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Perspective

Key considerations and current perspectives of epidemiological studies on human papillomavirus persistence, the intermediate phenotype to cervical cancer

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SUMMARY

Persistent infection with human papillomavirus (HPV) causes essentially all precancerous cervical lesions and cervical cancer in females and thus is an important intermediate phenotype to cervical cancer. A majority of infected individuals naturally clear HPV viral infection, but the virus persists in a subset of infected hosts and the mechanism for this differential outcome is not well described. Most of the epidemiological studies have been cross-sectional in nature, and even with longitudinal studies, the definition of HPV persistence or clearance has not been well defined. There is no consensus on the correct time interval between HPV DNA tests, or how to utilize HPV persistence information in clinical management because there is no treatment for HPV. While most studies are performed with the endpoint of cancer, the intermediate phenotype has been overlooked. Epidemiological studies of HPV persistence suffer with several challenges in definitions, study designs, and analyses that undermine its importance in identifying and understanding the interactions between the viral and host genomes in the process of HPV infection pathogenesis. We have evaluated the current status of HPV persistence and provide perspectives on how the field would benefit from a research focus on intermediate phenotype in epidemiological studies.

1. Introduction

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted infections (STIs) in both men and women. The virus is highly contagious and studies have estimated HPV transmission probability to be as high as 40–60% following unprotected sexual intercourse.[1] Annually, 160 million incident infections of HPV are estimated worldwide; however, as you progress to dysplasia and then to cervical cancer (529 409) the rates significantly decrease[2–4] (Figure 1). Incident HPV infections seem to be age-dependent, where HPV infections peak soon after the age when most young women become sexually active (average age of 20 years), and this is usually followed by a gradual decline. Approximately 80% of the female population is exposed to HPV sometime in their lifetime, but the infection is usually transient, with 70–90% of infected individuals 'clearing' the virus (HPV DNA undetectable by assays) within 12–24 months, and only a small proportion will progress to cervical cancer (Figure 1).[5,6] The main consequence of persistent infection with HPV is the development of precancerous cervical lesions that may progress to malignancy in the next 5–15 years after infection, and this subsequently could result in invasive cervical cancer.[7]

HPV persistence has been consistently and strongly associated with precancerous lesions.[8,9] A recent 16-year longitudinal study also confirmed the critical role of persistent carcinogenic HPV infections in predicting the risk of cervical cancer in women.[10] Based on this study, the 16-year risk of cervical cancer was 6.2% among women infected with any carcinogenic (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) HPV, and 13.5%, 10.3%, or 4.0% for women infected with HPV16, HPV58, or other carcinogenic HPVs (without HPV 16 and 58), respectively. However, the rates were lower with other HPV type infections: 2.1%, 1.1%, and 0.26% among women infected with possibly/probably carcinogenic HPV types, other non-carcinogenic HPV infections, and HPV-negative women, respectively. HPV infections occurring at older ages could have little impact on cancer cases compared to persistent infections occurring at an earlier age, due to the years required for cancer development. These observations further emphasize the need for earlier detection of disease progression by monitoring the persistent infection with HPV, before the development of lesions. The research focus should include persistent HPV infection since this is the known precursor of cervical cancer. In this paper, we will focus on HPV persistence, which we define as 'intermediate phenotype', and provide perspectives in epidemiological study designs and analyses.

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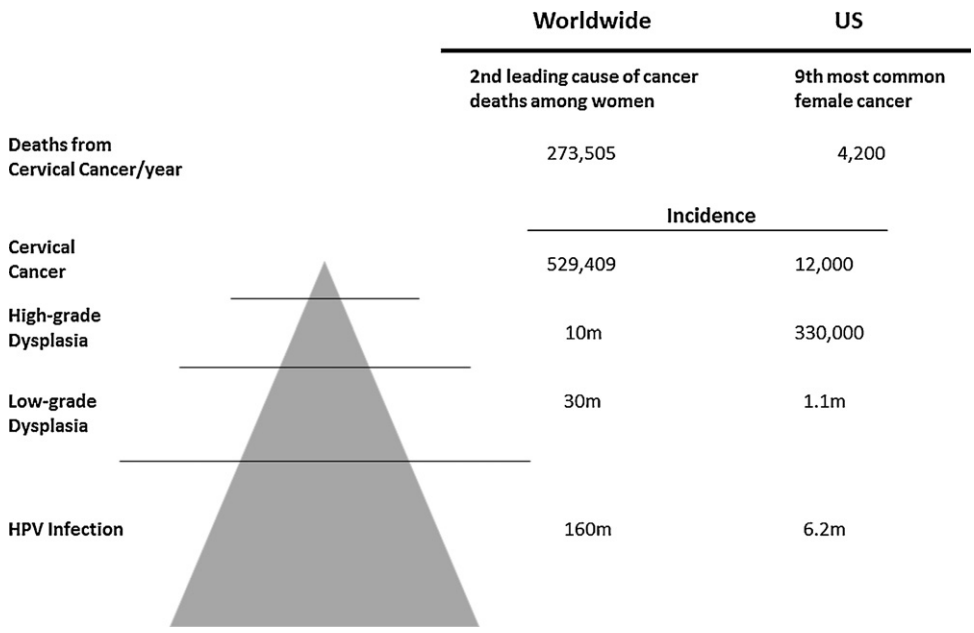


Figure 1. HPV infection and cervical abnormalities epidemiology in the USA and worldwide.[2–4]

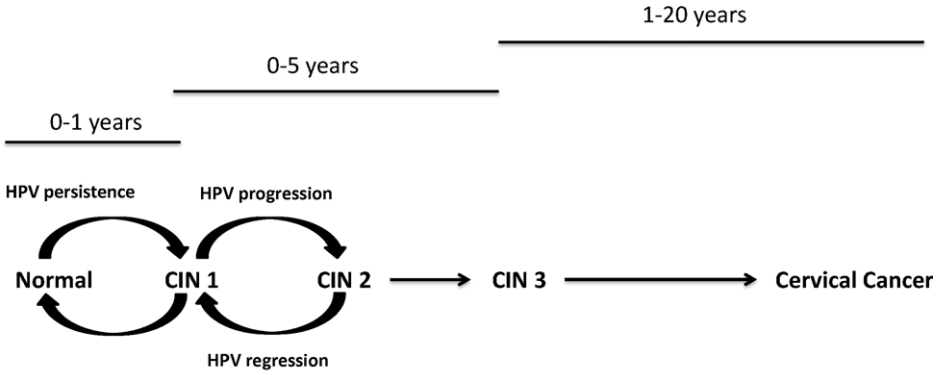
2. Why HPV persistence is the intermediate phenotype

Epidemiologic and virologic data demonstrate that 13–15 high-risk, or oncogenic HPVs are the primary and necessary causal agents of cervical cancer;[11–13] HPV infection is attributable to 99.9% of cervical cancers, and oncogenic HPV types 16/18 are responsible for 70% of all cervical cancers.[14] Individual HPV infections are not independent from each other in either sex, meaning that acquisition of multiple HPV types occurs more often than expected.[15]However, no two HPV types are more likely to be acquired together than any other HPV types in several populations.[15–17] While these cross-sectional studies show strong associations, HPV is required to persist to cause necessary cellular changes in the host to progress to cancer. Since most HPV infections do not clear, persistent HPV infection is a prerequisite and thus we consider it as an 'intermediate phenotype'. An infection persisting for more than 4 years has only a small chance of remission.[18] Women who have a persistent infection can develop cervical intraepithelial neoplasia (CIN) lesions; however a proportion of high-grade cervical lesions may never progress to cervical cancer and can even regress without treatment[4,19,20] (Figure 2). However, the status of HPV is not known during the cervical lesion regression process, i.e., whether regression is linked to clearance of HPV. Most epidemiological studies have described CIN or cervical cancer as the outcome in the host and only a few have incorporated HPV persistence as the main outcome. HPV pathogenesis with respect to the cervical carcinogenesis in the host should be viewed in two separate phases; first, the biology related to the virological and host immunological process of HPV persistent infection; and second, the functionally important stages in cervical cancer progression. It should be noted that the first phase is a prerequisite to the second phase. The limited studies on HPV persistence suggest that multiple HPV infections,[21] smoking,[22] and multiple lifetime sexual partners[23,24] are the main factors associated with persistent HPV infection. However, some of the epidemiological and biological factors associated with cervical cancer could be a surrogate for HPV persistence, where it is confounded by the selection bias of HPV persistent individuals among cancer patients. For instance, several studies have shown human leukocyte antigens (HLA) to be associated with cervical cancer,[25] but of note, HLA and other immune-related genes may be more involved with persistence or clearance of HPV.[26] The importance of HPV persistence as the intermediate phenotype has been acknowledged in the clinical setting, resulting in the inclusion of HPV testing in several screening programs. In a large longitudinal study, Castle et al. recently described that while both baseline Pap and HPV tests predicted the development of CIN3 within the first 2 years of follow-up, only HPV testing predicted CIN3 in 10 to 18 years.[27] Precancerous lesions and cervical cancer have often been the public health focus and recently HPV testing has been recommended in clinical screening.[7] Similarly, persistent HPV should also be carefully considered in research settings to understand the dynamics of how some are susceptible to persistent infection while most are able to overcome.[28] Phenotypically, it is extremely important for epidemiological studies to accurately define the intermediate phenotype, determine the correct HPV types, and systematically be able to analyze the complex data.

3. Challenges in research studies of HPV persistence, the intermediate phenotype

3.1. Epidemiological study designs

There has been considerable heterogeneity in study design and methodological approaches in various cohort studies examining the natural history and persistence of HPV. The most common epidemiological study design for HPV is a cross-sectional design that estimates the prevalence at any time-point, but does not provide information regarding HPV persistence and clearance. Prevalent cases may have had the infection for a few days to years, making these women significantly different to those with an incident infection during follow-up. In that sense, longitudinal data are more powerful and are better predictors for the outcome of interest. Ideally, a study assessing persistence of HPV in the population needs to follow women before their first HPV infection and for an extended period in order to be certain that the HPV infections are truly incident infections and not latent. Including only those with incident HPV infections allows the researchers a clearer understanding of when the individual was infected. While everyday sampling is theoretically possible with self-sampling approaches, it is logistically not quite feasible; thus, data from



Cervical Histological Lesion	Regression to normal	Lesion persists but unchanged	Progress to CIN3	Progress to CC
CIN1	60%	20-30%	5-10%	1%
CIN2	40%	40%	15-20%	5%
CIN3	33%			10-12%

Figure 2. HPV persistence and progression to cervical abnormalities timeline and estimated likelihoods of regression and progression of cervical histological lesions (data based on various resources).[4,19,20]

shorter visit periods will be more informative. There have been very few long-term longitudinal studies that have actually followed persistent HPV and assessed the risk of cervical cancer among women.[10,29]

3.2. Definitions of HPV persistence

Correctly defining the intermediate phenotype is critical for accurate study design and analysis. 'HPV viral persistence' is often defined as detection of the same HPV type at two or more intervals.[8,9,30–32] In a recent meta-analysis, Rositch et al. reported that the definition of HPV persistence was mediated by study region, detection method, and HPV type.[31] They estimated that approximately half of the HPV infections persist past 6–12 months. These findings coincide with the current ASC/ASCCP/ASCP guidelines that recommend a 1-year repeat screening interval for women over 30 years who are HPV-positive with normal cytology.[31] If a woman is going to clear an HPV infection, it will likely occur within a year, and future follow-up is needed if HPV persists longer than 1 year. This meta-analysis emphasizes the need for a concise definition of HPV persistence and time interval between HPV DNA tests in order to more effectively determine clinical and treatment outcomes.

After detection of the HPV virus, 'HPV viral clearance' is often defined as not detecting the same HPV type at two subsequent visits following an HPV-positive visit, thus requiring 'two consecutive visits'.[30] The main issue with these definitions is that there is no standard biologically relevant duration. While most studies use 'two consecutive visits' for clearance to rule out false-negatives, this process may also inflate the time to clearance since the exact date of clearance is unknown. It is also possible that negative HPV diagnostic results could indicate shedding of HPV virus at quantities below the limit of detection or the latent phase of HPV. Latency will change the definition of clearance, persistence, and reinfection and this concept of HPV is still poorly understood and further research is warranted.

3.3. HPV testing methods

There are several HPV genotyping methods currently being used. Molecular methods have been developed for HPV detection, including those based on signal amplified hybridization, PCR, DNA sequencing, type-specific probes, reverse line-blot hybridization, in situ hybridization, Southern blot hybridization, and immunological techniques, including ELISA and Western blot. The gold standard is based on PCR, but still the results can vary across methods. PCR-based methods are quite sensitive to minimal amounts of HPV, and even if the virus is not totally cleared from the system, it is still capable of detecting trace amounts of virus. Yet, it is not quite possible to characterize and differentiate a new infection from reinfection with the same HPV type, as some virus could remain latent in basal cells at undetectable levels.[30] Other parameters of the virus, such as viral variants, viral load, expression, and genomic integration capacity,[30] specifically in relation to co-infections, will need to be assessed as possible markers in future studies.

3.4. Analytical methods

A major analytical challenge has been the prevalent cases and censoring of events, since, as in any prospective study, it is not clear how long HPV persists after the end of the study. To account for some of these, the Cox model has been the standard approach; however this model cannot simultaneously analyze time to clearance of several types of HPV because it does not address possible correlations between incident HPV infections. The Cox model with the Wei–Lin–Weisfeld (WLW) extension accounts for the correlation between HPV subtypes within a person and has population-level interpretations.[33] While the Cox model with the frailty term also accounts for the correlation between HPV subtypes within a person, it has individual-level interpretations, which is difficult to comprehend in epidemiological studies.[33,34] Other methods, such as the model based on transitional probability,[35] the framework model based on the clustered longitudinal binary data structure,[36] and the discrete-time semi-Markov models,[37] have also been used to account for both prevalent and incident infections.

Additionally, missing HPV data in longitudinal studies can have a significant impact on correctly identifying persistence and clearance. Certain assumptions are made based on the data, but in several instances, a premature censoring or exclusion of

Table 1
HPV clearance and definitions of prevalence, incidence, clearance, left censoring, and right censoring

			V1		V2		V3		V4	V5		
Scenario 1	baseline	I	-		+		+	*	-	-	end of follow-up	C
Scenario 2		P-LC	+		+	*	-		-	-		C
Scenario 3		P-LC	+		+		+	*	-	-		C
Scenario 4		P-LC	+		+		+		+	+		RC
Scenario 5		I	-		-	¥	+		+	-		RC
Scenario 6		I	-	¥	+		-		+	-		RC
Scenario 7		I	-		+		-	*	M	-		C

P-LC, prevalence, left censored; I, incidence (¥); RC, right censored; C, cleared (*); V1–V5, visits 1 to 5 as examples; M, missing.

the individual episode are often required to outweigh the benefit of a larger sample size. For example, if the lower threshold of assay detection is in question, one would want HPV-negative results at two consecutive testing intervals, and the ideal time to clearance will be the midpoint between the last positive visit and the first negative visit (Table 1, scenario 1). In another example (Table 1, scenario 7), the individual could be positive for HPV at visit 2, negative at visit 3, data missing at visit 4, and negative at visit 5. One would assume that the HPV type remains the same as the previous visit, which indicates that the HPV virus cleared in this individual and this assumption holds at visit 5. However, missing data scenarios could be quite complex and thus, the interpretation could be very subjective. For consistent use of the intermediate phenotype, a clear consensus should be made on the biological definition of HPV persistence.

4. Conclusions

Throughout this paper we have described how the focus on cervical cancer and precancerous lesions are too little too late because the virus has already evaded the immune system and initiated the integration process. In clinical settings, HPV screening would be the first step, and confirmation of persistent infection from follow-up HPV testing would complement cytology testing. While a prophylactic HPV vaccine to four types is available, challenges remain for researchers to understand the pathogenesis of HPV, specifically among those already infected, those who become infected with other oncogenic types, and those who do not get vaccinated[38]. There is consensus that persistence of HPV is the known, main factor for progression to precancer and cancer. Thus, research based on the well-defined intermediate phenotype could provide valuable information for prevention and alternatives to progression of cancer. The technology to detect the virus is feasible in most settings and with careful follow-up plans would greatly assist scientists with their research and clinicians with their screening programs to help recognize, monitor, and manage the burden of the disease.

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