Practice of Epidemiology

Bias Due to Correlation Between Times-at-Risk for Infection in Epidemiologic Studies Measuring Biological Interactions Between Sexually Transmitted Infections: A Case Study Using Human Papillomavirus Type Interactions

Talía Malagón*, Philippe Lemieux-Mellouki, Jean-François Laprise, and Marc Brisson

* Correspondence to Dr. Talía Malagón, Division of Cancer Epidemiology, Department of Oncology, Faculty of Medicine, McGill University, 546 Pine Avenue West, Montreal, QC H2W 1S6, Canada (e-mail: talia.malagon@mcgill.ca).

The clustering of human papillomavirus (HPV) infections in some individuals is often interpreted as the result of common risk factors rather than biological interactions between different types of HPV. The intraindividual correlation between times-at-risk for all HPV infections is not generally considered in the analysis of epidemiologic studies. We used a deterministic transmission model to simulate cross-sectional and prospective epidemiologic studies measuring associations between 2 HPV types. When we assumed no interactions, the model predicted that studies would estimate odds ratios and incidence rate ratios greater than 1 between HPV types even after complete adjustment for sexual behavior. We demonstrated that this residual association is due to correlation between the times-at-risk for different HPV types, where individuals become concurrently at risk for all of their partners' HPV types when they enter a partnership and are not at risk when they are single. This correlation can be controlled in prospective studies by restricting analyses to susceptible individuals with an infected sexual partner. The bias in the measured associations was largest in low-sexual-activity populations, cross-sectional studies, and studies which evaluated infection with a first HPV type as the exposure. These results suggest that current epidemiologic evidence does not preclude the existence of competitive biological interactions between HPV types.

bias (epidemiology); coinfection; cross-protection; microbial interactions; papillomavirus infections; sexually transmitted diseases; time factors; viral interference

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; IRR, incidence rate ratio; POR, prevalence odds ratio; STI, sexually transmitted infection.

Persons with a sexually transmitted infection (STI) generally have a higher prevalence and incidence rate of infection with other STIs (1–12). These observations have sparked inquiry into the causal effects a first STI might have on the probability that a second STI can establish an active infection upon contact with an infected sexual partner. This causal effect on transmission would entail a biological interaction between the STIs. It is important to ascertain the existence of biological interactions from a public health perspective, because interventions against one STI could also affect the other STIs with which it interacts (13–15). If previous infection with a first STI increases the risk of becoming infected with a new STI (facilitative interaction), then interventions against the first STI could also reduce the incidence of other STIs. For example, if STIs increase the probability of becoming infected with human immunodeficiency virus (HIV), then STI control and management could help control HIV incidence (16). If previous infection with a first STI reduces the risk of becoming infected with other STIs (competitive interaction), then interventions targeted against the first STI could increase the incidence of infections with other STIs due to the reduced competition. For example, human papillomavirus (HPV) vaccination might increase the incidence of infection with nonvaccine HPV types through type replacement (15).

In practice, measures of association between STIs are biased estimates of their causal effects on each other's transmission. It is largely recognized that because both the exposure (the first STI) and the outcome (the second STI) are sexually acquired, the effect estimate will be confounded by common risk factors [17–19]. Notably, sexual behaviors and networks which increase the risk of being infected with one STI also increase the risk of being infected with a second STI, so their incidence is expected to be associated.

Furthermore, STIs' sexual mode of transmission leads to an additional bias caused by the correlation between the times-at-risk for infection with different STIs. To our knowledge, this issue has not been considered when analyzing STI associations. When individuals create and break off sexual partnerships, their at-risk status changes concurrently over time for all STIs. An individual's incidences of multiple STIs will be correlated over time because 1) when an individual has no partners or has uninfected partners, he/she simultaneously is not at risk for any STI and has a null risk of incident infection with any STI and 2) when an individual has partners who are infected with multiple STIs, he/she is simultaneously at risk for all of the partners' STIs during their partnership. This issue is separate from sexual risk confounding, because adjustments for sexual behavior markers (e.g., recent/lifetime numbers of sexual partners [8, 11, 12]) control for interindividual differences in infection risk that are assumed to remain constant over time. These markers do not control for the intraindividual correlation between the times an individual is at risk for different STIs due to partnership creation. Eliminating the correlation requires restricting analyses to times when individuals are at risk of being infected with the outcome STI due to sexual contacts with infected partners. However, this restriction is not often feasible in studies of STIs.

Mathematical transmission-dynamic models can help us analyze results of epidemiologic studies by explicitly incorporating the dependence of outcomes inherent to STI dynamics. They are thus valuable complements to traditional statistical analyses, which are often not adapted for dependent outcomes [20].

In this paper, we use HPV as an example with which to examine this bias. Antibodies developed against one type of HPV cross-react with related HPV types [21, 22], so infection with a first HPV type might plausibly decrease the subsequent risk of new HPV infections (competitive interaction). However, various cross-sectional and prospective studies have shown that HPV types cluster together [23–28] and that persons currently infected with one type have a higher risk of incident infection with another type, even after adjustment for sexual behaviors [9–12, 26–28]. The remaining association is generally interpreted as residual confounding by sexual activity or immune responses, and the existence of competitive interactions and type replacement following HPV vaccination are generally deemed unlikely [26–29]. However, none of these analyses have considered the correlation between the times-at-risk for all HPV types. Using a transmission-dynamic model of 2 HPV types, we aim to illustrate how the correlation between the times-at-risk for infection biases the estimation of causal effects between HPV types in cross-sectional and

prospective studies even when we assume that *they do not interact*. We identify which epidemiologic research designs minimize or eliminate this bias to estimate causal effects. We then generalize results to the situations of 1) STIs with different natural histories, 2) STIs in populations with different sexual behaviors, and 3) the assumption that competitive and facilitative interactions do exist.

METHODS

We built a mathematical model to simulate the transmission of 2 generic HPV types in an illustrative population. In this population, we simulated epidemiologic studies which measure the association between 2 HPV types. If the measured association between HPV types in these studies did not match the underlying modeled effect of the first HPV type on the second, we considered the association to be a biased estimate of the biological interaction. Further details on model structure, parameters, and equations can be found in the Web Appendix (available at http://aje.oxfordjournals.org/), including Web Figures 1 and 2 and Web Table 1.

Model structure

The modeled population is heterosexual, open, and stable. Age is not modeled. Individuals spend on average 40 years in the modeled population, representing their most sexually active years (approximately ages 15–54 years). Individuals are stratified into high and low sexual activity levels, which differ according to their rate of sexual partner acquisition. An assortativity parameter (ε) represents the proportion of sexual partnerships individuals make exclusively with members of their same sexual activity level, while the rest of the partnerships ($1 - \varepsilon$) are made proportionately between levels. We model the duration of sexual partnerships between individuals and the rate of sex acts within partnerships.

HPV type (1 or 2) is marked by the subscript i. Individuals in the model may be in one of 9 mutually exclusive health states representing the 9 possible combinations of susceptible (S_i), infected (I_i), or recovered/immune (R_i) states with both HPV types (Figure 1). We assumed that 1) infections with each HPV type are cleared independently [9, 12]; 2) individuals only acquire immunity once they clear an infection; 3) immunity is type-specific and wanes over time; 4) susceptible individuals can only be infected when they are in a sexual partnership with an infected partner; and 5) transmission probabilities of both HPV types are independent per sex act. Individuals susceptible to both HPV types ($[S_1S_2]$) who have a coinfected sexual partner ($[I_1I_2]$) have a joint probability of being infected with both HPV types ($\delta_1 \times \delta_2$), only type 1 ($\delta_1 \times (1 - \delta_2)$), or only type 2 ($(1 - \delta_1) \times \delta_2$) per sex act. These independence assumptions were made so that in the base case, HPV types do not affect each other's natural history (no interactions). However, the time periods during which individuals are at risk of getting HPV types 1 and 2 are correlated in the model, because they are not at risk for either type unless they are in a partnership with an infected person.

In sensitivity analyses, we allowed HPV types to interact by modifying each other's transmission probability.
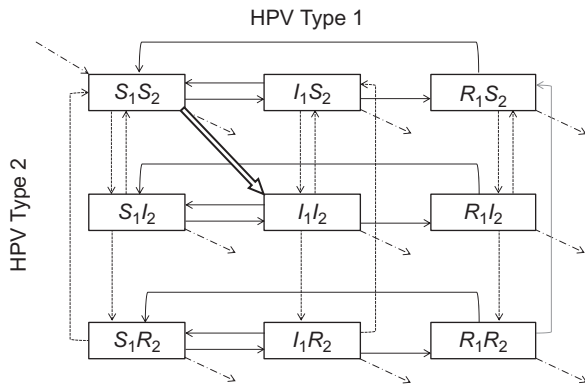
Figure 1. Different combinations of susceptible (*S*), infected (*I*), and recovered/immune (*R*) statuses for 2 types of human papillomavirus (HPV), designated type 1 and type 2 (indicated by a subscript), with the first letter corresponding to HPV type 1 health status and the second corresponding to HPV type 2 health status. The boxes show mutually exclusive health states, with possible transitions indicated by arrows. Boxes in the left-hand column represent HPV type 1-susceptible individuals; boxes in the middle column represent HPV type 1-infected individuals; and boxes in the right-hand column represent HPV type 1-immune individuals. Boxes in the first row represent HPV type 2-susceptible individuals; boxes in the second row represent HPV type 2-infected individuals; and boxes in the third row represent HPV type 2-immune individuals. Dashed-dotted arrows correspond to population entry and exit points, solid black arrows to HPV type 1 natural history transitions, dashed arrows to HPV type 2 natural history transitions, and the white outlined arrow to natural history transitions affecting both types of HPV. See the Web Appendix for differential equations and parameters for transition rates between health states.

Individuals currently infected with a first HPV type have their per-sex-act probability of infection with the second HPV type i multiplied by a factor χ_i. This factor represents the effect of interaction mechanisms hypothetically triggered during the active infection with the first HPV type (e.g., competition for host resources, disrupted epithelial tissues). Individuals immune to a first HPV type have their per-sex-act probability of infection with the second HPV type i multiplied by a factor φ_i. This factor represents the effect of interaction mechanisms hypothetically caused by long-term immunity to the first HPV type (e.g., cross-immunity). Values of <1, >1, and 1 for these factors correspond to competitive interactions, facilitative interactions, and no interactions, respectively.

Parameterization

For our base-case scenario, which used HPV as an example, we selected model parameters to illustrate HPV epidemiology (Table 1). Parameter values were based on empirical observations (30–35) and on model-based estimates of transmission probabilities (36). Importantly, we assumed no interactions between HPV types ($\chi_i = \varphi_i = 1$). For illustrative purposes, we made the simplifying assumptions that both HPV types had identical natural history parameters and that mixing between sexual activity levels was proportionate ($\varepsilon = 0$). However, because there is high uncertainty surrounding mixing patterns and mixing is probably assortative by sexual behavior (37), we varied this parameter in sensitivity analyses. We defined the low sexual activity level as 2 or fewer sexual partners in the past year (30).

Table 1. Base-Case Parameter Values and Sensitivity Analysis Ranges Used in a Model of Partnership Formation, Times-at-Risk for Human Papillomavirus Infection, and Their Interactions

Parameter	Value	Source of Derivation	Range of Sensitivity Analysis
Proportion of individuals in high sexual activity level	0.07	Chandra, 2011 (30)[a]	
Rate of partner change for low sexual activity level (per year)	0.80	Chandra, 2011 (30)[b]	0.25–2.50
Rate of partner change for high sexual activity level (per year)	5.00	—[c]	
Proportion of partnerships made exclusively with persons at the same sexual activity level (degree of assortativity ε)	0.00	(Proportionate mixing)	0.00–1.00
Rate of sex acts in partnerships (per year)	75.40	Mercer, 2013 (34)	
Proportion of time spent single	0.25	Demers, 2012 (35)[d]	
Duration of infection, years	1.40	Insinga, 2010 (31)[e]	0.25–25.00
Proportion of infections conferring natural immunity	0.60	Carter, 2000 (32)	
Duration of natural immunity, years	10.00	Wang, 2004 (33)[f]	
Probability of infection transmission per sex act with an infected person	0.13	Bogaards, 2010 (36)[e,g]	0.001–1.00
Interaction effects (χ_i and φ_i)	1.00	(No interactions)	0.05–7.76

Abbreviation: HPV, human papillomavirus.

[a] Based on having had more than 2 sexual partners in the last year.
[b] Average number among persons with 2 or fewer sexual partners in the last year.
[c] Manually set to achieve a 6% type-specific prevalence of HPV.
[d] Based on the proportion of individuals reporting being in a stable partnership.
[e] Averaged over HPV types.
[f] We assumed an exponential loss of immunity and found the rate of waning that led to 45% of individuals losing their seropositivity after 6.4 years.
[g] Calculated so that the average per-infected-partnership probability of infection was 0.79.

We manually set the high sexual activity level's rate of sexual partner change to achieve a type-specific HPV prevalence of 6% (prevalence of HPV-16 generally ranges between 3% and 11% (38–40)).

Epidemiologic studies of interactions between HPV types

We simulated 3 types of cross-sectional and prospective epidemiologic studies of HPV infection in the model population. In each study, we measured the association between HPV types 1 and 2. We considered the difference between the measured association and the modeled interaction effects (χ_i, φ_i) to be a bias in the estimation of the causal effect. The 3 types of studies differed in their definitions of exposure and outcome:

1. Cross-sectional studies in which the exposure is defined as prevalent infection with HPV type 1 and the outcome is prevalent infection with HPV type 2 (25–28). We calculated the prevalence odds ratio (POR) for type 2 infection by type 1 infection status (equation 1).

$$\text{POR} = \frac{\text{(no. infected with HPV type 1 and HPV type 2)}/}{\text{(no. infected with HPV type 1 and uninfected with HPV type 2)}}\Big/\frac{\text{(no. uninfected with HPV type 1 and infected with HPV type 2)}/}{\text{(no. uninfected with HPV type 1 and HPV type 2)}}$$

$$= \frac{[I_1 I_2]/([I_1 S_2] + [I_1 R_2])}{([S_1 I_2] + [R_1 I_2])/([S_1 S_2] + [S_1 R_2] + [R_1 S_2] + [R_1 R_2])}. \tag{1}$$

2. Prospective studies in which the exposure is defined as prevalent infection with HPV type 1 and the outcome is incident infection with HVP type 2 (9–12). We calculated the incidence rate ratio (IRR) for HPV type 2 infection by HPV type 1 infection status (equation 2).

$$\text{IRR by infection status} = \frac{\text{HPV type 2 infection rate in HPV type 1-infected individuals}}{\text{HPV type 2 infection rate in HPV type 1-uninfected individuals}}$$

$$= \frac{\text{HPV type 2 incident cases in } [I_1 S_2]/\text{person-years } [I_1 S_2]}{\text{HPV type 2 incident cases in } [S_1 S_2] + [R_1 S_2]/\text{person-years } ([S_1 S_2] + [R_1 S_2])}. \tag{2}$$

3. Prospective studies in which the exposure is defined as being seropositive for HPV type 1 and the outcome is incident infection with HPV type 2 (41–44). We calculated the IRR for HPV type 2 infection by HPV type 1 immunity status (equation 3).

$$\text{IRR by immunity status} = \frac{\text{HPV type 2 infection rate in HPV type 1-immune individuals}}{\text{HPV type 2 infection rate in HPV type 1-nonimmune individuals}}$$

$$= \frac{\text{HPV type 2 incident cases in } [R_1 S_2]/\text{person-years } [R_1 S_2]}{\text{HPV type 2 incident cases in } [S_1 S_2] + [I_1 S_2]/\text{person-years } ([S_1 S_2] + [I_1 S_2])}. \tag{3}$$

We assumed that prospective analyses are performed in individuals susceptible to HPV type 2 infections.

These crude associations calculated in the overall population are expected to be biased estimates of the interaction effects χ_2 and φ_2, due to sexual risk confounding and to the correlation between the times-at-risk of infection. To remove these biases, we performed the following analyses. First, we adjusted associations for sexual activity level using Mantel-Haenszel estimators. Because sexual activity level determines all sexual behaviors, adjusted associations have no residual confounding due to sexual risk factors. Second, we restricted analyses to only individuals in sexual partnerships. This partially controls for the correlation by removing immortal person-time from single individuals not at risk for any HPV infection. Third, we restricted analyses to only those individuals who had HPV type 2-infected partners (at-risk partnered individuals). This restriction should completely remove the correlation between the times-at-risk for infection with both HPV types, as it conditions on all individuals being at risk.

Sensitivity analyses

Natural history and sexual behavior. We varied natural history and sexual behavior parameters in sensitivity analyses. Infection prevalences were allowed to vary in response to parameter changes.

Interactions. We varied the interaction parameters χ_i and φ_i to evaluate whether the different studies could correctly estimate the effects of hypothetical interactions. We varied interaction parameters separately ($\varphi_i = 1$ when χ_i is varied and vice versa). HPV prevalences were allowed to vary in response to interactions.
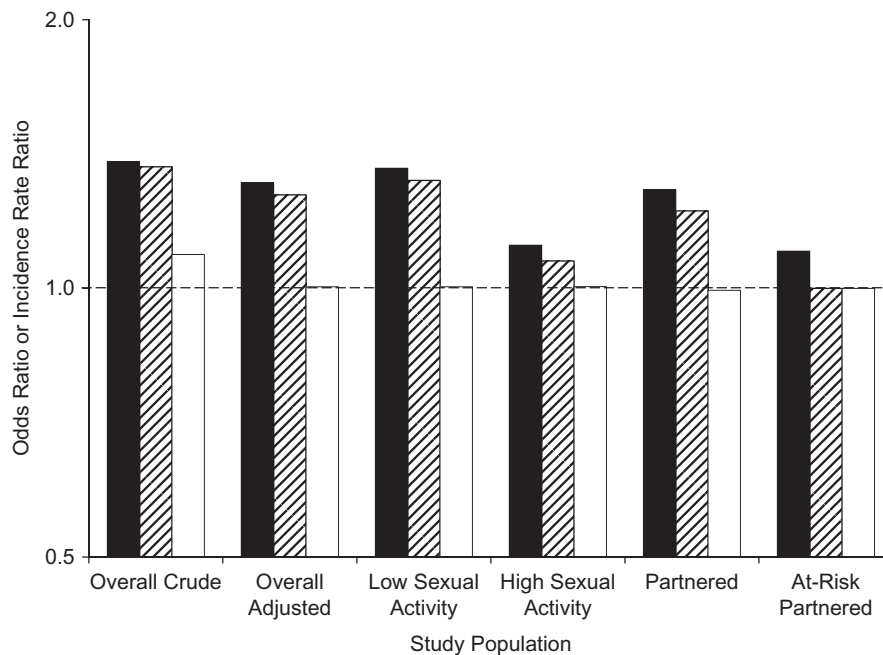
Figure 2. Measures of association between 2 types of human papillomavirus (HPV), designated type 1 and type 2, in the absence of interactions. The prevalence odds ratio (POR) (black columns) compares odds of a prevalent HPV type 2 infection in HPV type 1-infected versus -uninfected individuals. The incidence rate ratio (IRR) by infection status (striped columns) compares the HPV type 2 incidence rate in HPV type 1-infected versus -uninfected individuals. The IRR by immunity status (white columns) compares the HPV type 2 incidence rate in HPV type 1-immune versus -nonimmune individuals. The POR and IRRs are calculated in the full population including both single and partnered individuals ("Overall Crude"), in the full population with adjustment for sexual activity level ("Overall Adjusted"), in persons of each sexual activity level ("Low Sexual Activity" and "High Sexual Activity"), only among persons in current sexual partnerships ("Partnered"), and only among persons in current sexual partnerships with HPV type 2-infected partners ("At-Risk Partnered"). All associations (except for the overall crude association) were adjusted for sexual activity level. The y-axis is on a base-2 log scale.

RESULTS

### Base-case scenario assuming no interactions between HPV types

When we assumed no interactions between HPV types ($\chi_i = \varphi_i = 1$), the difference between the measured POR/IRRs and 1 constituted the bias in the estimation of the interaction effect.

The crude POR and IRRs measured in the overall population were 1.39, 1.37, and 1.09, respectively, in the absence of any interactions (Figure 2, Web Table 2). Adjustment for sexual activity level almost completely removed the bias for the IRR by immunity status (IRR = 1.00) but only slightly reduced the bias for the POR and the IRR by infection status. Residual bias in the POR and IRR by infection status was higher in the low sexual activity level (POR = 1.36, IRR = 1.32) than in the high sexual activity level (POR = 1.12, IRR = 1.07). Restricting the analysis to only individuals with sexual partners did not substantially reduce these associations. When the analysis was restricted to individuals at risk (those in sexual partnerships with HPV type 2-infected partners), both prospective IRRs estimated no association between HPV types 1 and 2 (IRRs = 1.00), but the POR was still greater than 1. This restriction thus eliminates the

prospective correlation between the times-at-risk for HPV infections, but it does not control for correlation between the times-at-risk from past sexual partnerships, which affects cross-sectional measures of association.

Sensitivity analyses assuming no interactions between HPV types

Natural history. Adjusted measures of association in the overall population were greater than or equal to 1 in the absence of any interactions under most biologically plausible parameter values for infection duration and transmission probability. The bias in the POR was larger when we assumed fairly short (<4 years) or long (>10 years) average durations of infection (Figure 3A). The adjusted IRR by infection status was also greater than or equal to 1 under most parameter values for duration and transmission, but it could be less than 1 when high transmission probabilities (>65%) were combined with infection durations longer than 1.4 years (Figure 3B). This is because at high transmission probabilities HPV types are almost always transmitted during the same sex act from coinfected partners, diminishing the positive prospective correlation. The adjusted IRR by immunity status was very close to 1 under most parameter
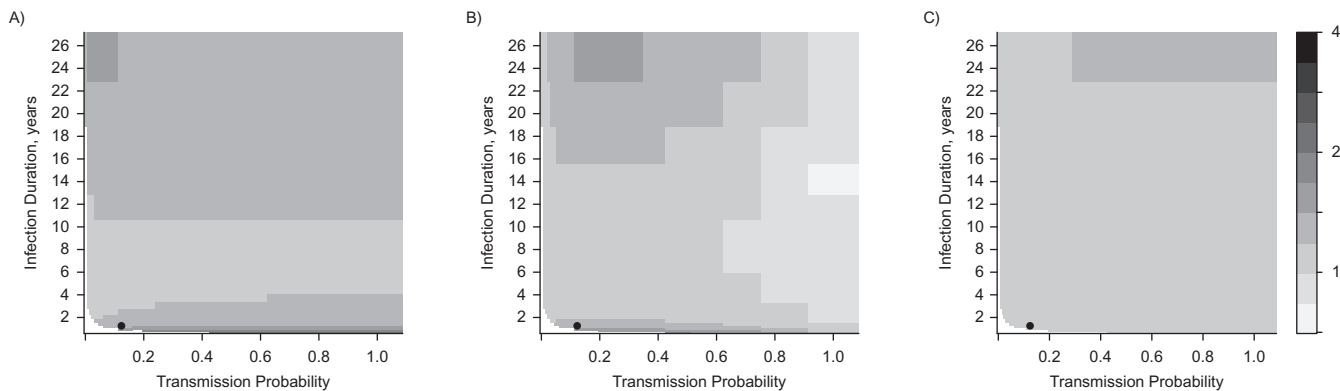
Figure 3. Adjusted measures of association between 2 types of human papillomavirus (HPV), designated type 1 and type 2, under different combinations of infection duration (in years) and transmission probabilities (per sex act with an infected individual). No interactions between HPV types are assumed. A) Prevalence odds ratio (POR); compares HPV type 2 infection prevalence odds in HPV type 1-infected versus -uninfected individuals. B) Incidence rate ratio (IRR) by infection status; compares the HPV type 2 incidence rate in HPV type 1-infected versus -uninfected individuals. C) IRR by immunity status; compares the HPV type 2 incidence rate in HPV type 1-immune versus -nonimmune individuals. All PORs and IRRs are measured in the overall population and adjusted for sexual activity level using Mantel-Haenszel estimators. White areas represent combinations of transmission probabilities and infection durations for which sustained transmission becomes impossible and the infections are eliminated from the model population. The black circle represents the base-case scenario.

values and only became noticeably larger than 1 when infection durations were very long (>20 years) (Figure 3C).

Sexual behavior. The bias in the POR and IRR by infection status in the low sexual activity level was extremely sensitive to sexual behavior (Figure 4). The bias in the POR and IRR by infection status was larger in the low sexual activity level (Figure 4D–4F) than in the high sexual activity level (Figure 4A–4C), and this difference substantially increased when the sexual mixing became more assortative within sexual activity levels (ε approaches 1) and when the low sexual activity level had lower rates of partner change (<1.0 partner change/year). This is because the decreasing infection prevalence and higher assortativity among low-sexual-activity individuals leads to more of their partners being co-uninfected, which substantially increases the correlation between their times-at-risk of infection with both HPV types. The IRR by immunity status was very close to 1 for both sexual activity levels under most sexual behavior parameter values, and the bias only appreciably increased in the low sexual activity level when there was a very high within-level assortativity combined with a very low rate of partner change (<0.5/year).

## Sensitivity analyses assuming interactions between HPV types

When we assumed interactions between HPV types, the difference between the measured POR/IRRs and the true modeled interaction effects χ_2 or φ_2 constituted the bias (Figure 5).

If we assumed that current infection with HPV type 1 strongly increased the probability of being infected with HPV type 2, the adjusted POR and IRR by infection status in the overall population correctly measured the direction of the interaction (>1) but could underestimate the facilitative effect (Figure 5A and 5C; interaction effect $\chi_2 > 1$). This underestimation occurs because, as facilitative interactions become stronger, the duration of partnerships and the time spent single become limiting factors in acquiring new HPV types, reversing the direction of the bias. When we assumed that current infection with HPV type 1 reduced the probability of being infected with HPV type 2 (Figure 5A and 5C; interaction effect $\chi_2 < 1$), the POR and IRR by infection status estimated a less protective association than the actual modeled effect. The IRR by immunity status was not a valid estimator of interaction effects caused by current infection (Figure 5E).

If immunity to HPV type 1 reduced the probability of being infected with HPV type 2 (Figure 5B and 5D; interaction effect $\varphi_2 < 1$), the POR and the IRR by infection status in the overall population estimated a strongly positive association of more than 1 even though current infection was assumed to have no effect ($\chi_2 = 1$). Persons immune to HPV type 1 [R_1S_2] are in the unexposed category for these ratios and contribute cases and person-time to the denominator (equation 2). If there is cross-immunity, they will thus contribute to a lower-than-expected rate of HPV type 2 infection in HPV type 1-uninfected individuals. The IRR by immunity status in the overall population correctly estimated the direction of the modeled interaction effect φ_2 but underestimated its magnitude (Figure 5F, gray dots).

When we restricted analyses to at-risk individuals in sexual partnerships with HPV type 2-infected partners, the IRR by infection status correctly estimated the modeled interaction effect χ_2 caused by infection (Figure 5C, black dots), and the IRR by immunity status correctly estimated the modeled interaction effect φ_2 caused by immunity (Figure 5F, black dots).
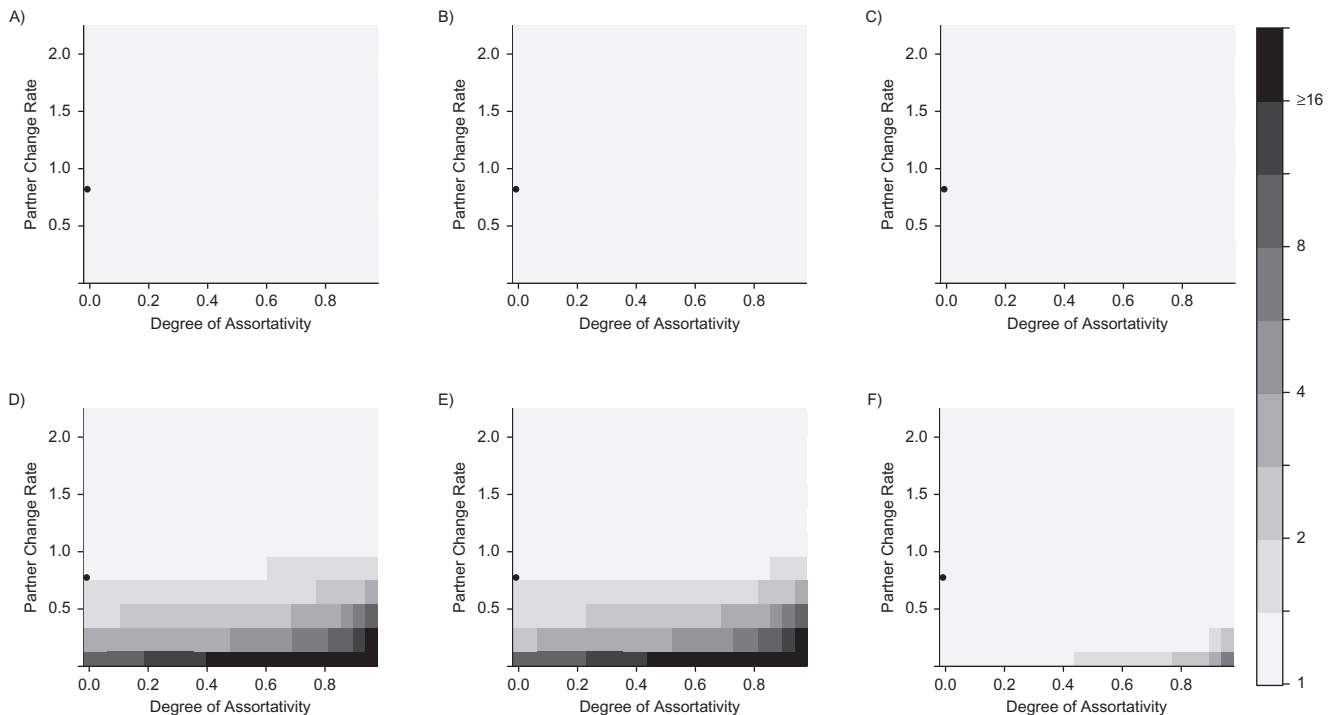
Figure 4. Sexual-activity-level–stratified measures of association between human papillomavirus (HPV) types 1 and 2 under different combinations of the partner change rate (per year) in the low sexual activity level and the degree of assortativity between sexual activity levels. A) Prevalence odds ratio (POR) in the high sexual activity level. B) Incidence rate ratio (IRR) by infection status in the high sexual activity level. C) IRR by immunity status in the high sexual activity level. D) POR in the low sexual activity level. E) IRR by infection status in the low sexual activity level. F) IRR by immunity status in the low sexual activity level. No interactions between HPV types are assumed. Black areas indicate values greater than or equal to 16 for the measured associations. The degree of assortativity corresponds to the proportion of sexual contacts made exclusively with members of the same sexual activity level (0 = proportionate, 1 = completely assortative). The black circle represents the base-case scenario.

DISCUSSION

In this study, we found that the correlation between the times-at-risk for HPV infections can substantially bias the estimation of interactions between HPV types in cross-sectional and prospective studies. Relative risk measures in most cases estimated associations between HPV types greater than 1 in the absence of any interaction. This correlation persisted after perfect adjustment for sexual activity. The infection IRR by immunity status was much less affected by this correlation than the POR and IRR by infection status, probably because immunity markers are correlated with past infection risk and are less strongly correlated with current risk for new HPV infections. Interactions between HPV types could not be validly estimated in our cross-sectional analyses. When analyses were restricted to time periods in which individuals were at risk for the outcome HPV type infection due to sexual contact with an infected partner, then the IRR by infection status validly estimated interaction effects caused by current infection with a first HPV type, and the prospective IRR by immunity status validly estimated interaction effects caused by immunity to a first HPV type. When we assumed that immunity with a first HPV type reduced the risk of being infected with a second HPV type per infected

contact (competitive interaction), the POR and IRR by infection status measured a strong association (>1) between HPV type incidences.

The observed clustering of HPVs in cross-sectional studies is often interpreted by epidemiologists as evidence against competitive cross-immune interactions that could lead to type replacement. However, our results indicate that cross-immunity could contribute to the clustering of HPV types because cross-immune individuals are protected against both types and would be included in the "co-uninfected" category in cross-sectional and prospective studies. Prospective epidemiologic studies which use HPV seropositivity rather than infection as the exposure are likely to provide more valid estimates of antibody-mediated cross-protective interactions between HPV types. In marked contrast with studies using other designs, prospective studies using seropositivity as the exposure generally find that persons who are seropositive for a first HPV type have no significantly increased risk of new infections with related HPV types or have a decreased risk of such infections (41, 44–46). Therefore, the possibility of competitive cross-immune interactions between HPV types cannot be dismissed, as they could be masked or underestimated in these studies due to residual confounding and the correlation between times-at-risk for infection.
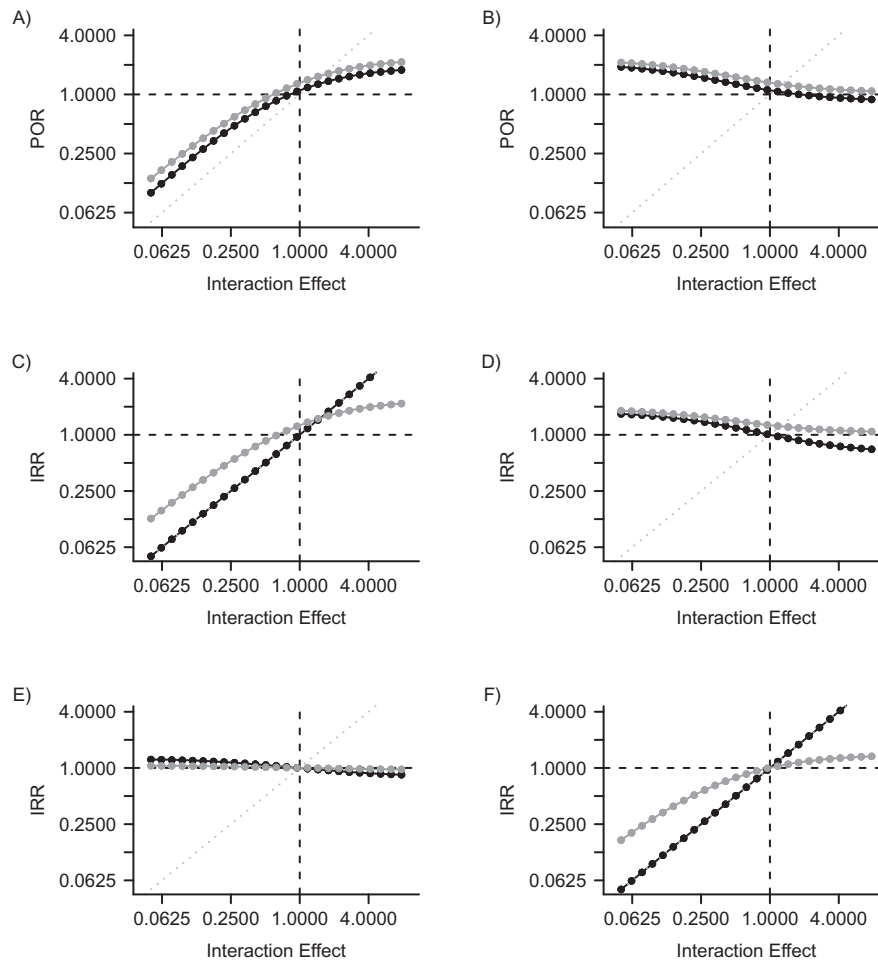
Figure 5.   Estimated adjusted measures of association between 2 types of human papillomavirus (HPV), type 1 and type 2, when interactions are modeled. The *y*-axis corresponds to associations measured in simulated epidemiologic studies. The *x*-axis represents the actual modeled underlying interactions. A) Prevalence odds ratio (POR) for HPV type 2 infection in HPV type 1-infected versus -uninfected individuals according to interaction effects caused by current infection (χ_2). B) POR for HPV type 2 infection in HPV type 1-infected versus -uninfected individuals according to interaction effects caused by immunity (φ_2). C) Incidence rate ratio (IRR) for HPV type 2 infection in HPV type 1-infected versus -uninfected individuals according to interaction effects caused by current infection (χ_2). D) IRR for HPV type 2 infection in HPV type 1-infected versus -uninfected individuals according to interaction effects caused by immunity (φ_2). E) IRR for HPV type 2 infection in HPV type 1-immune versus -nonimmune individuals according to interaction effects caused by current infection (χ_2). F) IRR for HPV type 2 infection in HPV type 1-immune versus -nonimmune individuals according to interaction effects caused by immunity (φ_2). Gray points correspond to associations measured in the overall population, adjusted for sexual activity level. Black points correspond to associations measured in analyses restricted to at-risk partnered individuals (persons in partnerships with HPV type 2-infected partners). Left-hand panels (A, C, and E) show the influence of interaction effect χ_2—the relative probability of HPV type 2 infection per sex act with an infected partner among persons who are HPV type 1-infected compared with persons who are HPV type 1-susceptible. Right-hand panels (B, D, and F) show the influence of interaction effect φ_2—the relative probability of HPV type 2 infection per sex act with an infected partner among persons who are HPV type 1-immune compared with persons who are HPV type 1-susceptible. Values to the left of 1.0000 on the *x*-axis correspond to modeled competitive interactions, while values to the right of 1.0000 correspond to modeled facilitative interactions. The dotted gray line represents the theoretical line of equality, where epidemiologic measures of association would validly estimate the actual modeled interactions. Both axes use a base-2 log scale.

To our knowledge, this study is the first to identify and illustrate the correlation between times-at-risk of infection as a source of bias in epidemiologic data and to suggest study designs which reduce the bias. Researchers in previous studies had also found that PORs greater than 1 can be measured when assuming no interactions between HPV types and that competitive HPV interactions could reproduce epidemiologic data (47, 48), but they had not explored the source of this

bias or included sexual risk heterogeneity. Our results suggest that integrating sexual risk heterogeneity and mixing based on empirical data would be necessary for transmission models to validly estimate the interactions between HPV types from epidemiologic data.

Our sensitivity analyses suggest that the correlation between the times-at-risk for infection can be generalized to other STIs, such as HIV or herpes simplex virus, which

have low transmission probabilities but long durations of infection (49, 50). Ulcerative STIs could plausibly increase HIV acquisition by disrupting the host's mucosal integrity and increasing the availability of HIV-susceptible cells (6); however, the magnitude of this interaction may not have been correctly estimated in epidemiologic studies due to bias (17). An overestimation of the effect of STIs on HIV acquisition might partly explain why clinical trials of interventions against STIs have shown disappointing effectiveness against HIV incidence (51).

Our study shows that the correlation between the times-at-risk for infection in prospective studies can be controlled by restricting analyses to times at which individuals are at risk for the outcome STI due to sexual contacts with an infected partner. However, this restriction is very challenging, as it requires detailed information on individuals' sexual partners. This control could be achieved with a discordant couples' study (52), but this design is often unfeasible due to the large sample sizes required for adequate statistical power and the ethical need to provide treatment for nonviral STIs. Ascertainment of the real-time STI status of individuals is also challenging because of test measurement errors and the need to frequently retest people (18).

Interestingly, we found that associations between HPV types were consistently larger in persons with lower rates of partner change. Persons with low rates of partner change have lower rates of infection, but their infections tend to be more strongly correlated over time because they spend more time not at risk between partners and stay longer in partnerships. This heterogeneity by sexual activity has been observed empirically, with individuals or populations with lower levels of sexual activity showing higher associations between STIs (1–3, 9, 23, 27).

Our study presents some limitations. Firstly, we used perfect measures of infection incidence to isolate the bias due to correlation between times-at-risk of infection with different HPV types. However, there will be information/confounding biases in empirical data which should be considered (17–19, 53). Secondly, we made the simplifying assumption that immunity is acquired after an individual clears infection. However, people may seroconvert while they are still infected with a type of HPV (32). We could not evaluate the influence of simultaneously infected/seropositive individuals in studies. This classification issue did not affect our main results with no interactions because infected and immune individuals have the same rates of infection with new types, but it would affect interaction sensitivity analyses. However, because the temporal overlap is short relative to the duration of seropositivity (only 3%–10% of seropositives are infected) (54), this should not have substantially affected our results. Thirdly, in order to eliminate the influence of stochastic error and truly isolate the systematic bias due to correlation between the times-at-risk, we chose to use a simple deterministic model. However, the magnitude of the bias was extremely sensitive to sexual partner change rates and mixing patterns. Base-case results are therefore not quantitative predictions of the empirical magnitude of bias in epidemiologic studies. To confirm that our results were not a result of our simple model structure, we calculated the POR for 2 HPV types using an individual-based stochastic model of HPV transmission, calibrated to the Canadian population (55), which had 4 levels of sexual activity, assortative mixing by age and sexual activity, and no interactions between HPV types (Web Figure 3). Results were qualitatively similar between models: Estimated PORs were substantially superior to 1 without interactions after adjustment for sexual activity, and they were highest in lower sexual activity levels and at ages with lower partner change rates. The PORs were much larger, however, in a stochastic model with more sexual risk heterogeneity. Our base-case results thus likely underestimate the true magnitude of the bias in empirical data.

There are many important challenges in measuring biological interactions between STIs (17–19). These interactions should be evaluated in epidemiologic studies because of their importance for the success of public health interventions against STIs. However, associations between STIs suffer from various biases which make the estimation and interpretation of causal effects problematic. We showed that both cross-sectional and prospective measures of association between STIs are likely to be substantially biased due to the correlation between the times-at-risk for STIs. While this bias cannot be controlled in cross-sectional studies, prospective studies can be designed to validly measure interactions if analyses are restricted to time periods in which individuals are at risk for the outcome STI. Realization of such studies nonetheless presents significant practical challenges and may not be feasible in most circumstances. Therefore, using STI transmission models to interpret results from epidemiologic studies could help us better understand biological interactions between STIs.

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