Effect of HSV-2 infection on subsequent HIV acquisition: an updated systematic review and meta-analysis

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Summary

Background HIV and herpes simplex virus type 2 (HSV-2) infections cause a substantial global disease burden and are epidemiologically correlated. Two previous systematic reviews of the association between HSV-2 and HIV found evidence that HSV-2 infection increases the risk of HIV acquisition, but these reviews are now more than a decade old.

Methods For this systematic review and meta-analysis, we searched PubMed, MEDLINE, and Embase (from Jan 1, 2003, to May 25, 2017) to identify studies investigating the risk of HIV acquisition after exposure to HSV-2 infection, either at baseline (prevalent HSV-2 infection) or during follow-up (incident HSV-2 infection). Studies were included if they were a cohort study, controlled trial, or case-control study (including case-control studies nested within a cohort study or clinical trial); if they assessed the effect of pre-existing HSV-2 infection on HIV acquisition; and if they determined the HSV-2 infection status of study participants with a type-specific assay. We calculated pooled random-effect estimates of the association between prevalent or incident HSV-2 infection and HIV seroconversion. We also extended previous investigations through detailed meta-regression and subgroup analyses. In particular, we investigated the effect of sex and risk group (general population *vs* higher-risk populations) on the relative risk (RR) of HIV acquisition after prevalent or incident HSV-2 infection. Higher-risk populations included female sex workers and their clients, men who have sex with men, serodiscordant couples, and attendees of sexually transmitted infection clinics.

Findings We identified 57 longitudinal studies exploring the association between HSV-2 and HIV. HIV acquisition was almost tripled in the presence of prevalent HSV-2 infection among general populations (adjusted RR 2·7, 95% CI 2·2–3·4; number of estimates [N_e]=22) and was roughly doubled among higher-risk populations (1·7, 1·4–2·1; N_e=25). Incident HSV-2 infection in general populations was associated with the highest risk of acquisition of HIV (4·7, 2·2–10·1; N_e=6). Adjustment for confounders at the study level was often incomplete but did not significantly affect the results. We found moderate heterogeneity across study estimates, which was explained by risk group, world region, and HSV-2 exposure type (prevalent *vs* incident).

Interpretation We found evidence that HSV-2 infection increases the risk of HIV acquisition. This finding has important implications for management of individuals diagnosed with HSV-2 infection, particularly for those who are newly infected. Interventions targeting HSV-2, such as new HSV vaccines, have the potential for additional benefit against HIV, which could be particularly powerful in regions with a high incidence of co-infection.

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Introduction

HIV and herpes simplex virus type 2 (HSV-2) are two global health problems. In 2015, 36·7 million people were estimated to be living with HIV/AIDS globally.[1] In 2012, an estimated 417 million people aged 15–49 years had HSV-2 infection.[2] 70% of HIV infections are in sub-Saharan Africa, which also has the highest HSV-2 prevalence.[1,2] HSV-2 causes genital herpes, which, when symptomatic, is characterised by periodic recurrences of painful genital ulcers.[3] Although genital herpes is asymptomatic or unrecognised in about 80–90% of individuals, asymptomatic viral reactivation and shedding are common.[3] Thus, although individuals with HSV-2 are most infectious when they are symptomatic, most transmissions are thought to occur when the source partner is asymptomatic.[4] Genital HSV-2 infection can considerably affect relationships through feelings of shame and stigma and concerns about risk of transmission.[5,6]

HIV and HSV-2 are both lifelong sexually transmitted infections (STIs) that are associated with similar risk factors (eg, age, sex, partner change rate, condom use, male circumcision).[7–10] Additionally, evidence exists of direct and reciprocal biological interactions between HIV and HSV-2.[11,12] Active HSV-2 infection, regardless of symptoms, involves high concentrations of activated CD4-positive T cells, which are target cells for HIV, in the genital area and can lead to breaks in the mucosal layer

Research in context

Evidence before this study

Herpes simplex virus type 2 (HSV-2) and HIV are both lifelong infections sharing common risk factors and widely co-occur geographically. Two meta-analytic systematic reviews of longitudinal studies have been published to date and have shown the adjusted risk of HIV acquisition among individuals with prevalent HSV-2 infection to be two to three times the risk in those without. This increased risk is thought to be caused by the recruitment of activated CD4-positive T cells to the genital area, which are target cells for HIV, and breaks in the protective epithelial layer during active HSV-2 infection. However, the most recent of these reviews is now more than a decade old and only included 19 studies, limiting the scope for assessing the effect of heterogeneity and study quality. The substantial number of new studies published on the subject since then allows for more in-depth investigation, including, for the first time, quantification of the effect of exposure to incident in addition to prevalent HSV-2 infection on pooled estimates of the association with HIV acquisition. For this systematic review and meta-analysis, we searched PubMed, MEDLINE, and Embase between Jan 1, 2003, and May 25, 2017, for studies of the association between incident or prevalent HSV-2 infection and HIV infection. Studies were included if they were a cohort study, controlled trial, or case-control study (including case-control studies nested within a cohort or controlled trial); were designed to assess the effect of preceding HSV-2 infection on HIV acquisition; and used a type-specific antibody assay to determine HSV-2 infection (serostatus). 57 longitudinal studies met our inclusion criteria, which was 38 more than the last systematic search and review (by Freeman and colleagues).

Added value of this study

We report that the pooled adjusted risk of HIV acquisition after incident HSV-2 infection is almost five times the risk without HSV-2 infection and almost twice the risk associated with exposure to prevalent HSV-2 infection. These findings provide a strong indication for a biological effect of HSV-2 infection on HIV, because the frequency and severity of genital ulceration, viral shedding, and associated inflammation in the genital tract are highest in new HSV-2 infections and tend to decrease with time after infection. The association was higher among general populations than among higher-risk populations. Study-level adjustment for confounders was often incomplete, but did not meaningfully affect the association when comparing crude and adjusted pooled estimates. Heterogeneity across study estimates was moderate. We extended previous reviews through detailed assessment of heterogeneity using meta-regression and sub-pooling and through extensive assessment of potential biases. We found limited evidence of publication bias.

Implications of all the available evidence

Our results provide evidence in support of a direct effect of HSV-2 infection on HIV acquisition, which is strengthened by our finding of significantly higher HIV risk associated with incident HSV-2 infection than with prevalent HSV-2 infection. At the population level, new interventions targeting HSV-2, such as new vaccines or microbicides, could have an additional indirect benefit on HIV as a consequence of the interactions between HIV and HSV-2. Such synergies could greatly enhance the effect of combination prevention for HIV infection, particularly in settings with high HIV prevalence. The magnitude of this public health benefit now needs to be carefully estimated for different settings by use of mathematical models informed by the most recent evidence of the associations between HSV and HIV.

through which HIV can enter.[13] Because genital ulceration and viral shedding occur most frequently in the first year of HSV-2 infection,[14–16] the increase in HIV susceptibility might be highest for incident HSV-2 infections. Co-infection with HIV increases HSV-2 genital shedding and transmissibility, while HSV-2 infection correlates with increased HIV viraemia and transmissibility.[17–20]

Development of multipurpose prevention products that could protect against multiple STIs (eg, topical microbicides and oral pre-exposure prophylaxis) would provide exciting opportunities to simultaneously reduce the burden of disease of more than one infection.[21–23] Quantifying the effect of HSV-2 infection on HIV acquisition has important public health implications, particularly in high-prevalence settings where co-infection is common, because prevention of HSV-2 infection (with single-purpose or multipurpose prevention tools) might indirectly prevent HIV infection. Although trials of use of daily suppressive antiviral therapy against HSV-2 have not shown reduced risk of HIV acquisition or transmission,[24–26] perhaps because agents were not used at sufficient doses or for sufficient duration, new vaccines against HSV-2 that are currently under development[27] could hold more promise for HIV prevention.

Two systematic reviews and meta-analyses of the association between HSV-2 infection and subsequent HIV acquisition have been done: one in 2002[12] and the other in 2006[11] (the one in 2006 was briefly updated in an editorial[28]). These reviews reported a two to three times increase in the risk of HIV infection with baseline prevalent HSV-2 infection;[11,12] estimates of the association tended to be lower for high-risk populations than for low-risk populations. Here we update and substantially augment these reviews to modernise our understanding of the interaction between HSV-2 infection and HIV, in line with the pace of advancing prevention efforts against HSV-2 and HIV.

Methods

Search strategy and selection criteria

For this systematic review and meta-analysis, we searched PubMed, MEDLINE, and Embase between Jan 1, 2003, and May 25, 2017, to identify studies of the relative risk (RR) of HIV acquisition after exposure to HSV-2 infection either at baseline (ie, prevalent infection) or during follow-up (ie, incident infection), published since the review by Freeman and colleagues.[11] For PubMed, we searched for articles and abstracts using the terms ("HIV", "human immunodeficiency virus", "human immunedeficiency virus", "human immune deficiency virus", OR "human immuno deficiency virus") AND ("HSV", "herpes simplex", "herpes virus type 2", "herpes virus 2", "herpesvirus 2", "genital herpes" OR "herpes genitalis"). Articles in PubMed were also searched with the Medical Subject Headings terms ("herpes simplex" OR "simplexvirus") AND ("human immunodeficiency virus", "HIV infection", "HIV antibodies", "HIV seronegativity", OR "HIV seroprevalence"). We included studies if they were a cohort study, controlled trial, or case-control study (including case-control studies nested within a cohort or controlled trial); were designed to assess the effect of preceding HSV-2 infection, compared with HSV-2 negativity, on HIV acquisition (ie, excluding case-control studies based on samples from only one point in time and studies that only looked at the effect of pre-existing HIV infection on HSV-2 acquisition); and determined the HSV-2 infection status of study participants with an antibody-type-specific assay.

Individuals were defined as HSV-2 negative (unexposed) if they remained HSV-2 seronegative throughout follow-up (preferably) or were HSV-2 seronegative at baseline (for those studies in which HSV-2 testing was not done during follow-up; repeat testing that was done but not reported was noted as a possible source of reporting bias for the subsequent assessment of study quality). For full details of the search, selection criteria, and data extraction, see the appendix.

For studies measuring incident HSV-2 infection, we classified RR estimates of HIV acquisition after exposure to incident HSV-2 infection into five subcategories for timing sequence. These subcategories reflected uncertainty in the exact timing of HSV-2 and HIV seroconversion: (1) HSV-2 seroconversion was observed in a previous time interval and thus HSV-2 infection happened before HIV (definitely before); (2) HSV-2 seroconversion was observed in the same time interval as HIV seroconversion and so HSV-2 infection might have happened before or after HIV infection (indeterminably close); (3) HSV-2 seroconversion was observed in a previous or in the same time interval as HIV seroconversion (before and indeterminably close); (4a) some HSV-2 seroconversion might have occurred after HIV infection (maybe after and indeterminably close or before); and (4b) some HSV-2 seroconversion was observed after HIV infection (after and indeterminably close or before; appendix).

Two authors (KJL and JARE) did the systematic review and meta-analysis, according to PRISMA[29] and MOOSE[30] guidelines. Conflicts about inclusion were resolved through discussion between reviewers.

Data analysis

Based on previous evidence suggesting differential susceptibility to HIV and HSV-2 by sex,[7–9] after exposure to incident HSV-2 infection,[14–16] and by risk behaviour,[10] we defined a priori that our primary outcomes would be pooled RR estimates of the association between incident HIV infection and pre-existing prevalent or incident HSV-2 infection, for both women and men and by risk group (general population *vs* higher-risk populations), also allowing for comparisons with previous pooled estimates.[11] Higher-risk populations included female sex workers and their clients, men who have sex with men, serodiscordant couples, and attendees of STI clinics. No other stratification was used. To minimise biases due to reverse causation, pooled RR estimates for the association between HIV infection and exposure to incident HSV-2 infection were restricted to study estimates when HSV-2 seroconversion was definitely known to have occurred before HIV seroconversion.

To assess study quality, we used the Newcastle-Ottawa Scale[31] to define nine criteria assessing selection of study participants, sample representativeness, exposure or outcome ascertainment, and confounding for the extracted information about participant and study characteristics. A star was awarded for each predetermined criterion that was met (appendix). This assessment was done at the estimate level, not the study level. The effect of the number of stars awarded and other measures of study quality related to study characteristics on RR estimates was subsequently explored with meta-regression and subgroup analyses.

We investigated the effect of heterogeneity across independent RR estimates using the I^2 statistic.[32] Sources of heterogeneity were explored with univariate meta-regression analysis of independent adjusted RR estimates, which estimated the fraction of the between-study variance in adjusted RR estimates (ie, R^2) explained by participant characteristics (eg, world region), study characteristics (eg, study design), and study quality (eg, number of Newcastle-Ottawa Scale stars). Multivariate meta-regression analysis was also done with estimates for exposure to prevalent HSV-2 infection, but not with estimates for exposure to incident HSV-2 infection because of the small number of estimates (N_e). Additionally, we did separate subgroup analyses of adjusted RR estimates for the incident and prevalent HSV-2 exposures (appendix).

See Online for appendix

Publication bias was assessed quantitatively at the estimate level, not the study level, in two ways. First, we produced funnel plots[33] and did Egger's test for publication bias[34] for both crude RR and adjusted RR estimates and for exposure to prevalent compared with exposure to incident HSV-2 infection (appendix).

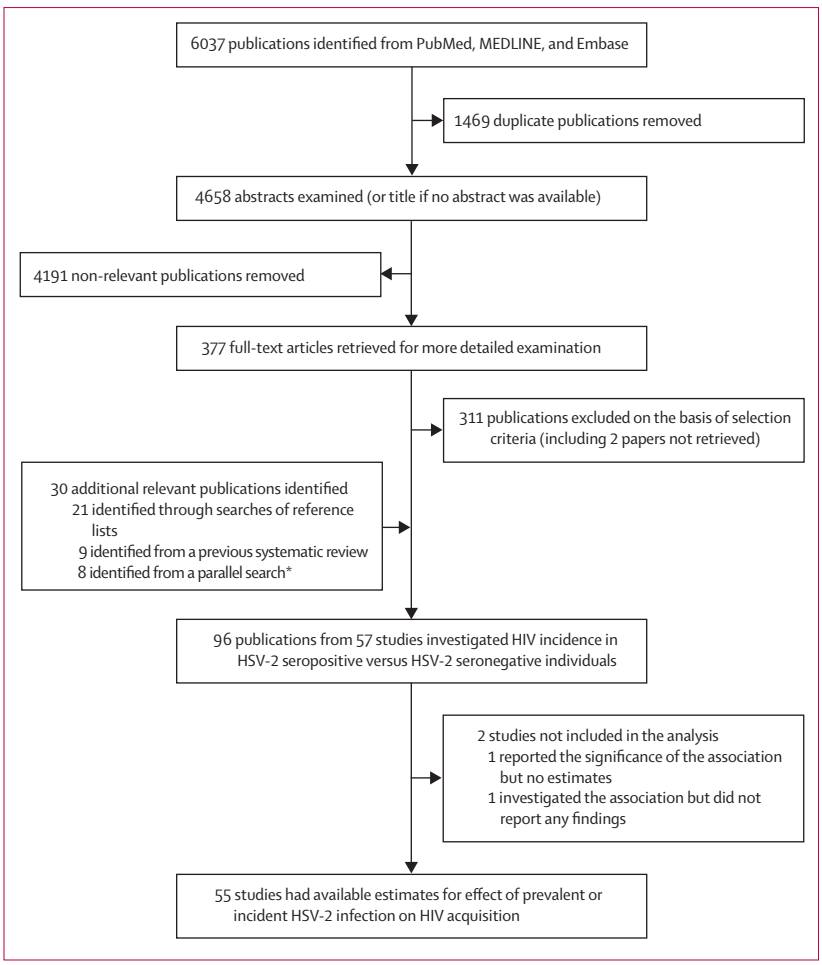

Figure 1: **Study selection**
*The search was done in parallel by two reviewers (KJL and JARE). The results for one reviewer are shown; the results were very similar between the two reviewers. Each reviewer found eight publications to be relevant that the other reviewer did not. HSV-2=herpes simplex virus type 2.

Second, we assessed with meta-regression analysis whether crude RR estimates calculated from the available data were less likely to be significant than those provided directly in the paper. Third, we qualitatively assessed whether there was evidence of selective reporting of significant results.

All meta-analyses, meta-regressions, subgroup analyses, and forest plots were done with R version 3.2.2. We derived pooled RR estimates with natural log-transformed study estimates and SEs with random-effect models, based on the DerSimonian-Laird inverse-variance method,[35] using the metafor package in R.[36] Pooled estimates were then back-transformed to the original scale (further details in the appendix).

Role of the funding source

This study was funded by WHO through the Unified Budget, Results and Accountability Framework (UBRAF) from the Joint United Nations Programme on HIV/AIDS (UNAIDS). SLG from WHO commissioned the study, contributed to the direction of the work, and commented on the drafts. KJL and JARE had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We identified 96 relevant publications from 57 independent studies in the systematic review (figure 1). 54 studies reported on the RR of HIV acquisition after exposure to prevalent HSV-2 infection and 28 studies reported on the RR of HIV acquisition after exposure to incident HSV-2 infection (table 1; appendix). More studies reported on men than on women and on female sex workers, men who have sex with men, and other higher-risk groups than on general populations (table 1). Most studies were done in Africa, in populations where baseline HSV-2 prevalence was greater than 30%, and were observational cohort studies with follow-up exceeding 1 year. Additionally, most studies tested for HIV every 6 months or more frequently and defined the unexposed group for exposure to prevalent HSV-2 infection as participants who were HSV-2 (sero)negative at baseline. Key potential confounders that were adjusted for included age and sexual behaviour, while several studies inappropriately adjusted for genital ulcer disease.

39 studies reported 55 adjusted RR estimates for our primary outcome: HIV acquisition after exposure to prevalent or incident HSV-2 infection (timing of HSV-2 infection definitely before HIV infection) among general and higher-risk populations, by sex (figure 2). No significant differences were seen in the associations among general populations by sex. The overall pooled adjusted RR for general populations was 2·7 (95% CI 2·2–3·4; N_e=22; I^2=59%) for exposure to prevalent HSV-2 infection and 4·7 (2·2–10·1; N_e=6; I^2=64%) for exposure to incident HSV-2 infection. The results for higher-risk populations were similar to those for general populations, but the magnitude of the associations was lower: the overall pooled adjusted RR estimate for higher-risk populations was 1·7 (1·4–2·1; N_e=25; I^2=45%) for exposure to prevalent HSV-2 infection and 2·9 (1·7–5·0; N_e=2; I^2=0%) for exposure to incident HSV-2 infection.

50 studies reported on 64 crude RR estimates for our primary outcome (appendix). Pooled crude RR estimates overall and by sex were similar to pooled adjusted RR estimates, although the pooled crude RR estimates for exposure to incident HSV-2 infection were somewhat higher than the adjusted RR estimates for general populations and were lower than the adjusted RR estimates for higher-risk populations. Crude estimates were generally more heterogeneous than adjusted estimates (figure 2; appendix).

In the univariate meta-regression analysis of 48 independent adjusted estimates (N_e=40 for prevalent HSV-2 infection; N_e=8 for incident HSV-2 infection), only risk group (higher-risk population *vs* general

	Prevalent HSV-2 infection*			Incident HSV-2 infection†		
	Number of studies (n=54)	Number of estimates		Number of studies (n=28)	Number of estimates	
		Crude RR	Adjusted RR		Crude RR	Adjusted RR
Participant characteristics						
Mean or median age‡§						
≤25 years	15	18	17	10	9	12
>25 years	39	68	54	20	31	35
Not reported	2	4	1	1	1	0
Sex‡						
All women	28	41	34	11	14	24
General population	11	17	15	4	10	13
Female sex workers	8	9	13	2	1	7
Other higher-risk populations¶	10	15	6	5	3	4
All men	28	37	31	17	23	19
General population	10	14	18	6	11	13
Men who have sex with men	13	16	9	9	11	3
Other higher-risk populations‖	5	7	4	3	1	3
Women and men combined**	8	12	7	5	4	4
WHO region						
Africa	35	64	56	16	27	38
Americas	8	11	5	5	7	3
Europe	1	1	0	1	1	0
Eastern Mediterranean	0	0	0	0	0	0
Southeast Asia	5	7	7	4	4	6
Western Pacific	4	4	2	2	2	0
World (not including Africa)	1	3	2	0	0	0
HSV-2 prevalence‡						
≤30%	13	14	12	8	8	6
>30%	42	75	59	21	33	41
Not reported	1	1	1	0	0	0
Study characteristics						
Study year (mid-point)‡						
Pre-2000	16	31	32	11	20	20
2000 onwards	33	54	34	16	19	25
Not reported	6	5	6	2	2	2
Study design						
Cohort	27	34	38	15	14	21
Case-control††	7	8	4	6	7	2
Controlled trial	20	48	30	7	20	24
Study design for analysis of controlled trial data						
Prospective	17	33	18	6	10	12
Nested case-control††	3	15	12	2	10	12
Controlled trial intervention group‡						
Intervention	6	8	2	1	2	2
Control	6	8	3	1	2	2
Combined	20	32	25	7	16	20
Overall number of participants for study‡						
≤1000	31	49	36	13	23	15
>1000	24	41	36	16	18	32

(Table 1 continues on next page)

	Prevalent HSV-2 infection*			Incident HSV-2 infection†		
	Number of studies (n=54)	Number of estimates		Number of studies (n=28)	Number of estimates	
		Crude RR	Adjusted RR		Crude RR	Adjusted RR
(Continued from previous page)						
Follow-up duration‡						
≤1 year	14	22	15	7	11	9
>1 year	37	64	52	18	26	37
Not reported	4	4	5	4	4	1
Length of time between tests for HIV						
≤6 months	36	55	41	17	22	27
>6 months	6	17	16	4	12	12
Mixture of short and long intervals	3	4	4	2	3	5
Not reported	9	14	11	5	4	3
HSV-2 assay cutoff (only studies with Focus HerpeSelect as known assay)						
1·1/manufacturer's recommendation/unknown	14	22	15	8	9	13
>1·1	9	11	14	4	4	9
Definition of prevalent HSV-2 infection exposure‡						
Baseline	47	79	60	NA	NA	NA
Baseline and >60 days before HIV seroconversion	1	2	7	NA	NA	NA
Baseline or >2 years before HIV seroconversion	1	1	1	NA	NA	NA
Before, or at same visit as, HIV seroconversion	3	5	2	NA	NA	NA
Same interval as HIV seroconversion	1	1	0	NA	NA	NA
At visit 6 months before HIV seroconversion	1	1	1	NA	NA	NA
Anytime	1	1	1	NA	NA	NA
Definition of incident HSV-2 infection exposure‡						
≤60 days before HIV seroconversion	NA	NA	NA	1	0	1
60 days before HIV seroconversion	NA	NA	NA	1	0	5
≤6 months before HIV seroconversion	NA	NA	NA	2	2	1
>6 months before HIV seroconversion	NA	NA	NA	1	1	1
≤2 years before HIV seroconversion	NA	NA	NA	1	1	1
Before, or at same visit as, HIV seroconversion	NA	NA	NA	2	2	1
Visit before HIV seroconversion	NA	NA	NA	1	1	1
Same interval as HIV seroconversion	NA	NA	NA	1	1	0
Anytime	NA	NA	NA	21	33	34
Not reported	NA	NA	NA	1	0	2
Definition of unexposed group‡						
HSV-2 negative at baseline	28	40	24	0	0	0
HSV-2 negative throughout follow-up	21	39	40	28	38	44
Not reported	6	11	8	1	3	3
Extraction of crude estimate‡‡‡						
Reported	32	45	NA	14	20	NA
Calculated from available data	23	45	NA	11	21	NA
Adjusted for male circumcision status (men or women and men combined)‡§§¶¶						
Yes	9	NA	14	5	NA	6
No	15	NA	21	9	NA	17
Unknown	3	NA	3	0	NA	0
Adjusted for condom use‡§§¶¶						
Yes	15	NA	25	8	NA	18
No	23	NA	43	12	NA	27
Unknown	4	NA	4	1	NA	2

(Table 1 continues on next page)

	Prevalent HSV-2 infection*			Incident HSV-2 infection†		
	Number of studies (n=54)	Number of estimates		Number of studies (n=28)	Number of estimates	
		Crude RR	Adjusted RR		Crude RR	Adjusted RR
(Continued from previous page)						
Adjusted for female hormonal contraceptive use (women or women and men combined)‡§§¶¶						
Yes	6	NA	13	4	NA	10
No	16	NA	26	8	NA	16
Unknown	2	NA	2	1	NA	2
Adjusted for any sexual behaviour (excluding condom use)‡§§¶¶						
Yes	29	NA	48	16	NA	33
No	8	NA	21	3	NA	12
Unknown	3	NA	3	1	NA	2
Adjusted for genital ulcer disease‡§§¶¶						
Yes	9	NA	18	8	NA	16
No	29	NA	51	11	NA	30
Unknown	3	NA	3	1	NA	1
Adjusted for number of sexual partners‡¶¶						
Yes	20	NA	33	11	NA	24
No	16	NA	32	8	NA	18
Unknown	5	NA	7	3	NA	5
Adjusted for age‡¶¶						
Yes	34	NA	63	14	NA	41
No	8	NA	9	5	NA	5
Unknown	0	NA	0	1	NA	1
Timing of incident HSV-2 infection relative to HIV acquisition‡‖‖						
1 (definitely before)	NA	NA	NA	7	8	14
2 (indeterminably close)	NA	NA	NA	2	2	0
3 (before and indeterminably close)	NA	NA	NA	12	12	11
4a (maybe after and indeterminably close or before)	NA	NA	NA	8	14	17
4b (after and indeterminably close or before)	NA	NA	NA	5	5	5
Type of estimate‡						
Hazard ratio	29	31	43	15	13	25
Incidence ratio	17	22	11	8	8	9
Odds ratio	21	37	18	11	20	13

HSV-2=herpes simplex virus type 2. RR=relative risk. NA=not applicable. STI=sexually transmitted infection. *The crude number of estimates for all studies was 90 and the adjusted number of estimates for all studies was 72. †The crude number of estimates for all studies was 41 and the adjusted number of estimates for all studies was 47. ‡Same study included in more than one subcategory. §Values might be estimated from ranges. ¶Women with higher-risk sexual behaviour, women working in food and recreational facilities, STI clinic attendees, bar workers, and women in an HIV serodiscordant partnership (grouped with female sex workers in figures). ‖Men with higher-risk sexual behaviour (likely to be men who have sex with men), STI clinic attendees, male trucking company employees, clients of female sex workers, Thai military conscripts (grouped with men who have sex with men in figures). **Estimates by sex could not be obtained. ††All case-control studies were subsequently analysed together. ‡‡Only studies providing crude estimates or sufficient information to calculate a crude estimate. §§Includes probable adjustment, and variable not included in multivariate model because of non-significance. ¶¶Only studies providing adjusted estimates. ‖‖Five subcategories for the timing sequence of HSV-2 and HIV seroconversion are defined in Methods.

Table 1: **Description of studies and RR estimates of the association between HIV incidence and exposure to HSV-2 infection by participant and study characteristics**

population; R^2=31%), world region (Africa *vs* outside Africa; R^2=24%), definition of HSV-2 unexposed group (HSV-2 negative at baseline *vs* HSV-2 negative throughout follow-up; R^2=24%), and HSV-2 exposure type (prevalent *vs* incident; R^2=17%) significantly explained the variation across study estimates (all p<0·05; table 2). The risk of HIV acquisition was about twice as large for general populations compared with higher-risk populations (RR 0·53, 95% CI 0·38–0·75), for Africa compared with outside Africa (0·57, 0·39–0·82), for incident HSV-2 infection compared with prevalent HSV-2 infection (1·96, 1·16–3·31), and when the definition for the unexposed group was not reported compared with when the definition for the unexposed group was HSV-2 negative throughout follow-up (1·84, 1·08–3·14). Variation across study estimates was not explained by confounder adjustment or any of the other factors explored, including star rating based on the Newcastle-Ottawa Scale. In a multivariate meta-regression analysis restricted to estimates for prevalent

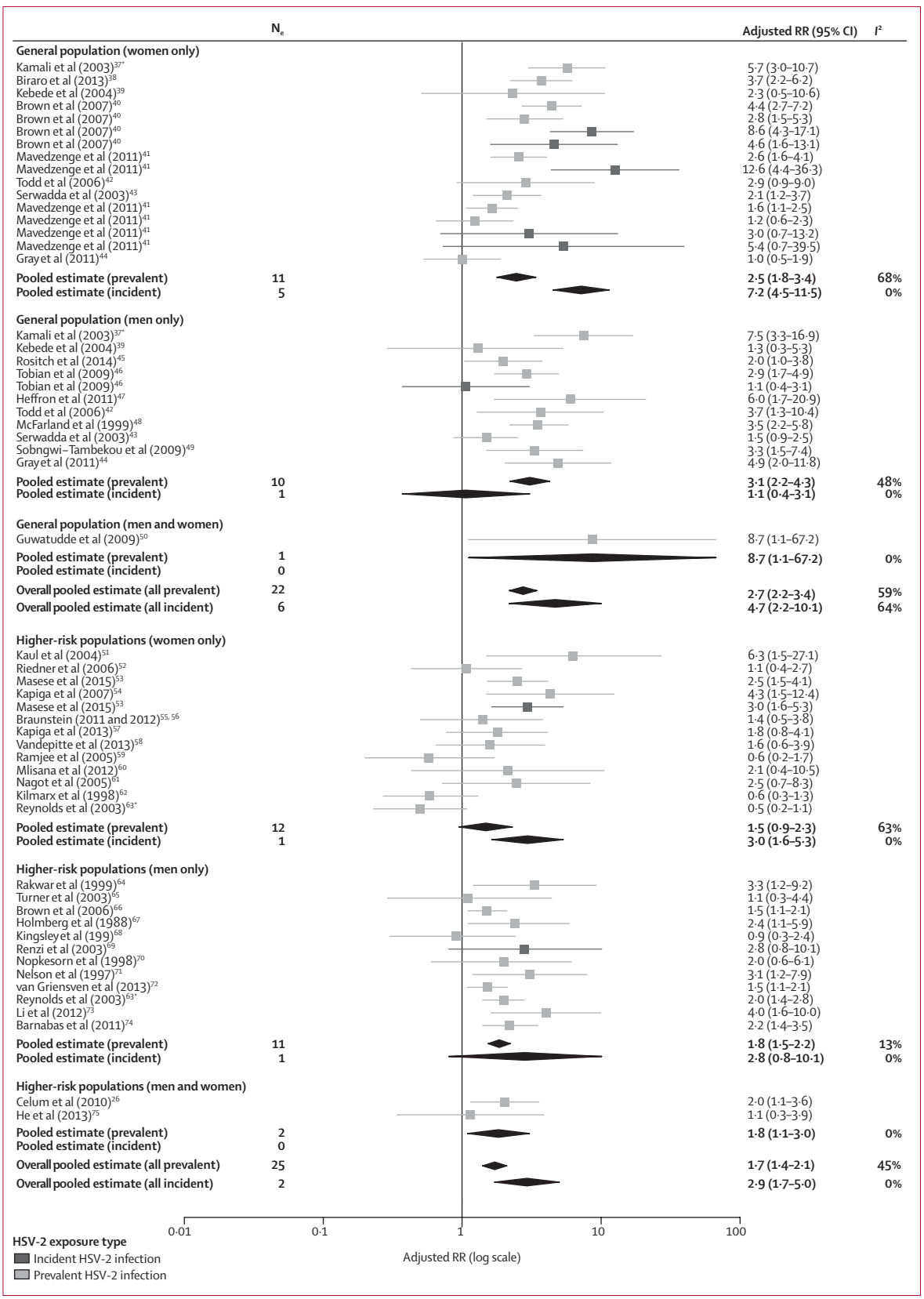

Figure 2: **Pooled adjusted RR estimates of the association between HIV incidence and exposure to HSV-2 infection**

Estimates for effect of both prevalent and incident HSV-2 infection on HIV acquisition (timing 1; ie, HSV-2 seroconversion was observed in a previous time interval and so definitely occurred before HIV seroconversion) are shown. Estimates are shown for women and men combined when they could not be obtained separately by sex. Multiple estimates for the same study corresponding to different study countries or areas are shown when these could not be combined or when it was not appropriate to do so (ie, countries spanning two sub-regions); however, all estimates are independent (ie, for non-overlapping study populations) within each HSV-2 exposure subcategory. N_e=number of estimates. RR=relative risk. HSV-2=herpes simplex virus type 2. *Data from these studies were obtained from reference 11.

HSV-2 infection, risk group, world region, and definition of the HSV-2 unexposed group were all significant modifiers of the association with HIV infection (results not shown). However, we could not disentangle the effect of world region from that of risk group because no estimates for general populations were from outside Africa.

Figure 3 shows the pooled adjusted RR estimates from our subgroup analysis for exposure to prevalent or incident HSV-2 infection by key risk factors. Prevalent HSV-2 infection was associated with a significantly (95% CI did not overlap) higher risk of HIV acquisition in general populations (adjusted RR 2·7, 95% CI 2·2–3·4; N_e=22; I^2=59%) than in higher-risk populations (1·7, 1·4–2·1; N_e=25; I^2=45%; same as shown in figure 2) and in Africa (2·5, 2·1–3·0; N_e=34; I^2=52%) than outside Africa (1·5, 1·2–2·0; N_e=13; I^2=56%), but not for any other characteristic explored.

For exposure to incident HSV-2 infection, the risk of HIV acquisition was significantly higher in younger (≤25 years; adjusted RR 7·6, 95% CI 4·4–13·3; N_e=3; I^2=0%; figure 3) than in older (>25 years; 2·5, 1·6–3·9; N_e=4; I^2=3%) individuals; however, no study estimates were available for younger individuals in higher-risk populations, whereas two of four estimates for older individuals were from higher-risk populations. The risk of HIV acquisition after exposure to incident HSV-2 infection was also significantly higher when HIV testing was done every 6 months or less (5·0, 3·1–8·1; N_e=7; I^2=38%) than when tests were done at a mixture of short and long intervals (1·1, 0·4–3·1; N_e=1; I^2=0%), although this analysis was based on only one study. No other significant differences were observed, although the magnitude of the association tended to be larger for women than for men. Notably, most study estimates came from women in general populations in Africa. Our subgroup analysis showed that pooled adjusted RR estimates were increased, although not significantly, when exposure to incident HSV-2 infection was known to have or might have occurred after HIV (timing 4a and 4b *vs* timing 1; figure 3).

The study characteristics relevant to the evaluation of study quality, and the results of the Newcastle-Ottawa Scale assessment, are summarised in the appendix. Of the 55 adjusted estimates included in our principal meta-analysis, a seven-star or eight-star rating was the most common (N_e=31). The most common reasons for loss of a star were defining the HSV-2 unexposed group by use of baseline status and no matching or adjustment for number of sexual partners. However, a star could also be lost if the required information for assessment was not reported in the paper, which is not necessarily the same as poor study quality. Conversely, a star could have been awarded for adequate participant retention (low loss to follow-up), which was assessed on the basis of the information in the publication but which might not have mentioned all dropouts.

	Number of estimates	Adjusted RR (95% CI)	Variance explained R^2 (%)	p value
Participant characteristics				
Mean or median age				
≤25 years	14	1·00	0%	0·40
>25 years	33	1·34 (0·88–2·04)	..	..
Not reported	1	1·26 (0·32–4·88)	..	..
Sex				
Women	23	1·00	0%	0·97
Men	22	1·02 (0·68–1·53)	..	..
Combined*	3	0·90 (0·36–2·25)	..	..
Risk group				
General population	22	1·00	31%	0·0004
Higher-risk population	26	0·53 (0·38–0·75)	..	..
World region (derived from WHO region)				
African region	34	1·00	24%	0·003
Outside Africa	14	0·57 (0·39–0·82)	..	..
HSV-2 prevalence				
≤30%	10	1·00	6%	0·08
>30%	37	0·97 (0·63–1·51)	..	..
Not reported	1	0·24 (0·07–0·85)	..	..
Study characteristics				
Study year (mid-point)				
Pre-2000	18	1·00	0%	0·85
2000 onwards	24	1·13 (0·74–1·71)	..	..
Not reported	6	1·05 (0·51–2·14)	..	..
Study design				
Cohort	25	1·00	0%	0·69
Controlled trial	15	1·20 (0·77–1·86)	..	..
Case-control (including nested case-control)	8	0·98 (0·56–1·71)	..	..
Controlled trial intervention group				
Control	0	..	..	..
Intervention	0	..	..	..
Combined	19	NA	..	..
Follow-up duration				
≤1 year	12	1·00	0%	0·97
>1 year	32	0·95 (0·59–1·52)	..	..
Not reported	4	0·94 (0·39–2·25)	..	..
Length of time between tests for HIV				
≤6 months	31	1·00	0%	0·98
>6 months	6	0·99 (0·55–1·78)	..	..
Mixture of short and long intervals	3	0·86 (0·37–2·04)	..	..
Not reported	8	1·07 (0·62–1·84)	..	..
HSV-2 assay cutoff (only those studies with Focus HerpeSelect as known assay)				
1·1/manufacturer's recommendation/ unknown	12	1·00	19%	0·20
>1·1	7	1·57 (0·79–3·10)	..	..

(Table 2 continues on next page)

	Number of estimates	Adjusted RR (95% CI)	Variance explained R^2 (%)	p value
(Continued from previous page)				
HSV-2 exposure type				
Prevalent	40	1·00	17%	0·01
Incident	8	1·96 (1·16–3·31)	..	..
Definition of unexposed group				
HSV-2 negative throughout follow-up	21	1·00	24%	0·005
HSV-2 negative at baseline	19	0·76 (0·52–1·11)	..	..
Not reported	8	1·84 (1·08–3·14)	..	..
Adjusted for male circumcision status (men or women and men combined)				
No	15	1·00	0%	0·48
Yes	7	1·33 (0·84–2·10)	..	..
Not reported	3	1·08 (0·57–2·05)	..	..
Adjusted for condom use				
No	27	1·00	0%	0·88
Yes	17	1·10 (0·72–1·68)	..	..
Not reported	4	1·13 (0·52–2·48)	..	..
Adjusted for female hormonal contraceptive use (women or women and men combined)				
No	17	1·00	0%	0·70
Yes	7	1·25 (0·58–2·69)	..	..
Not reported	2	1·82 (0·34–9·81)	..	..
Adjusted for any sexual behaviour (excluding condom use)				
No	9	1·00	0%	0·48
Yes	36	1·39 (0·81–2·37)	..	..
Not reported	3	1·46 (0·58–3·69)	..	..
Adjusted for genital ulcer disease				
No	36	1·00	10%	0·14
Yes	9	1·54 (0·97–2·45)	..	..
Not reported	3	1·61 (0·60–4·34)	..	..
Adjusted for number of sexual partners				
No	19	1·00	0%	0·46
Yes	23	0·85 (0·55–1·31)	..	..
Not reported	6	0·68 (0·36–1·26)	..	..
Adjusted for age				
No	6	1·00	0%	0·99
Yes	42	1·00 (0·54–1·85)	..	..
Estimate type				
Hazard ratio or incidence ratio	39	1·00	0%	0·25
Odds ratio	9	0·74 (0·45–1·23)	..	..
Study quality (as defined by number of stars awarded with Newcastle-Ottawa scale)				
Increase of 1 star (continuous variable)	48	1·00 (0·85–1·17)	0%	0·96

Only independent adjusted RR estimates were included. RR=relative risk. HSV-2=herpes simplex virus type 2. NA=not applicable. *Estimates by sex could not be obtained.

Table 2: **Results of univariate meta-regression analysis of adjusted RR estimates**

There was little indication of publication bias from the funnel plots (appendix): most study estimates were evenly distributed around the overall pooled crude and adjusted RR estimates. However, the fewer RR estimates available for exposure to incident HSV-2 infection than for exposure to prevalent HSV-2 infection made the assessment for incident HSV-2 infection more difficult. In a meta-regression analysis, crude RR estimates calculated from the available data were lower than those reported in the studies (0·79, 95% CI 0·57–1·09; R^2=2·5%; p=0·15), although the difference was not significant. Our qualitative assessment found some evidence of selective reporting of estimates based on significance (eg, studies reporting crude but not adjusted estimates or only mentioning the significance of an association without presenting any estimates; appendix).

Discussion

This systematic review and meta-analysis provided new insight into the effect of HSV-2 infection on risk of HIV acquisition by analysis of 57 longitudinal studies of different study designs. We found good evidence that HIV incidence in general populations is roughly tripled by exposure to prevalent HSV-2 infection (adjusted RR 2·7, 95% CI 2·2–3·4), with an even larger increase in HIV risk after exposure to incident HSV-2 infection (4·7, 2·2–10·1). The greater cofactor effect for incident HSV-2 infection than for prevalent HSV-2 infection might be because newly acquired HSV-2 infection is associated with an increased frequency and severity of genital ulceration, viral shedding, and inflammation in the genital tract, symptoms and manifestations that decrease with time after infection.[14–16] These biological mechanisms and gradient in risk strengthen the argument for a genuine biological effect of HSV-2 infection on HIV acquisition risk.

In addition to differences by prevalent versus incident HSV-2 infection, heterogeneity in the magnitude of the association across adjusted RR estimates was also explained by population risk group. The associations remained significant but were somewhat lower among higher-risk populations than among general populations, perhaps because these populations have an increased risk of HIV independent of HSV-2 or because higher-risk individuals infected with HSV-2 might be more likely to use condoms or abstain from sex when symptomatic.

The results of our systematic review and meta-analysis were generally in line with, and strengthen results from, previous meta-analyses.[11,12] The 38 studies published since the last systematic search and review,[11] and the large body of study information extracted, allowed us to comprehensively review existing evidence on the association between HSV-2 and HIV infections and assess the effects of a wide range of factors related to participant and study characteristics, including study quality, which have not been previously explored. We also produced the first pooled estimates of the association between exposure to incident HSV-2 infection and subsequent HIV acquisition.

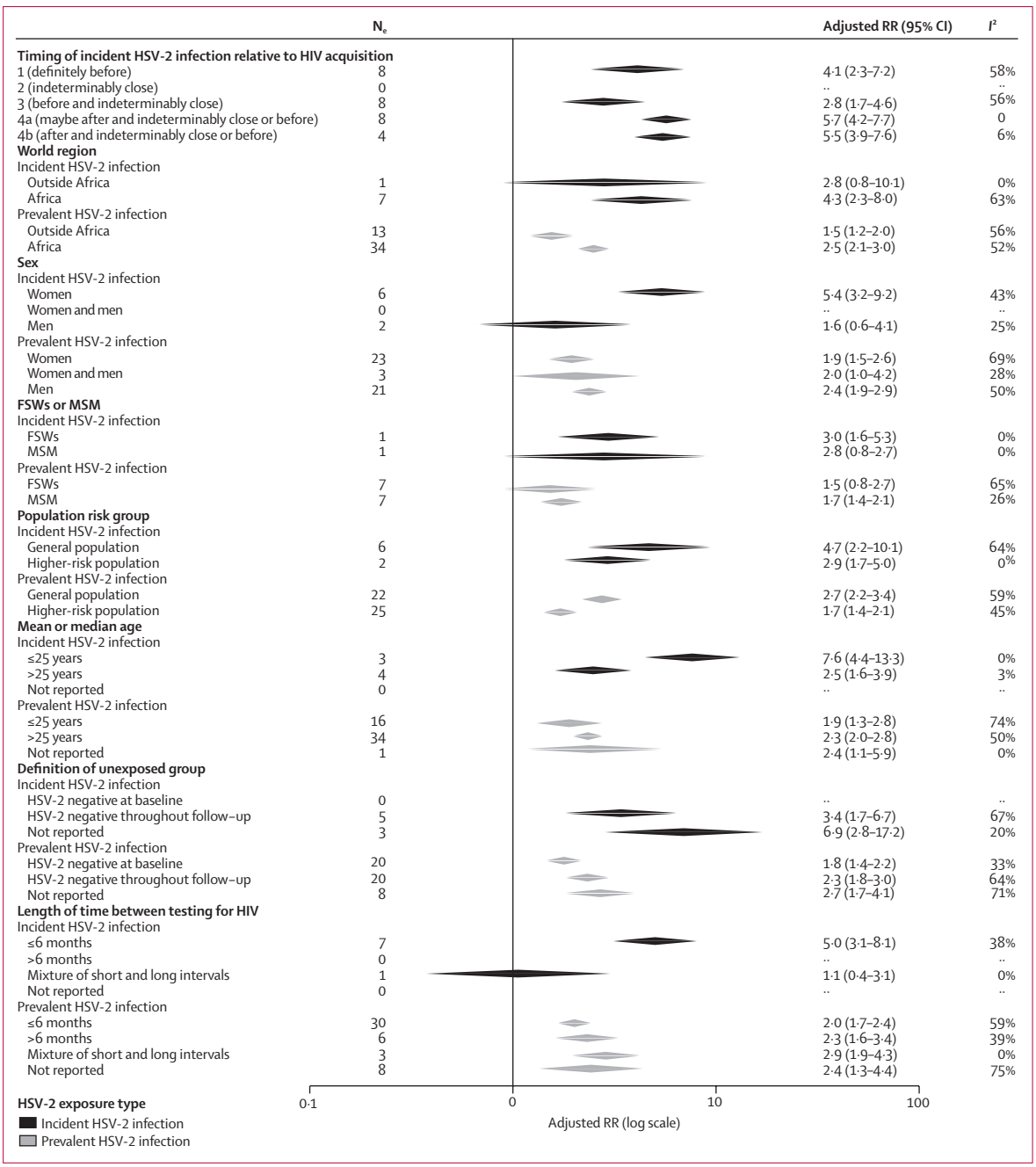

Figure 3: **Sub-pooled adjusted RR estimates of the association between HIV incidence and exposure to HSV-2 infection**
Estimates for effect of both prevalent and incident HSV-2 infection on HIV acquisition (timing 1; ie, HSV-2 seroconversion was observed in a previous time interval and so definitely occurred before HIV seroconversion) are shown. Estimates were added for sub-pooling if they were available by subcategories, although only independent study estimates were included within a subcategory. RR=relative risk. HSV-2=herpes simplex virus type 2. N_e=number of estimates. FSWs=female sex workers. MSM=men who have sex with men.

HSV-2 and HIV are lifelong infections that affect genital sites and have similar risk factors, such as sexual behaviour, which increases the risk of spurious association in observational studies because of confounding. We minimised the risk of confounding at the study level by focusing our analysis on adjusted estimates. However, the risk of residual confounding could not be totally eliminated because many potential confounding factors were often not controlled for, even in adjusted estimates. The presence of HSV-2 infection (particularly incident HSV-2 infection) might be a marker of having had sex with a partner infected with HIV

because of the higher prevalence of HSV-2 among HIV-infected than among non-HIV-infected individuals.[76–78] Except for serodiscordant-couple studies, in which partner HIV status was known, few studies controlled for partner characteristics. Additionally, some studies inappropriately adjusted for genital ulcer disease, which might have biased pooled estimates toward the null value because HSV-2 commonly causes genital ulcers and these act as a point of entry for HIV.[13] Nevertheless, we did not find any notable difference between crude and adjusted pooled estimates, and, in the meta-regression analysis, we found that adjustment for key confounders was not associated with the effect size. Confounding could also have arisen from combining estimates from heterogeneous studies, which is an important reason for doing detailed meta-regression and subgroup analyses. Fewer estimates for incident HSV-2 than for prevalent HSV-2 increased the risk of confounding when combining heterogeneous studies, but also precluded a multivariate meta-regression analysis for incident HSV-2 infection. No estimates were available for general populations outside Africa for either prevalent or incident HSV-2 infection, meaning that our results might not be generalisable to general populations outside this setting. Furthermore, our finding of a higher risk of HIV with HSV-2 among general populations than among higher-risk populations could have been confounded by world region (or vice versa).

Another potential threat to validity was misclassification bias of the exposure to HSV-2 infection. Misclassification bias can occur if HSV-2 exposure is defined solely according to HSV-2 antibody status at baseline and some unexposed individuals seroconvert to HSV-2 during the study. Although exposure status was defined solely on baseline HSV-2 infection status in half of the studies estimating the association between prevalent HSV-2 infection and HIV acquisition, we found only weak indication that the association between HSV-2 and HIV was lower in those studies. However, in our meta-regression analyses, estimates were significantly increased for unknown definition of the unexposed comparison group, which might be a proxy for poor study quality more generally.

To minimise the risk of reverse causation, we only included longitudinal studies and categorised estimates for effect of incident HSV-2 infection on HIV acquisition according to the timing of HSV-2 infection compared with HIV seroconversion. For our principal meta-analysis and meta-regression, we restricted inclusion of estimates for incident HSV-2 infection to when incident HSV-2 infection was known with greatest certainty to have occurred before HIV seroconversion (ie, timing 1), excluding any estimates for which HSV-2 infection was known to or might have occurred after HIV infection. By erring on the side of caution, we might have inadvertently excluded estimates for when HSV-2 infection occurred before HIV in studies where testing was not done sufficiently frequently to disentangle the sequence of infection. However, those estimates that included known or possible HIV infection before HSV-2 acquisition were not significantly different to our estimate for timing 1.

Our qualitative assessment of selective reporting of crude and adjusted estimates based on significance found some evidence of publication bias. However, publication bias was not significant from either funnel plots or our meta-regression comparing reported crude estimates with crude estimates derived from available data. We did not find any evidence in our meta-regression that study quality influenced the association between HSV-2 and HIV, except where the definition of HSV-2 negative was not reported, which was associated with a significantly increased risk of HIV acquisition due to HSV-2.

Understanding the effect of HSV-2 infection on HIV risk is essential for several reasons. From a clinical perspective, knowledge of this association informs the advice and information given to individuals diagnosed with genital herpes, who might be at increased risk of acquiring HIV. Much, if not most, of HSV-associated HIV transmission is thought to occur outside symptomatic episodes, including among individuals who harbour HSV-2 infection but have never had symptoms of genital herpes. Thus, from a population perspective, understanding the interaction between HSV-2 and HIV is also important for informing public health interventions for the control of both infections, because an intervention targeting HSV-2 might have additional, indirect benefits on HIV.

Current prevention and treatment options for HSV-2 infection are imperfect and limited by the often asymptomatic presentation of HSV-2 infection.[3] However, development of new interventions is underway. Multipurpose prevention technologies (eg, microbicides) that target both HIV and HSV-2 infection hold promise, but developments have been hampered by low compliance and acceptability among women.[22] The best option is likely to be an effective vaccine against HSV-2 infection. Efforts to develop an HSV-2 vaccine are underway.[27] HSV-2 infection is a common infection globally, but has a particularly high incidence in specific settings where HIV is endemic, such as sub-Saharan Africa, and among higher-risk groups, who are important in concentrated HIV epidemics. Therefore, addressing the interactions between HSV-2 and HIV could produce substantial health and economic gains. This meta-analysis is an important step towards clearer quantification of the potential magnitude of that benefit.

Contributors

KJL, SLG, and M-CB designed the study with input from all coauthors. KJL and JARE contributed equally to the literature search, data extraction, forest plots, meta-regression, and subgroup analysis. KJL wrote the first version of the manuscript with input from JARE. M-CB supervised JARE, gave advice on the direction of the analysis, and guided the different aspects of the analysis. SLG oversaw the study and provided advice on the different stages as required. JTS contributed expertise on HSV-2. KMET and PV supervised KJL and contributed technical expertise. All authors contributed to the interpretation of results and revised the different versions of the manuscript.

Declaration of interests
KJL reports personal fees from WHO during the conduct of the study. JARE reports grants from the US National Institutes of Health (NIH) during the conduct of the study and grants from the Wellcome Trust outside of the submitted work. KMET reports personal fees and other support from Aquarius Population Health outside the submitted work. M-CB reports non-financial support from WHO and grants from the NIH through the HIV Prevention Trials Network (HPTN), both during the conduct of the study. SLG, JTS, and PV declare no competing interests.

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