky JM, Moreira ED Jr, Penny ME, et al. External genital human papillomavirus prevalence and associated factors among heterosexual men on 5 continents. J Infect Dis 2011;203:58–65.
32. Sichero L, Pierce Campbell CM, Ferreira S, Sobrinho JS, Luiza Baggio M, Galan L, et al. Broad HPV distribution in the genital region of men from the HPV infection in men (HIM) study. Virology 2013; 443:214–7.
33. Smith JS, Backes DM, Hudgens MG, Bailey RC, Veronesi G, Bogaarts M, et al. Prevalence and risk factors of human papillomavirus infection by penile site in uncircumcised Kenyan men. Int J Cancer 2010;126: 572–7.
34. Nyitray AG, Smith D, Villa L, Lazcano-Ponce E, Abrahamsen M, Papenfuss M, et al. Prevalence of and risk factors for anal human papillomavirus infection in men who have sex with women: a cross-national study. J Infect Dis 2010;201:1498–508.
35. Lu B, Wu Y, Nielson CM, Flores R, Abrahamsen M, Papenfuss M, et al. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. J Infect Dis 2009;199:362–71.
36. Vaccarella S, Lazcano-Ponce E, Castro-Garduno JA, Cruz-Valdez A, Diaz V, Schiavon R, et al. Prevalence and determinants of human papillomavirus infection in men attending vasectomy clinics in Mexico. Int J Cancer 2006;119:1934–9.
37. Schabath MB, Villa LL, Lazcano-Ponce E, Salmeron J, Quiterio M, Giuliano AR, et al. Smoking and human papillomavirus (HPV) infection in the HPV in Men (HIM) study. Cancer Epidemiol Biomarkers Prev 2012;21:102–10.
38. Zou L, Bao YP, Li N, Dai M, Ma CP, Zhang YZ, et al. Life-style and genital human papillomavirus in a cross-sectional survey in Shanxi Province, China. Asian Pac J Cancer Prev 2011;12:781–6.

Published OnlineFirst September 12, 2014; DOI: 10.1158/1055-9965.EPI-14-0365

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Prevalence, Incidence, Clearance, and Associated Factors of Genital Human Papillomavirus Infection among Men: A Population-Based Cohort Study in Rural China

Mengfei Liu, Zhonghu He, Chanyuan Zhang, et al.

Cancer Epidemiol Biomarkers Prev 2014;23:2857-2865. Published OnlineFirst September 12, 2014.