Impact of viral load and the duration of primary infection on HIV transmission: systematic review and meta-analysis

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Objectives: HIV 'treatment as prevention' (TasP) describes early treatment of HIV-infected patients intended to reduce viral load and transmission. Crucial assumptions for estimating TasP's effectiveness are the underlying estimates of transmission risk. We aimed to determine transmission risk during primary infection, and describe the relation of HIV transmission risk to viral load.

Design: A systematic review and meta-analysis.

Methods: We searched PubMed and Embase databases for studies that established a relationship between viral load and transmission risk, or primary infection and transmission risk, in serodiscordant couples. We analysed assumptions about the relationship between viral load and transmission risk, and between duration of primary infection and transmission risk.

Results: We found 36 eligible articles, based on six different study populations. Studies consistently found that higher viral loads lead to higher HIV transmission rates, but assumptions about the shape of this increase varied from exponential increase to saturation. The assumed duration of primary infection ranged from 1.5 to 12 months; for each additional month, the $\log_{10}$ transmission rate ratio between primary and asymptomatic infection decreased by 0.40.

Conclusion: Assumptions and estimates of the relationship between viral load and transmission risk, and the relationship between primary infection and transmission risk, vary substantially and predictions of TasP's effectiveness should take this uncertainty into account.   © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

Introduction

Treatment as prevention (TasP) is a promising approach to curbing the HIV epidemic, but estimates of its effectiveness vary. A crucial assumption in models estimating the effectiveness of TasP is the high rate of HIV transmissions during primary HIV infection. How much primary infection contributes to overall transmission is a matter of debate [1], and estimates of the proportion of HIV transmission during the primary phase range from 2 to 90% [2]. Powers *et al.* [3] recently argued that primary infection is a major driver of the epidemic, causing up to 40% of new infections, a rate that may be high enough to compromise TasP strategies. Williams *et al.* [4], however, suggested that only 2% of all HIV transmissions occur during primary infection, which suggests that the epidemic could be ended with TasP.

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Transmission during primary infection has been estimated directly (from serodiscordant couples with recently infected index partners) and indirectly (using high viral load values during primary infection as a proxy for primary infection). Combining evidence from two studies, one using direct and one indirect estimation of transmission, Hollingsworth *et al.* [5] concluded that the indirect approach underestimates rates of transmission during primary infection.

We conducted a systematic literature review to analyse direct and indirect estimates of transmission during primary infection and compare the results of the two approaches to determine if the measured viral load values could explain observed transmission during primary infection.

Materials and methods

Search strategy, eligibility criteria and study selection
We systematically searched Medline and Embase databases on 7 March 2012, using free text words and Medical Subject Headings in Pubmed and Emtree-terms in Embase and variations of the following search terms: HIV, transmission rate, transmission probability, viral load and primary infection. We also examined the references of included papers. Our detailed search strategy is shown in the web appendix, http://links.lww.com/QAD/A451. Eligible studies assessed the risk of HIV transmission on the basis of viral load values, or compared HIV transmission during the primary and the asymptomatic stage of infection. We included studies of serodiscordant couples and systematic reviews of such studies. We excluded studies of nonhuman populations and studies on nonsexual transmission (e.g. mother-to-child transmission, blood transfusion). Two reviewers independently screened abstracts and selected full text articles in accord with the above criteria. Disagreements were resolved by consensus.

Data collection
Two reviewers used a standardized sheet to extract the following: characteristics of the study (study locations, study period, follow-up time, monitoring frequency, inclusion/exclusion criteria, number of participants); characteristics of couples [sex and age of both partners, viral load, $CD4^+$ cell count and antiretroviral therapy (ART) status of index partner]; factors that determine the risk of HIV transmission (sexually transmitted diseases of both partners, circumcision status of male partner, reported number of unprotected sex acts); and the relation between HIV transmission and viral load values and stage of infection. We resolved disagreements by consensus.

Standardization of study results
We standardized the unit of transmission risk to compare the results from different studies: we report transmission rates per year or transmission rate ratios. Transmission probabilities per sex act were transformed into transmission rates. We assumed an average of four sex acts per month, which is consistent with previous studies [6]. Sexual activity was assumed to be independent of viral load and phase of infection. The transmission rate per year r is therefore related to the per act transmission probability p by the formula

$$r = -n*\log(1-p),$$

where $n = 48$ is the average number of sex acts per year. A detailed description of these calculations is shown in the web appendix, http://links.lww.com/QAD/A451. All analyses were done in R version 2.14.2 [7].

Meta-analysis of two types of studies
We separately analysed the relationship between viral load and transmission risk, and the relationship between primary infection and transmission risk. We compared results of these separate analyses to determine if high viral load values could explain the high transmission risk during primary infection.

Relationship between viral load and transmission risk
For each study, we described the relationship between HIV transmission rates per year and $\log_{10}$ viral load. We graphically compared different patterns of this relationship (e.g. proportional hazards models, step functions, saturating curve). We conducted a random-effects meta-analysis of the transmission hazard ratio per $\log_{10}$ increase in viral load. If multiple studies were conducted on the same study population, we included the largest study in the meta-analysis. As a sensitivity analysis, we reanalysed data from Fideli *et al.* [8] to separate the heterogeneity caused by different assumptions and the heterogeneity caused by different data.

Relationship between primary infection and transmission risk
We extracted the assumed duration of primary infection and calculated corresponding transmission rate ratios between primary and asymptomatic infection. The result was presented graphically. For one study population (Rakai [9]), which had been analysed with different assumed durations of the primary infection, we used a regression model to relate the rate ratio to the assumed duration of primary infection.

Viral load and primary infection compared
We compared estimated transmission rates on the basis of viral load values (approach 1) with observed transmission rates during primary infection (approach 2), as follows: in approach 1, we assumed the viral load was 3 $\log_{10}$ higher during the primary infection than during the

asymptomatic infection, an assumption consistent with a previous study by Pilcher *et al.* [10] in which it was assumed that viral load values remained elevated for 2 months. On the basis of our meta-analysis of the relationship between viral load and transmission risk, we calculated the estimated increase in transmission during primary infection based on viral load values. We used the regression model of the relationship between primary infection and transmission risk to calculate the number of expected transmissions during primary infection (again 2 months), and then compared that number with the number of expected transmissions during the asymptomatic phase. We also used other association patterns (i.e. step functions and saturating curves) to describe the relation between viral loads and HIV transmission.

Results

Identification of relevant studies

We found 788 potentially eligible articles (765 from search and 23 from references) and included 36 in our analysis (Table 1) [5,6,8−41]. Of 788 potentially eligible articles, we excluded 173 duplicates and 515 that were conducted in nonhuman populations, did not focus on sexual HIV transmission, studied other diseases than HIV, were case reports or did not report on individual viral load values. We excluded 64 more studies that were not based on serodiscordant couples ($n = 39$), did not calculate a transmission rate or probability based on viral load or primary infection ($n = 20$), or reported results from previous studies ($n = 5$). Twenty articles described the relationship between viral load level and risk of HIV
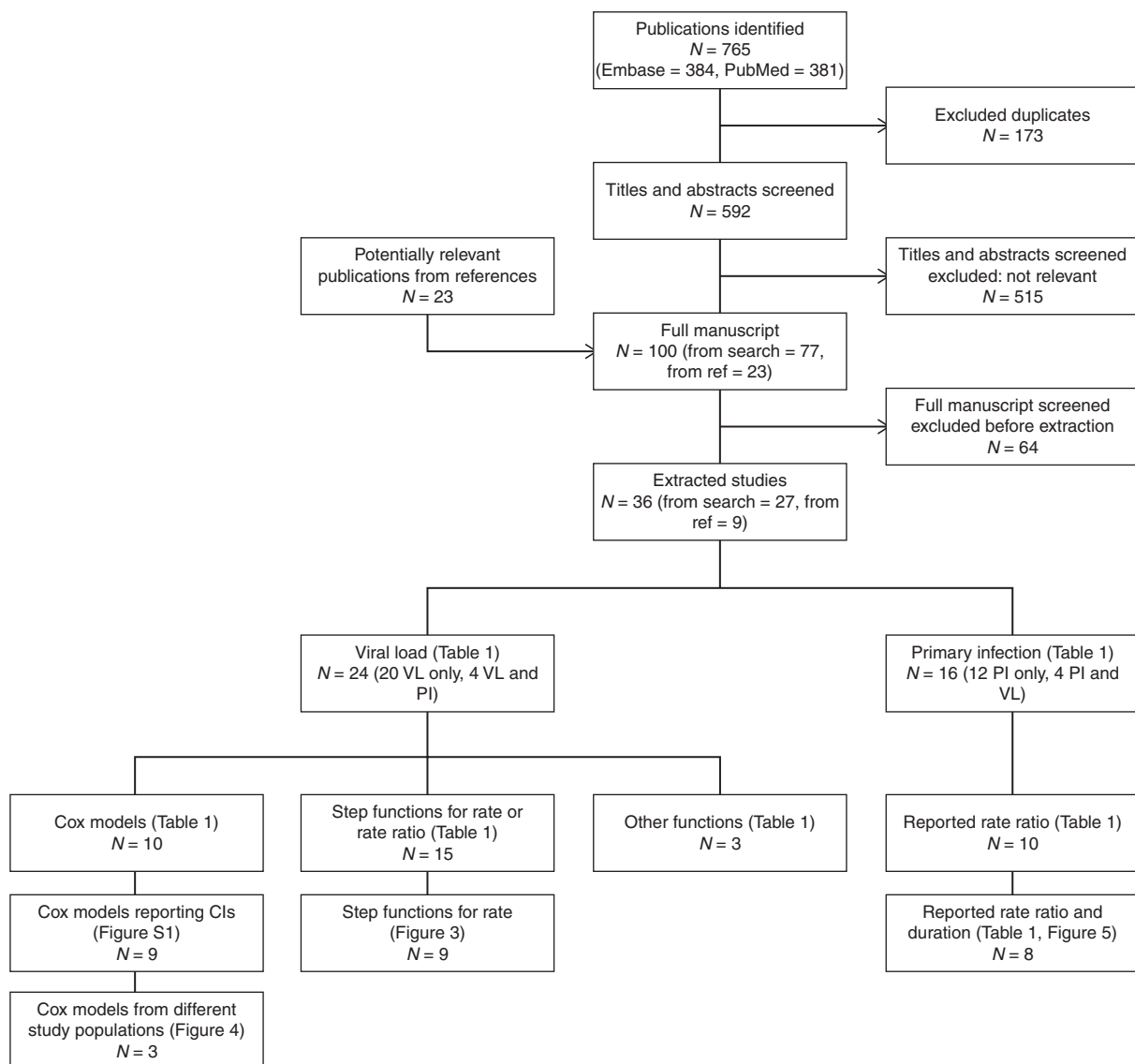

Fig. 1. Identification and selection of studies. CIs, confidence intervals; *N*, number of studies; PI, primary infection; VL, viral load.

transmission during the asymptomatic phase of HIV infection; 12 articles compared the risk of HIV transmission during primary infection with the risk during the asymptomatic phase, but did not consider viral load levels; four studies reported on both (Fig. 1). The 20 articles that used primary data were based on only six different primary study populations of serodiscordant couples: the European Study Group on Heterosexual Transmission of HIV, the Medical Research Council Programme on AIDS in Uganda, a cohort study in Tanzania, the Partners in Prevention HSV/HIV Transmission Study Team in seven countries in Eastern and Southern Africa, the Zambian HIV Research Project and the Rakai Project Study Group in Uganda (Table 1) [6,8,9,12,13,15,17–26,28,35–37]. Thirteen of the included articles were reviews and meta-analyses [5,10,11,14,16,27,29–34,38], and another three articles estimated the effect of primary infection on HIV transmission from a mathematical model fitted to HIV prevalence data during the early epidemic [39–41].

Study populations
The six study populations consisted of heterosexual HIV-serodiscordant couples. Studies included between 29 and 3381 couples and observed between six and 426 transmissions (Table 1) and were conducted after 1987, in different samples of the general population. In the Partners in Prevention study group, index partners were Herpes Simplex virus (HSV)-2 seropositive and had a $CD4^+$ cell count of at least 250 cells/μl. Other inclusion criteria were married or cohabiting couples, age ranges, known circumcision status, minimum follow-up duration, reported monogamy, reported unprotected sex or a high risk of HIV infection.

Relationship between viral load and transmission risk
Most studies made one of three different assumptions about the relationship between HIV viral load values and the risk of HIV transmission (Table 1): step functions wherein viral load values are grouped into categories [6,8,9,11–22]; Cox proportional hazards models wherein the transmission rate r depends on the $\log_{10}$ viral load x according to the formula $r(x) = c*b^x$, with constants b and c [6,8,19,20,23–28]; and a variation of the Cox proportional hazards model in which the per-act transmission probability was assumed to be proportional instead of the rate, which results in the function $r(x) = -n*\log(1 - c*b^x)$ for the transmission rate, with constants b and c and annual number of sex acts n [29,30]. For high viral load values, Cox models assume that HIV transmission rates will continue to increase. Step functions assumed a constant transmission rate for high viral loads or reported transmission rates only for $\log_{10}$ viral load values below 7. Fraser et al. [31] assumed a saturating curve (Hill function). Figure 2 (top) illustrates the different patterns, using the most recent study in each

category. Although transmission rates all increase with higher viral load values, their shapes are quite different, and differences become more pronounced for high viral load values. Figure 2 (bottom) shows the results of a reanalysis of data by Fideli et al. [8], which illustrates that the heterogeneity between models persists for high viral load values when analysing the same data.

Cox proportional hazard models assume a constant increase in risk of HIV infection per $\log_{10}$ increase in viral load. Across the nine studies, the HIV transmission rate ratio per $\log_{10}$ increase in viral load ranged from 1.5 to 2.9 (Figure S2, http://links.lww.com/QAD/A451). Our meta-analysis of the largest studies of each study population ($n = 3$) resulted in a combined rate ratio of 2.09 [95% confidence interval (CI) 1.47–2.97] per $\log_{10}$ increase in viral load (Fig. 3).

Relationship between primary infection and transmission risk
The assumed duration of primary infection varied from 1.5 to 12 months; corresponding HIV transmission rate ratios that compared primary with asymptomatic infection varied from 43.7 to 0.8 (Table 1; Fig. 4, top). Two studies [5,32] estimated both duration and rate ratio. On the basis of the Rakai data, Hollingsworth et al. [5] found that primary infection lasted 87 days, and Pinkerton [32] found that it lasted 49 days. Studies that assumed primary infection lasted for a year consistently found that it had little effect on transmission rates. For the Rakai study, we found a linear relationship between duration of primary infection and log rate ratio: each month increase in duration of the primary infection decreased the log rate ratio between primary and asymptomatic infection by 0.40 (Fig. 4, bottom).

Viral load and primary infection compared
Viral load values during primary infection do not entirely explain the higher risk of HIV transmission during this period. Our meta-analysis (Fig. 3) shows that a 3 $\log_{10}$ increase in viral load values (consistent with Pilcher et al. [10] who assumed that the primary infection lasts for 2 months) corresponds to a 9.1-fold higher risk of HIV transmission during primary infection than during the asymptomatic period ($= 2.09^3$). If we use the highest reported rate ratio of 2.89 [23], the transmission rate ratio between primary and asymptomatic infection reaches 24 ($= 2.89^3$). When we assumed other relationships between viral load and transmission rates (e.g. saturating curves), the estimate was lower.

In contrast, observed HIV transmissions during primary infection were higher than we expected based on viral load values. On the basis of the meta-regression (Fig. 4, bottom), the rate ratio for a primary infection of 2 months was 37 ($= e^{3.61}$).

Table 1. Characteristics of included studies.

Reference	Time period	Serodiscordant couples	Person-years of follow-up	Number of transmissions	Transmission risk depends on VL/PI	Transmission rate $r(x)$ as a function of $x = \log_{10}(VL)$	Duration of primary infection (days)	Transmission rate ratio between primary and asymptomatic infection
European Study Group on Heterosexual Transmission of HIV								
Leynaert *et al.* [35]	1987–1992	499	NA	94	PI		90	3.15
Medical Research Council Programme on AIDS in Uganda								
Carpenter *et al.* [37]	1989–1999	29	85	6	PI		365	2.4
Tanzania cohort study								
Hugonnet *et al.* [36]	1991–1995	61	122	9	PI		365	2.4
Partners in Prevention HSV/HIV Transmission Study Team								
Baeten *et al.* [12]	2004–2008	1096	1685	64	VL	Step function		
Baeten *et al.* [6]	2004–2008	2521	3509	113	VL	Step function, $r(x) = c \ast b^x$		
Celum *et al.* [13]	2004–2007	3360	4868	132	VL	Step function		
Donnell *et al.* [15]	2004–2008	3381	4467	142	VL	Step function		
Hughes *et al.* [23]	2004–2008	3297	NA	86	VL	$r(x) = c \ast b^x$		
Lingappa *et al.* [19]	2004–2008	3381	4756	108	VL	Step function, $r(x) = c \ast b^x$		
Rakai Project Study Group								
Gray *et al.* [17]	1994–1998	411	752	89	VL	Step function		
Gray *et al.* [18]	1994–1998	174	328	38	VL	Step function		
Kiwanuka *et al.* [24]	1997–2002	268	812	92	VL/PI	$r(x) = c \ast b^x$	365	0.8
Quinn *et al.* [20]	1994–1998	415	760	90	VL	Step function, $r(x) = c \ast b^x$		
Wawer *et al.* [9]	1994–1999	235	558	68	VL/PI	Step function	150	11.71
Zambia HIV Research Project								
Fideli *et al.* [8]	1994–2000	317	NA	109	VL	Step function, $r(x) = c \ast b^x$		
Malhotra *et al.* [25]	1995–2006	567	NA	240	VL	$r(x) = c \ast b^x$		
Merino *et al.* [26]	1995–2006	566	1697	240	VL	$r(x) = c \ast b \wedge x$		
Song *et al.* [28]	1995–2006	568	NA	240	VL	Step function		
Tang *et al.* [21]	1995–2002	292	NA	141	VL	Step function		
Tang *et al.* [22]	1995–2006	429	1336	205	VL	Step function		
No primary data								
Attia *et al.* [11]						Step function		
Boily *et al.* [33]						$r(x) = -n \ast \log(1 - c \ast b \wedge x)$	90–150	9.17
Chakraborty *et al.* [29]						Step function		
Corey *et al.* [14]						Step function	150	4.98
Fox *et al.* [16]						Hill function	180	NA
Fraser *et al.* [31]							Not reported	NA
Gisselquist *et al.* [38]							87	26.04
Hollingsworth *et al.* [5]							60	NA
Jacquez *et al.* [39]						$r(x) = c \ast b \wedge x$		
Modjarrad *et al.* [27]								
Pilcher *et al.* [10]							54	NA
Pinkerton [32]							49	43.68
Powers *et al.* [34]							90–150	6.59
Shiboski *et al.* [40]							Not reported	NA
Shiboski *et al.* [41]							690–1530	NA
Wilson *et al.* [30]						$r(x) = -n \ast \log(1 - c \ast b \wedge x)$		

Step function: $r(x) = \sum_{i=1}^{n} a_i I_{\{b_{i-1} \leq x < b_i\}}$, Hill function: $r(z) = \frac{\beta_{max} z^{\beta_k}}{z^{\beta_k} + (\beta_{50})^{\beta_k}}$; $z = 10^x$. PI, primary infection; VL, viral load.
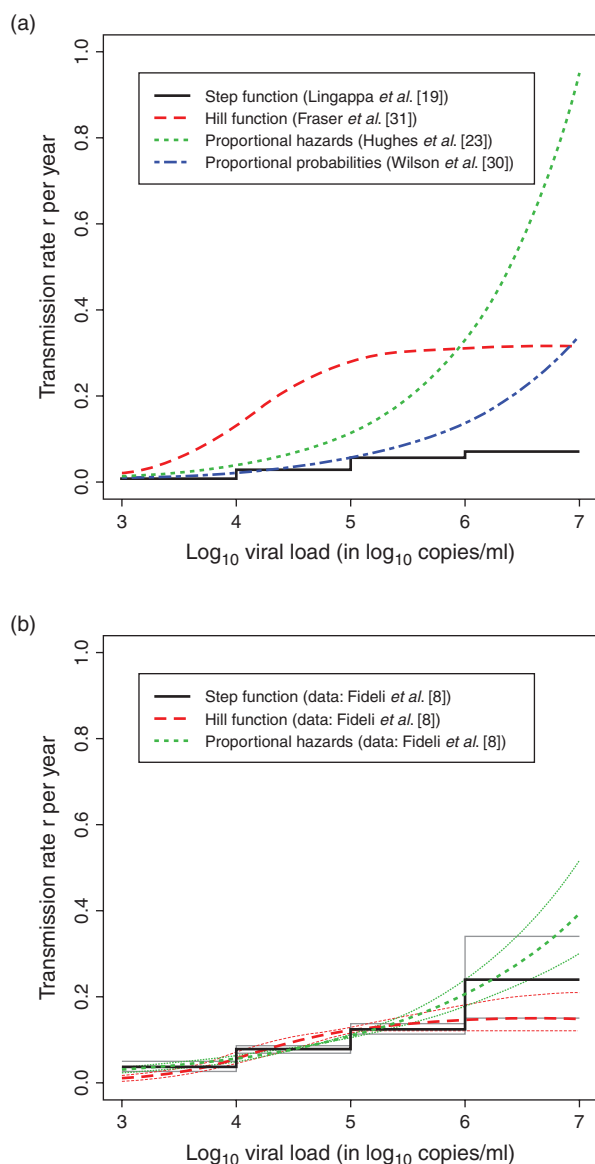
(a)

(b)

Fig. 2. Examples of the four assumed relationships between viral load and transmission rates. (a) The most recent study of each example is presented. Bottom: Reanalysis of the data by Fideli *et al.* [8], which shows that the heterogeneity in transmission risk for lower viral load values is mostly due to different data sources, but for high viral load values, the heterogeneity is also due to different assumptions.

Discussion

The relationship between viral load values and HIV transmission rate was described in four different ways: step functions, Cox proportional hazard models, proportional probability models and Hill functions. Risk of HIV transmission increased with viral load values, but for high viral load values, the pattern of the relationship is unclear. For high viral load values, transmission rates across the studies ranged from saturating to exponentially

increasing. Between studies, assumed duration of primary infection varied from 1.5 to 12 months. Studies that assumed longer primary infections estimated lower rates of HIV transmission during primary infection. Predictions based on high viral load values alone underestimated the risk of HIV transmission during primary infection.

The discrepancy in transmission risk between studies that focused on viral load and studies that focused on primary infection could be because viral load values were measured during the asymptomatic phase, when the virus may be less infectious [42]. We could not determine if viral load was measured during primary or asymptomatic infection. High estimated transmission risk during primary infection might also be caused by coinfections [5,9,32], greater susceptibility in newly exposed uninfected partners [9] or biological differences between strains [32]. None of these hypotheses has been tested.

The high risk of HIV transmission during primary infection (beyond what may be explained by high viral load values during primary infection) is supported by Wawer *et al.* [9]. After adjusting for viral load values, Wawer *et al.* [9] found that HIV transmission rates during primary infection was 4.98 (95% CI 2.00−12.39) times higher than during asymptomatic infection. In contrast, Kiwanuka *et al.* [24] assumed that primary infection lasted a year and estimated that the transmission rate ratio adjusted for viral load was 0.8 (0.4−1.4). Other authors [43,44] used different methods to determine the influence of primary infection on the HIV epidemic. For instance, Brenner *et al.* [43] used phylogenetic clustering analysis and found that early infection accounted for approximately half of onward transmissions.

Earlier meta-analyses compared HIV transmission during primary and asymptomatic infection without accounting for different durations of primary infection. Boily *et al.* [33] and Powers *et al.* [34] meta-analysed the estimated rates during primary infection from the Rakai study and the European study group (with different durations of primary infection), and found that transmission rates were 6.6 and 9.2 times higher during primary infection than in the asymptomatic period. The Rakai study and the European study are difficult to compare because they are designed differently. The Rakai study included couples who started out sero-negative, and in which one partner seroconverted during follow-up. Leynaert *et al.* [35] estimated time of infection on the basis of decline in $CD4^+$ cell counts, and thus was less reliable than the Rakai study. Although the Rakai study consisted of only 23 couples and transmissions were not genetically confirmed, it is still the best data to assess transmission rates during primary infection. Whether this study provides solid evidence about the transmission rate during primary infection is a matter of debate [1]. Studies by Hugonnet *et al.* [36] and by Carpenter *et al.* [37] estimated
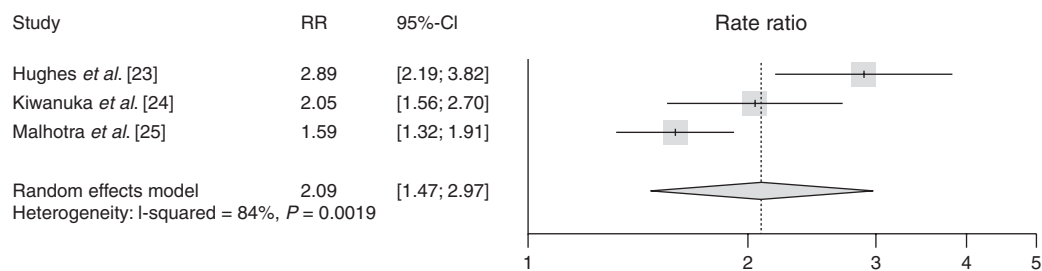
Fig. 3. Meta-analysis of transmission rate ratio per log$_{10}$ increase in viral load. For each study population, we used the largest study reporting rate ratio per log$_{10}$ increase in viral load. Figure S2, http://links.lww.com/QAD/A451 summarizes all studies on rate ratio per log$_{10}$ increase.
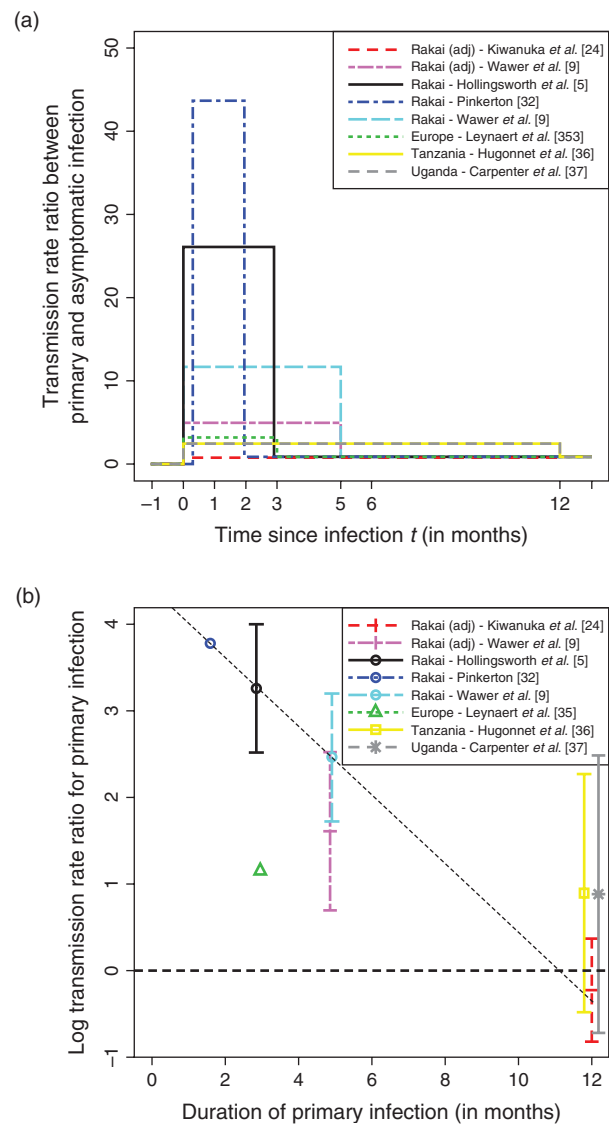

Fig. 4. Duration of primary infection and transmission rate ratio between primary and asymptomatic infection. Top: Transmission rates in the first year after infection. Bottom: Transmission rate ratio between primary and asymptomatic infection and linear regression for Rakai studies that did not adjust for viral load values.

transmission rates during primary infection under the assumption that primary infection lasted a year. These two studies might be interesting to reanalyse with different durations.

Several models assessed the effect of a test and treat strategy on HIV transmission, with conflicting results. The model used by Granich *et al.* [45] showed that, in a high HIV prevalence setting, annual HIV testing and immediate treatment could reduce HIV incidence to below one per 1000 person–years. Powers *et al.* [3] showed that the proposed intervention (annual HIV testing and immediate treatment) did not reduce HIV transmission as much as Granich *et al.* [45] had estimated. The validity of these findings and the differences between them were discussed at length [1,4,46]. Granich *et al.* [45] assumed a rate ratio of 10 for the first 2 months, while Powers *et al.* [3] assumed a risk ratio of 30.3 over 4.8 months. Our review suggests that these assumptions are opposite extremes: the rate ratio of 10 would be appropriate if primary infection lasted for 6 months instead of 2, while a rate ratio of 30 would be reasonable if the primary infection lasted for 2 months instead of 4.8. Although we are not sure how long primary infection lasts, our review shows that duration and transmission rate during this period are negatively associated and bivariate estimates are necessary.

We standardized transmission risk before we analysed the data to compare the results of different studies. We thus made assumptions about sexual behaviour, which are probably inaccurate. We could not account for changes in sexual behaviour patterns over time [47] or inaccurate self-reporting of sexual contacts [48]. Because we used transmission rates per year instead of transmission probabilities per sex act, our assumptions about sexual behaviour did not substantially affect the results. Moreover, we only used these assumptions in five of 24 viral load studies, which reported probabilities instead of rates. For primary infection studies, we report rate ratios, and these are less sensitive to sexual behaviour than rates. We acknowledge these additional limitations: Most of the studies that used a step function stratified risk of HIV transmission according to variables (e.g. circumcision status, female to male versus male to female transmission,

baseline $CD4^+$ cell count value, baseline age), which made comparisons difficult. The number of steps and change points also varied between studies. Studies used different inclusion criteria. There was some heterogeneity in HIV transmission between studies. Transmission rates in the Partners in Prevention study were lower than in the Rakai study. It is unlikely that the functional dependence between transmission rate and viral load values depends on different transmission rates between studies. In a sensitivity analysis, in which we analysed data from Fideli *et al.* [8] under different assumptions about the relationship between viral load and transmission rates, we found that heterogeneity was smaller, but remained substantial.

Ours is the first review to consider assumed durations of primary infection. The strength of our study is its unique design: we systematically reviewed the literature and analysed rates of HIV transmission on the basis of different relationships between viral load and transmission rate. Our viral load models were not restricted to one parametric form, and we could fully compare estimates of transmission rates for high viral loads. We analysed rates of HIV transmission for different durations of primary infection. This is an improvement over previous reviews that meta-analysed transmission rates without adjusting for the underlying assumptions about the duration of primary infection.

Reliable estimates of transmission rates for high viral loads are urgently needed. All studies were based on six primary data sources. Collecting more primary data on HIV-negative couples who seroconvert, and on serodiscordant couples will improve estimates. Current studies capture additional transmission risk for an assumed duration of primary infection, but accurate estimation of this duration requires more complete data on initially seronegative couples. Available primary data on viral load should be reanalysed to estimate the shape of the increase in transmission rates when viral load increases.

Conclusion
The relation between viral load values and HIV transmission, and the duration and amount of transmission during primary infection are poorly understood. HIV transmission risk increases as viral load values increase, but the pattern of this relationship is unclear. If transmission rates increase exponentially with viral load values, then finding and treating patients with high viral load values should be a public health priority [49]. But if the function is saturating, then TasP may not reduce HIV incidence. Estimates of the relationship between HIV transmission risk and viral load, and the contribution of primary infection diverge because researchers make different assumptions about the shape of the relationship between transmission rate and viral load, and the duration of primary infection. TasP is widely advocated as a public health approach for HIV prevention, but we cannot assess

its effectiveness until HIV transmission rates in the primary phase, and effect of viral load on transmission, are accurately described.

Conflicts of interest
The authors have no conflicts of interest to declare.

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