Title: Association between sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy: The PARTNER STUDY

Date of revision: 26th March 2016

Authors

Alison J Rodger[1] MD, Valentina Cambiano[1] PhD, Tina Bruun[2] RN, Pietro Vernazza[3] MD, Simon Collins[4], Jan Van Lunzen[5] PhD, Giulio Maria Corbelli[6], Vicente Estrada[7] MD, Anna Maria Geretti[8] PhD, Apostolos Beloukas[8] PhD, David Asboe[9] FRCP, Pompeyo Viciana[10] MD, Félix Gutiérrez[11] PhD, Bonaventura Clotet[12] MD, Christian Pradier[13] MD, Jan Gerstoft[14] MD, Rainer Weber[15] MD, Katarina Westling[16] MD, Gilles Wandeler [17] MD, Jan M Prins [18] PhD, Armin Rieger[19] MD, Marcel Stoeckle[20] MD, Tim Kümmerle[21] PhD, Teresa Bini[22] MD, Adriana Ammassari[23] MD, Richard Gilson[1] MD, Ivanka Krznaric[24] PhD, Matti Ristola[25] PhD, Robert Zangerle[26] MD, Pia Handberg[27] RN, Antonio Antela[28] PhD, Sris Allan[29] FRCP, Andrew N Phillips[1] PhD and Jens Lundgren[2] PhD for the PARTNER Study Group

Affiliations

1. Research Department of Infection & Population Health, University College London, London, UK
2. Department of Infectious Diseases / CHIP, Rigshospitalet, University of Copenhagen, Copenhagen, DK.
3. Division of Infectious Diseases and Hospital Epidemiology, Cantonal Hospital, St. Gallen, Switzerland
4. HIV i-Base, London, UK.
5. University Medical Center Hamburg-Eppendorf, Hamburg-Eppendorf, Germany.
6. European AIDS Treatment Group, Bruxelles, Belgium.
7. Hospital Clinico San Carlos & Universidad Complutense, Madrid, Spain.

8. Institute of Infection and Global Health, University of Liverpool, Liverpool, UK.
9. Chelsea and Westminster NHS Foundation Trust, London, UK.
10. Hospital Virgen del Rocío, Sevilla, Spain.
11. Hospital General de Elche & Universidad Miguel Hernández, Alicante, Spain.
12. Hosp. Universitari Germans Trias i Pujol, Barcelona, Catalonia, Spain. UVIC-UCC. UAB
13. CHU Nice Hopital de l' Archet 1, Nice, France.
14. Rigshospitalet, Copenhagen, Denmark.
15. Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.
16. Department of Medicine, Unit of Infectious Diseases and Dermatology, Karolinska Institutet and Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.
17. Division of Infectious Diseases and Hospital Epidemiology, University Hospital Bern, Bern, Switzerland.
18. Academic Medical Center, Amsterdam, the Netherlands.
19. Medical University of Vienna, Vienna, Austria
20. Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland.
21. Department of Internal Medicine, University Hospital of Cologne, Cologne, Germany.
22. Ospedal San Paolo, Milan, Italy.
23. Ospedale L. Spallanzani, via Portuense 292 – 00149, Roma, Italy.
24. Praxis Driesener Straße, Berlin, Germany.
25. Helsinki University Central Hospital, Helsinki, Finland.
26. Medical University Innsbruck, Innsbruck, Austria.
27. Hvidovre Universitets Hospital, Hvidovre, DK.
28. Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain.
29. Coventry and Warwickshire Hospital, Coventry, UK.

Key words: Condomless Transmission ART HIV-positive

Corresponding author

Dr Alison Rodger

Research Department of Infection & Population Health

University College London (UCL)

Rowland Hill Street. London, NW3 2PF, UK

Tel: +44 20 7472 6754 Fax: +44 20 7794 1224 Email : alison.rodger@ucl.ac.uk

Word count: Abstract: 349 Text: 4039

ABSTRACT

Importance: A key factor in assessing the effectiveness and cost-effectiveness of antiretroviral therapy (ART) as a prevention strategy is the absolute risk of HIV transmission through condomless sex with suppressed HIV-1 RNA-Viral-Load (VL) for both anal and vaginal sex.

Objective: To evaluate the rate of within-couple HIV transmission (heterosexual and MSM) during periods of sex without condoms and the HIV positive partner had HIV-1 RNA load <200 copies/mL.

Design, Setting, and Participants: The prospective, observational PARTNER study enrolled in 75 European Sites, (September 2010 to May 2014), 1166 HIV serodifferent couples (HIV-positive partner on suppressive ART) who reported condomless sex. Eligibility criteria for inclusion of couple-years-of-follow-up were: condomless sex and HIV-1 RNA load <200 copies/mL. Anonymised phylogenetic analysis compared couples' HIV-1 polymerase and envelope sequences if a negative partner became infected to determine phylogenetically linked transmissions.

Exposure: Virally suppressive ART

Main outcome: Risk of within couple HIV transmission to the HIV negative partner

Results: Among 1166 enrolled couples, 888 (mean age:42, IQR:35-48, 548 heterosexuals [61.7%], 340 MSM [38.3%]) provided 1238 eligible couple-years-of-follow-up (median follow-up, 1.3 years; IQR 0.8-2.0 years). At baseline couples reported condomless sex for a median 2 years (IQR: 0.5-6.3). Condomless sex with other partner/s was reported by 33% (n=108) HIV-negative MSM and 4% (n=21) heterosexuals. During follow-up, couples had condomless sex a median of 37 times/year (IQR: 15-71), with MSM couples reporting ~22,000 condomless sex acts and heterosexuals~18,000. Although 11 negative partners became HIV-positive (10 MSM; 1 heterosexual; 8 reported condomless sex with other partners), no phylogenetically linked transmissions occurred over 1238 eligible couple-years-of-follow-up, giving a rate of within-couple HIV transmission of zero,

with an upper 95% confidence limit of 0.30/100 couple-years-of-follow-up. The upper 95% confidence limit for condomless anal sex was 0.71/100 couple-years-of-follow-up.

Conclusions and Relevance: Among serodifferent heterosexual and MSM couples in which the HIV-positive partner was using suppressive ART and who reported condomless sex, during median follow-up of 1.3 years per couple (1238 total couple-years-follow-up), there were no documented cases of within-couple HIV transmission (upper 95% confidence limit 0.30/100 couple-years-of-follow-up). Additional follow-up is necessary to provide more precise estimates of risk .

BACKGROUND

Several studies have demonstrated that HIV-positive people on antiretroviral therapy (ART) with low plasma HIV-1 RNA load have markedly reduced infectiousness for sexual transmission.[1-4] In particular, the HPTN 052 study, which was conducted primarily in heterosexual serodifferent couples, demonstrated a 96% reduction in HIV transmission risk in HIV-positive adults randomized to early ART initiation compared to the group that deferred treatment.[4] As a result, new WHO guidelines now recommend that ART should be offered to all HIV-positive people irrespective of CD4 count. [5]

There are however a number of gaps in currently available evidence to inform such guideline changes. The most significant issue is that no data are available concerning transmission rates for anal sex when the HIV-positive partner is on suppressive ART; even though without ART per-act estimates of HIV transmissibility are approximately 10 times higher for anal intercourse[6-9] compared to vaginal sex.[10] Secondly, in all the transmission studies in heterosexual couples published to date, including HPTN 052, most of the observed couple-years-of-follow-up have been in the context of reported consistent condom use (up to 93%),[2-4,11-13] which also effectively prevents HIV transmission.[15] Study results therefore demonstrate the added benefit of ART in addition to the use of condoms, not just from use of ART alone. Condomless sex (sex where condoms are not used) was reported for only 330 couple-years-of-follow-up across all previous studies combined[2-4, 11-13] which is insufficient follow up to give precise estimates for transmission in the context of ART alone when condoms are not used.[15] The absolute risk of sexual HIV transmission from condomless sex for a person on stable suppressive ART therefore remains uncertain.

The primary aim of the PARTNER study is to follow serodifferent partnerships that have penetrative sex without using condoms where the HIV-positive partner is on ART with a plasma HIV-1 RNA load <200 copies/mL in order to study risk of HIV transmission through anal and vaginal sex in the absence of condom use.

METHODS

Study Design: The PARTNER study was an observational multi-centre study of serodifferent couples, heterosexual and men who have sex with men (MSM), in which the positive partner is on ART. The methods for the PARTNER study have been published previously.[16]

Ethics approvals

Prior to the initiation of the study at each clinical research site, the protocol, all informed consent forms and the participant information materials were submitted to and approved by the site's Ethics Committee (IRB or IEC). In the UK, the study was reviewed and approved by the North West London REC 2 Ethics Committee (EC reference number 10/H0720/55). Ethics approval was obtained in-country for all other European sites involved in the study. In addition any amendments to the study protocol were submitted and approved by each site's Ethics Committee (IRB or IEC).

Study Population and eligibility criteria: From September 2010, we recruited serodifferent couples from 75 clinical sites in 14 European countries. HIV-positive people on ART aged older than 18 years were eligible to take part with their HIV-negative partners. The HIV-positive partner was expected to remain on ART and the partnership met the following criteria: (i) the partners reported penetrative sex without using condoms (condomless sex) together in the month before enrollment (during which period the HIV-negative partner was aware of the HIV status of the HIV-positive partner) and (ii) the partners expected to have sex together again in the coming months.

Study procedures: Participating clinics asked HIV-positive patients on ART about condomless sex with HIV-negative partners and if they wished to take part in an HIV transmission study. If both positive and negative partners agreed to take part, they signed separate informed consents, which included partner identification by name. The informed consent also included explicit reference to the fact that HIV-negative partners knew their

partner was HIV-positive and that there was a transmission risk from condomless sex. Clinic staff were asked to recommend consistent condom use at each study contact.

Follow-up was stopped if the partnership ended, the partners moved away or either person in the partnership withdrew consent, but not for changes in sexual behaviour or use of ART (although such changes could lead to the follow-up time not being eligible for the main analysis). Follow-up in heterosexual couples ended on 31st May 2014, and remains ongoing for MSM couples. Follow-up in this report was censored on 31st May 2014.

Data collection: Study data were collected on standardized case report forms after consent at baseline and then every 4 to 6 months. Detailed information was collected on socio-demographics (including participant self-identified ethnicity [using fixed categories defined by the investigators] to consider a possible association between ethnicity and transmission rate); self-reported adherence to ART rated from 0% to 100% over the previous month (positive partner); sexual activity between the partners (since last visit), frequency of intercourse, type of intercourse (receptive or insertive, vaginal or anal) and whether ejaculation occurred; sexually transmitted infections (STIs) and the presence of symptoms suggestive of an STI; and use of pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP). Injection drug use was assessed and if needles, syringes or any part of injection equipment was shared.

HIV-negative partners were also asked if they had condomless sex with anyone other than their HIV-positive partner since their last visit, the number of other partners and if any were HIV-positive or of unknown serostatus. For the HIV-positive partner, data on ART, CD4 count and current and recent plasma HIV-1 RNA load were recorded through a clinical case report form.

Laboratory testing and phylogenetic analysis

The HIV-negative partner was asked to test for evidence of HIV seroconversion every 6 to 12 months. Where possible, a combined antigen/antibody test was used to increase diagnostic sensitivity in early infection. Plasma

HIV-1 RNA was measured in the HIV-positive partner according to routine care every 6 to 12 months; testing was undertaken at the local diagnostic laboratory. If at any time the HIV-negative partner was found to have become HIV-positive, a venous blood sample in EDTA was taken from both partners to determine genetic relatedness of HIV-1 *pol* and *env* sequences.

Details of the methodology used for sequencing and analysis are given in the Supplementary File. Briefly, following Sanger sequencing of either plasma HIV-1 RNA or cellular HIV-1 DNA[17], maximum-likelihood (ML) and Bayesian Markov Chain Monte-Carlo (MCMC) inferences, as implemented in RAxML-HCP2 v8 and MrBayes v3.2.6 respectively, were determined as previously described[18]. Controls comprised the 10 closest sequences identified through BLAST searches of GenBank (http://blast.ncbi.nlm.nih.gov). Replicate partners' sequences (obtained from different sampling points, different specimen types, or repeat testing of the same sample), and sequences from confirmed HIV- transmission pairs obtained in a separate study[18] were included as positive controls. A seroconversion event was to be classed as linked if the partners' sequences grouped together on a monophyletic branch with high support, defined as a bootstrap value ≥0.90 (ML) or a posterior probability ≥0.95 (MCMC), and had a pairwise genetic distance of ≤0.015 nucleotide substitutions per *pol* site, as per published methodology[19, 20]. Sequences showing a genetic distance ≤0.045 were subjected to further inspection, to ensure potential linkage was not missed[19, 20].

Statistical Analysis

The primary analysis was estimation of the rate of transmission, calculated as the number of HIV infections that occurred during eligible couple-years-of-follow-up (see definition below) divided by eligible couple-years-of-follow-up; excluding infections which were shown to be phylogenetically distinct from the index patient's virus (i.e. transmission has not been from the original HIV-positive index partner). Couple-years-of-follow-up were determined as periods of time delimited by HIV tests, and corresponding questionnaires on sexual behavior, in the negative partner. These were eligible for inclusion in this analysis if: (i) couples had condomless sex during the period (reported at the end of the time period by the HIV-negative partner or, if this partner did not reply,

by the positive partner); (ii) there was no report of PEP or PrEP use; (iii) latest plasma HIV-1 RNA load in the positive partner was <200 copies/mL and not dated older than 12 months; and (iv) follow-up occurred before 31st May 2014 (i.e. the censoring date). In sensitivity analysis, we included periods of follow-up time in which the HIV-RNA was suppressed at the beginning of the period but, as revealed by an HIV RNA measure at the end of the period, had become elevated during the period. This allows inclusion of periods where a couple may continue having condomless sex until they know the HIV RNA is elevated. In order to calculate confidence intervals for the incidence rate of transmission we used exact Poisson methods. The rate of within-couple transmission was calculated restricting to couple-years-of-follow-up where a certain type of sex (e.g. receptive anal sex with ejaculation) was reported. For this analysis it was not required that this was the only type of sex the couple was having. However, in sensitivity analysis we calculated the rate and confidence interval taking a hierarchical approach to classifying transmission risk with types of sex. Having defined such a hierarchy of risk, in referring to a specific sex act, we estimated the upper limit of the rate if this type of sex is the highest risk sex being performed. The hierarchy (from the highest to the lowest risk) was: receptive anal sex with ejaculation, receptive anal sex without ejaculation, insertive anal sex, vaginal sex with ejaculation and vaginal sex without ejaculation.

To assess whether there were differences in the characteristics among HIV-negative and HIV-positive people across the different groups (heterosexual men, heterosexual women and MSM) we performed two-sided Kruskal Wallis test for continuous variables and two-sided Chi-Square tests for categorical variables. All tests of significance used $P<0.05$ as the threshold of statistical significance. Missing data were not imputed and the analysis was performed only on the available data. Data were analysed using SAS (version 9.3).

Sample size calculation

In planning the sample size it was known that the transmission rate was low[3] and the aim was to generate a more precise estimate of the rate than was available. The sample size was based on a hypothesized transmission rate of 1 per 1000 couple-years of condomless sex, with the choice of this very low rate based on

arguments laid out in the Swiss Statement[21]. Under this hypothesis, 2000 couple-years-of-follow-up was required to have an 85% chance that the upper limit of the 95% confidence interval for the transmission rate is < 0.44 per 100 couple-years-of-follow-up (0.2 per 100 couple-years-of-follow-up with a transmission rate of zero). The Executive Committee stopped follow-up of heterosexual couples at 31st May 2014 at the end of Phase1 in order to concentrate resources on MSM couples. Phase 2 with MSM couples only will be continued for a further 4 years to accrue additional data for anal intercourse. We have pre-specified that we will not undertake further analysis until the end of the second phase of the study in 2018.

RESULTS

Eligible couple-years-of-follow-up accrued

From the 1166 couples enrolled by 31st May 2014, 1004 couples had at least one follow-up visit by the censoring date and 888 couples contributed 1238 eligible couple-years-of-follow-up; 1251 when including periods of follow-up time in which the HIV-RNA was suppressed at the beginning of the period but during which the HIV-RNA became elevated. The main reasons for couples providing no eligible couple-years-of-follow-up (n=116) out of those with at least one follow-up visit were: no HIV test in the negative partner (n=20), use of PEP/PrEP (n=9), no condomless sex reported (n=15), and plasma HIV-1 RNA >200 copies/mL (n=55) and plasma measurement not available (n=17). Participants who contributed eligible couple years of follow-up (compared to those who did not) were slightly older, more likely to have undertaken vocational education (among heterosexuals), more likely to have reported sex with other men rather than "other" as HIV acquisition route (among MSM), had been having condomless sex for longer (among MSM) and more likely to have a CD4 count >350 mm^3 (among heterosexuals) (see Supplementary Table 5).

Median eligible years-of-follow-up per couple was 1.3 years (IQR: 0.8-2.0). The estimated dropout rate was 18 per 100 couple-years-of-follow-up when considering all the couples enrolled (n=1,166) and 11 per 100 couple-

years-of-follow-up when restricting to the 888 couples who contributed to eligible couple-years-of-follow-up. The reason for dropping out of the study, among couples who contributed eligible couple years of follow-up (888 couples) were: relationship broke up (n=69, 41%), moved away (n=15, 9%), consent withdraw/do not want to continue (n=18, 11%) and other/not clear (n=65, 39%). Of couples contributing eligible couple-years-of-follow-up, 340 were MSM, 269 heterosexual where the male partner was HIV-positive (male positive/female negative) and 279 heterosexual where the female partner was HIV-positive (male negative/female positive). Overall, 94% of the eligible couple-years-of-follow-up were during periods of very low plasma HIV-1 RNA <50 copies/mL, the other 6% were between 50-200 copies/mL.

Baseline couple characteristics

Characteristics at baseline of the participants who contributed to eligible couple years of follow-up are reported in Table 1. Median age was 40-45 years in all-participant groups. HIV-negative MSM reported having condomless sex with their positive partners for a median 1.4 years (interquartile range [IQR]: 0.5 to 3.5 years) prior to enrolment. For heterosexual couples this was 2.8 years (IQR: 0.6 to 7.5 years) for male negative/female positive and 3.6 years (IQR: 0.7 to 11.4 years) for male positive/female negative.

At baseline, HIV-positive partners had been on ART a median of 7.5 years (IQR: 3.3 to 14.2 years), 10.6 years (IQR: 4.3 to 15.6 years) and 4.8 years (IQR: 1.9-11.4 years) in women, heterosexual men and MSM, respectively. Self-reported adherence to taking ART was high across all HIV-positive groups, with 93% of heterosexual men, 94% of heterosexual women and 97% of MSM reporting adherence to ART of >90% and very few reporting they missed ART for more than 4 consecutive days; though this was more common in heterosexuals (6% men, 8% women) than in MSM (3%). MSM were also more likely to correctly self-report a suppressed HIV load, 94% MSM compared to 84% of heterosexual men and 87% of heterosexual women. The majority in all groups had CD4 count >350mm^3 at baseline.

Follow up clinical and behavioral data

During prospective follow-up (Table 2), 17% of HIV-negative MSM and 18% of HIV-positive MSM reported being diagnosed with an STI at some point, while in both HIV-negative and HIV-positive heterosexual men and women this was 6%.

Thirty three percent of HIV-negative MSM and 4% of HIV-negative heterosexual men and women reported condomless sex with other partners. Very few HIV-negative partners reported injecting drugs during follow-up, 3% MSM, 2% heterosexual men and 1% women.

Couples reported frequent condomless sex during follow up as illustrated by the number of condomless sex acts reported during follow up (Table 3). The median number of condomless sex acts per year within the partnership reported by the HIV negative partner were similar across all groups during eligible couple-years-of-follow-up, with MSM reporting a median of 42 condomless acts/year (IQR 18-75) compared to 35 (IQR 14-68) by in heterosexual men and 36 (IQR 13-71) by women. Overall, all groups reported large numbers of condomless sex acts during follow-up, with over 22,000 condomless sex acts in MSM, and 18,000 in heterosexual couples.

Figure 1 provides data on prevalence of the type of condomless penetrative sex (with the positive partner) reported by the HIV-negative partner. By definition, couples contributing eligible couple-years-of-follow-up reported condomless penetrative sex at some point during follow-up. Among heterosexual couples, 99% reported vaginal sex with or without ejaculation and 11.1% reported anal sex. For MSM, 67% of negative partners had receptive anal sex without ejaculation, 45% had receptive anal sex with their partner ejaculating inside them and 92% reported insertive anal sex.

The main reasons reported by HIV-negative partners for not using a condom were a belief that the risk of HIV transmission was very low (57% heterosexual men, 52% women, 63% MSM) and that sex was more enjoyable without condoms (38% heterosexual men, 41% women, 61% MSM). Fifteen percent of HIV-negative women reported not using a condom as they were trying for a pregnancy.

Rates of HIV transmission through condomless sex

A total of 11 of the originally HIV negative partners were observed to acquire HIV during eligible follow up, but there were no phylogenetically linked transmissions. Of the 11 people who became infected, 10 were MSM, 1 was heterosexual, 8 (73%) of these reported that they had recent condomless sex with others apart from their study partner.

Viral sequences were recovered successfully from all couples, comprising 22/22 (100%) subjects for *pol* and 20/22 (91%) subjects for *env*. Samples collected from the two partners of each couple were median 0 months' apart (IQR 0.0-5.9). The partners that were initially HIV-positive had subtype B infection in all cases. The partners that seroconverted during the study acquired subtype B infection in 9/11 cases; one subject (couple 5) acquired subtype A1 and a second subject (couple 6) acquired CRF14_BG (Supplementary Table 1). In the phylogenetic analyses, none of the partners' sequences clustered together, with consistent results observed across analyses (Figure 2 and Supplementary Figures 1 and 2). The partners' sequences showed pairwise genetic distances consistently >0.040. With couple number 7, the pairwise genetic distances of *pol* sequences were 0.043 and 0.040 for different sample types; however there was no phylogenetic evidence of linked clustering (Figure 2 and Supplementary Figures 1 and 2). Positive control sequences showed median genetic distances of 0.004 (IQR <0.001-0.008) (Supplementary Table 2), and were closely linked on monophyletic branches with bootstrap values ≥0.98 and posterior probabilities =1.00.

Given that there were no linked transmissions (even when considering periods during which the HIV-RNA became elevated), the estimated rate for transmission through any condomless sex with the positive partner on ART with HIV load <200 copies/mL was zero, with an upper 95% confidence limit of 0.30 per 100 couple-years-of-follow-up (0.29 when including periods of follow-up time in which the HIV-RNA was suppressed at the beginning of the period but during which the HIV-RNA became elevated). Figure 1 reports the rates of within-couple HIV transmission per 100 eligible couple-years-of-follow-up by sexual behaviour reported by the HIV-negative partner. For all sex in heterosexual couples the upper confidence limit was 0.97 per 100 couple-years-of-follow-up for male positive/female negative couples, and 0.88 for female positive/male negative couples. In

MSM the upper confidence limit for all sex was 0.84 per 100 couple-years-of-follow-up. For anal sex, the upper 95% confidence limit was 0.71 per 100 couple-years-of-follow-up (heterosexual and MSM data combined) and for receptive anal sex with ejaculation it was 2.23 per 100 couple-years-of-follow-up (heterosexual and MSM data combined), and 2.70 per 100 couple-years-of-follow-up for MSM only. The upper limit of the 95% CI is higher for anal sex due to the lower number of couple-years-of-follow-up accrued to date. When considering a hierarchical approach (i.e. the act-specific rates were restricted to couple-years of follow-up where that type of act was the highest risk type of sex reported), the 95% upper limit of the confidence interval increases: for receptive anal sex without ejaculation it increased from 8.14 to 11.95 per 100 couple-years-of-follow up for heterosexual women, and from 1.68 to 3.06 per 100 couple-years-of-follow up for MSM, while for vaginal sex it increased from 0.59 to 0.69 per 100 couple-years-of-follow up (Heterosexual men and women combined) (See Supplementary Table 4 for further details. A table detailing the rates and 95% upper confidence limited using this approach has been included in the supplementary material (Supplementary Table 4). All but 3 non-linked HIV-1 infections occurred among partners reporting condomless sex with other partners.

DISCUSSION

This study provides the first estimate of HIV transmission risk through condomless anal sex where the HIV-positive partner is on ART with suppressed plasma HIV viral-load and an estimate of the absolute rate of HIV transmission through condomless heterosexual sex. The estimate of the overall transmission rate, and of the transmission rate for anal sex, was zero. However 95% confidence limits suggest that with eligible couple years accrued so far we cannot exclude appreciable levels of risk, particularly for anal sex and when considered from the perspective of a cumulative risk over several years.

Only couples that continued to have condomless sex were included in this study to enable focus on situations where transmission risk without ART is highest. This contrasts with other transmission studies including HPTN

052 where reported condom use was high (93%[4]) and the low absolute rate of transmission in the ART group reflects both ART and condom use, thus assessing two prevention strategies in combination not just ART alone. It is important to know how low the risk of transmission is with the use of ART alone without simultaneous use of condoms and this study contains more than three times couple-years-of-follow-up for condomless sex than all the other previous studies combined including over 500 couple-years-of-follow-up of condomless anal sex. [15] Both MSM and heterosexual couples in this study reported regularly having sex without a condom during follow-up. Based on the number and type of sex acts and the cumulative probability of HIV transmission, we would have expected over 100 transmissions in the MSM group alone (see supplementary material) if the positive partner had not been on ART. [10]

Although these results cannot directly provide an answer to the question of whether it is safe for serodifferent couples to practice condomless sex, our study provides reliable data (especially for heterosexuals) for couples to base their personal acceptability of risk on. In the absence of ART, receptive anal sex with ejaculation is recognized as carrying a higher risk than other forms and, although we observed a transmission rate of zero for this risk behavior, we cannot exclude a clinically important rate of less than 2.2 per 100 couple-years-of-follow-up. This translates into an upper limit estimate of 20% risk over 10 years. As the upper limit of the confidence interval is simply a function of the amount of couple-years-of-follow-up for that sexual act, additional follow-up in MSM is therefore needed through the second phase of the PARTNER study (PARTNER2), in order to provide even more precise estimates for transmission risk in MSM in the context of ART. These data are needed to provide equality of evidence between MSM and heterosexual couples both to inform policy and also individual choice on condom use.

Although we found no linked transmissions within couples, 11 unlinked HIV transmissions did occur during eligible follow-up. One third of HIV-negative MSM in this study reported having condomless sex concurrently with other partners outside the main relationship. A high prevalence of sexual concurrency and in particular concurrent condomless anal sex has been reported in other studies in MSM. [22,23] Related to this, HIV-negative

MSM were also relatively commonly diagnosed with an STI. Acquisition of an STI was not associated with risk of HIV-1 transmission within the couples under study, although power was limited to exclude a possible true effect.

Our study has several limitations. We originally aimed to observe 2000 couple-years of follow-up, but only 1238 couple-years were eligible for the primary analysis. As the primary analysis concerns the estimation of a rate with a 95% confidence interval this does not impact on the interpretation. In addition, although there was a degree of drop out of study participants, the reasons for dropout do not give cause to consider that those who dropped out would have experienced a higher transmission rate while virally suppressed on ART. The follow-up time was relatively short although at study entry couples reported having condomless sex with their current partner for several months to years. Direct evidence that some individuals are particularly susceptible to early acquisition of HIV infection is currently lacking, but it remains possible that the transmission rate is higher in the initial period of condomless sex between a couple. It is important to note that although the transmission rate was also zero in the 23% of couples in the study where the partnership was relatively recent (< less than 6 months), we are unable to determine the risk of HIV-transmission in very new partnerships.

Conclusions

Among serodifferent heterosexual and MSM couples in which the HIV-positive partner was using suppressive ART and who reported condomless sex, during median follow-up of 1.3 years per couple, there were no documented cases of within-couple HIV transmission, with an upper 95% confidence limit of 0.30/100 couple-years-of-follow-up. This provides the first estimate of HIV transmission risk for MSM through condomless anal sex with suppressed plasma HIV viral-load. Additional longer-term follow-up is necessary to provide more precise estimates of risk.

Acknowledgements

The authors would like to acknowledge the contribution of the PARTNER study participants. We would also like to acknowledge Dr John Ambrose of the University of Liverpool for his support with sequencing

PARTNER Study Group

Spain: Hospital Virgen del Rocío, Sevilla: V Pompeyo, M Trastoy, R Palacio. Hospital General de Elche: F Gutiérrez, M Masiá, S Padilla, C Robledano. Hosp. Universitari Germans Trias i Pujol, Badalona: B Clotet, P Coll. Hospital La Paz, Madrid: J Peñ. Hospital Universitario San Carlos, Madrid: V Estrada, M Rodrigo, E Santiago. Hospital Universitario Reina Sofia De Cordoba: A Rivero. Hospital Clínico Universitario de Santiago de Compostela: A Antela, E Losada, C Lires, A Aguilera. Hospital Clínic de Barcelona, Barcelona: J Gatell. Centro Sanitario Sandoval, Madrid: J Guerrero. Hospital Ramon y Cajal, Madrid: F Dronda. Hospital Carlos III, Madrid: V Soriano.

United Kingdom: Chelsea and Westminster Hospital, London: D Asboe, N Nwokolo, J Sewell. Mortimer Market Clinic, London: R Gilson, N Esteban, S McNamara. Royal Free Hospital: A Rodger, K Sturgeon. Southmead Hospital, Bristol: M Gompels, L Jennings. Coventry and Warwickshire Hospital: S Allan. Edinburgh Infectious Diseases, University of Edinburgh: Clifford Leen, Sheila Morris**.** King's College Hospital: M Brady, L Campbell. Brighton: M Fisher. Leicester Royal Infirmary: J Dhar. Newham: R O'Connell. Birmingham Heartlands: D White. St Thomas Hospital, London: J Fox. St Mary's Hospital, London: S Fidler. Bradford: P Stanley. Earnsdale, Redhill: U Natarajan. Northampton: M Ghanem. North Middlesex University Hospital, London: J Ainsworth, A Waters. North Manchester General Hospital: E Wilkins. St James's, Leeds: J Minton, J Calderwood. Hastings: H Patel. Whipps Cross Hospital, London: M Lascar.

Germany: University Clinic, Hamburg Eppendorf: J Lunzen. University Hospital Cologne: T Kümmerle, G Fätkenheuer, E Rund, C Lehmann. Praxis Driesener Straße, Berlin: I Krznaric, P Ingiliz, J Motsch, A Baumgarten.

Medizinische Poliklinik, Munich: J Bogner. Universitäts-Hautklinik, Bochum: N Brockmeyer. ICH Study Center, Hamburg: H J Stellbrink. Gemeinschaftpraxis Jessen-Jessen-Stein, Berlin: H Jessen. University Hospital, Bonn: J Rockstroh.

Switzerland: University Hospital Basel: M Stoeckle, M Battegay. University Hospital Zürich: R Weber, C Grube, D Braun, H Günthard,. University Hospital Bern: G Wandeler, H Furrer, T Konrad, A Rauch. Cantonal Hospital, St. Gallen, Switzerland: P Vernazza, M Rasi. Ospedale Regionale Di Lugano: E Bernasconi. Cantonal Hospital Baselland: P Tarr.

Denmark: Rigshospitalet, Copenhagen: J Gerstoft, Nurses from 8622, T Quist. Hvidovre Hospital: P Handberg, B Clausen, L Mathiesen. Aarhus Universitetshospital, Skejby Oestergaard, L. Odense Universitetshospital: S Stenvang.

Finland: Helsinki University Central Hospital: M Ristola, P Kivelä.

Sweden: Karolinska Hospital, Stockholm: K Westling, E Frisén. Venhälsan, Stockholm; A Blaxhult.

Ireland: St. James' Hospital, Dublin: G Cortney.

Belgium: CHU Saint-Pierre, Bruxelles : N Clumeck. University Ziekenhuis, Gent: L Vandekerckhove.

The Netherlands: AMC. Amsterdam: J Prins. OLVG, Amsterdam: K Brinkman. Medisch Centrum Jan van Goyen, Amsterdam: D Verhagen. DC Klinieken, Amsterdam: A Eeden.

France: Hopital de l' Archet 1, Nice: C Pradier, J Durant, M Serini, S Bréaud. CHU Hotel-Dieu, Nantes : F Raffi. Hopital Tenon, Paris ; G Pialoux. Pari : M Ohayon. Aides : V Coquelin.

Austria: Medical University of Vienna : A Rieger, V Touzeau-Roemer. Medical University Innsbruck : R Zangerle, M Kitchen, M Gisinger, M Sarcletti. FA für Dermatologie/Venerologie, Linz: M Geit.

Italy: San Paolo Hospital, Milan: T Bini, L Comi, A Pandolfo, E Suardi. Ospedale Spallanzani, Roma: A Ammassari, P Pierro,G Carli, N Orchi. Università di Catania: M Celesia. Università degli Studi di Modena: C Mussini. Universitaria San Martino, Genova: A Biagio.

Portugal: Hospital Santa Maria, Lisabon: N Janerio.

Funding Source Statement

This paper summarises independent research funded by the National Institute for Health Research (NIHR) under its Programme Grants for Applied Research Programme (Grant Reference Number RP-PG-0608-10142). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

The funders had no role in design and conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication

Data Access, Responsibility, and Analysis

Valentina Cambiano and Jens Lundgren had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Author contributions

Alison Rodger, Andrew Phillips and Jens Lundgren conceived the study and obtained funding. Alison Rodger drafted the manuscript and wrote the final version of the paper. Valentina Cambiano analyzed the data. Tina

Bruun, Pietro Vernazza, Simon Collins, Jan Van Lunzen, Giulio Maria Corbelli, Vicente Estrada and Anna Maria Geretti contributed to the study design, interpretation of the data and writing of the paper. Anna Maria Geretti and Apostolos Beloukas performed the performed the sequencing and phylogenetic analysis. Tina Bruun oversaw study implementation. Alison Rodger, Pietro Vernazza, Jan Van Lunzen, Vicente Estrada, David Asboe, Pompeyo Viciana, Félix Gutiérrez, Bonaventura Clotet, Christian Pradier, Jan Gerstoft, Rainer Weber, Katarina Westling, Gilles Wandeler , Jan M Prins , Armin Rieger, Marcel Stoeckle, Tim Kümmerle, Teresa Bini, Adriana Ammassari, Richard Gilson, Ivanka Krznaric, Matti Ristola, Robert Zangerle, Pia Handberg, Antonio Antela and Sris Allan provided data for the study. All authors reviewed and approved the final manuscript.

Conflict of Interest Statements

Valentina Cambiano (personal fees from Merck Sharp & Dohmed Limited, outside the submitted work). Pietro Vernazza (consultancy fees from Gilead, Tibotec and BMS), Jan Van Lunzen (Board membership Gilead, BMS, Bionor and ViiV, and receiving grant funding from DFG, BMBF, BMS and Gilead), Giulio Maria Corbelli (Honoraria for consulting work from Abbvie, BMS, Gilead, Viiv), Vicente Estrada (Grant funding from Abbott, Gilead, ViiV, BMS, and consultancy fees from Abbott, Gilead), Christian Pradier (Pfeizer, Gilead, VIIV Health Care, MSD), Jan Gerstoft (Bristol-Meyers Squibb, Gilead, Medivir, AbbVie, Glaxo, Merck, and Janssen), Rainer Weber (travel grants Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dome, Pfizer, Roche, TRB Chemedica and Tibotec), Katarina Westling (Speaker and/or advisor fees from Abbvie, Bristol-Myers Squibb, Gilead Sciences and Janssen), Marcel Stoeckle (advisor fees and/or travel grants AbbVie, BMS, Gilead, Janssen, MSD, ViiV). Bonaventura Clotet (acted as a consultant, participated in advisory boards, received speaker fees and been an investigator for clinical trials for Abbott, AbbVie, Gilead, Janssen, Merck (MSD) and ViiV). Ivanka Krznaric (speaker or consultant for MSD, BMS, GSK, AbbVie, Janssen, ViiV, Gilead), Matti Ristola (Honoraria, consultancy fees, and support for attending scientific conferences from Abbvie, BMS, Gilead, GSK, Janssen, and Merck and grants for scientific activities from AbbVie and GSK), Andrew N Phillips (Grant

funding Gilead, ViiV), Anna Maria Geretti: Consultancy fees and Speaker's fees from Abbott Diagnostics, Abbvie, Gilead, GSK, Janssen, MSD, Pfizer, and ViiV, outside the submitted work. The University of Liverpool is the recipient of grant income from BMS, Gilead, Janssen and ViiV for studies of which AMG is the principle investigator, outside the submitted work

The following have no conflicts to declare: Alison J Rodger, Tina Bruun, Simon Collins, Apostolos Beloukas, David Asboe, Pompeyo Viciana, Felix Gutiérrez, Gilles Wandeler, Jan Prins, Armin Reiger, Tim Kümmerle, Teresa Bini, Adriana Ammassari, Richard Gilson, Robert Zangerle, Pia Handberg, Antonio Antela, Sris Allan, Jens Lundgren

REFERENCES

1. Quinn TC, Wawer MD, Sewankambo N, et al. Viral load and heterosexual transmission of HIV type 1. *N Engl J Med* 2000; 342:921–929.
2. Donnell D, Baeten JM, Kiarie J, et al. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. *Lancet* 2010; 375:2092–2098.
3. Attia S, Egger M, Müller M, Zwahlen M, Low N. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *AIDS* 2009; 23:1397–1404.
4. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; 365:493–505.
5. Policy brief: consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: what's new. World Health Organization. 2015 http://apps.who.int/iris/bitstream/10665/198064/1/9789241509893_eng.pdf?ua=1. Accessed December 31, 2014.
6. Vittinghoff E, Douglas J, Judson F, et al. Per-contact risk of human immunodeficiency virus transmission between male sexual partners. *Am J Epidemiol* 1999;150(3):306-11
7. Jin F, Jansson J, Law M, et al. Per-contact probability of HIV transmission in homosexual men in Sydney in the era of HAART. *AIDS* 2010;24(6):907-13.
8. Hallett TB, Smit C, Garnett GP, de Wolf F. Estimating the risk of HIV transmission from homosexual men receiving treatment to their HIV-uninfected partners. *Sexually Transmitted Infections* 2011;87(1):17-21.
9. Baggaley RF, White RG, Boily MC. Infectiousness of HIV-infected homosexual men in the era of highly active antiretroviral therapy. *AIDS* 2010;24(15):2418-20.
10. Baggaley RF, White RG, Boily MC. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. *Int J Epidemiol* 2010;39(4):1048-63

11. Melo MG, Santos BR, De Cassia Lira R, et al. Sexual transmission of HIV-1 among serodiscordant couples in Porto Alegre, southern Brazil. *Sex Transm Dis* 2008;35(11):912-5.
12. Castilla J, Del Romero J, Hernando V, Marincovich B, García S, Rodríguez C. Effectiveness of highly active antiretroviral therapy in reducing heterosexual transmission of HIV. *J Acquir Immune Defic Syndr* 2005;40(1):96-101.
13. Reynolds SJ, Makumbi F, Nakigozi G, et al. HIV-1 transmission among HIV-1 discordant couples before and after the introduction of antiretroviral therapy. *AIDS* 2011;25(4):473-7
14. Weller S, Davis K. Condom effectiveness in reducing heterosexual HIV transmission. *Cochrane Database Syst Rev* 2001;3:CD003255.
15. Rodger AJ, Bruun T, Vernazza P, et al for the PARTNER Study Group. Further research needed to support a policy of antiretroviral therapy as an HIV prevention initiative. *Antivir Ther* 2013;18(3):285-7.
16. Rodger A, Bruun T, Weait M, et al; PARTNER study group Partners of people on ART - a New Evaluation of the Risks (The PARTNER study): design and methods. *BMC Public Health* 2012;12:296
17. Geretti AM, Conibear T, Hill A, et al for the SENSE Study Group. Sensitive testing of plasma HIV-1 RNA and Sanger sequencing of cellular HIV-1 DNA for the detection of drug resistance prior to starting first-line antiretroviral therapy with etravirine or efavirenz. J Antimicrob Chemother 2014;69:1090-7.
18. Beloukas A, Magiorkinis E, Magiorkinis G, et al. Assessment of phylogenetic sensitivity for reconstructing HIV-1 epidemiological relationships. Virus Res 2012;166:54-60.
19. Hue S, Brown AE, Ragonnet-Cronin M, et al. Phylogenetic analyses reveal HIV-1 infections between men misclassified as heterosexual transmissions. AIDS 2014;28:1967-75.
20. Mbisa JL, Fearnhill E, Dunn DT, et al. Evidence of self-sustaining drug resistant HIV-1 lineages among untreated patients in the United Kingdom. Clin Infect Dis 2015;61:829-36
21. Vernazza P, Hirschel B, Bernasconi E, Flepp M. *Les personnes séropositives ne souffrant d'aucune autre MST et suivant un traitment antirétroviral efficace ne transmettent pas le VIH par voie sexuelle.* Bulletin des

médecins suisses 89 (5), 2008. English translation, including translator's affidavit. http://www.edwinjbernard.com/pdfs/SwissCommissionstatement_May2008_translationEN.pdf . Accessed 31 December 2015.

22. Tieu HV, Nandi V, Frye V, et al for the NYC M2M Study Team. Concurrent partnerships and HIV risk among men who have sex with men in New York City. *Sex Transm Dis* 2014;41(3):200

23. Rosenberg ES, Khosropour CM, Sullivan PS. High prevalence of sexual concurrency and concurrent unprotected anal intercourse across racial/ethnic groups among a national, Web-based study of men who have sex with men in the United States. *Sex Transm Dis* 2012;39(10):741-6.

Table 1: Characteristics at baseline of HIV-positive and negative partners enrolled in the PARTNER study and eligible for the primary analysis(n=888)

		HIV+ heterosexual M (n=269)	HIV+ heterosexual W (n=279)	HIV+ MSM (n=340)	HIV- heterosexual M (n=279)	HIV- heterosexual W (n=269)	HIV- MSM (n=340)	p-value* (HIV+)	p-value* (HIV-)
Age, median (IQR)		44.9 (40.1 - 48.6)	41.7 (35.5 - 46.8)	40.1 (34.6 - 46.5)	44.9 (37.6 - 50.6)	40.3 (34.3 - 46.7)	40.1 (31.9 - 46.5)	<.0001	<.0001
Ethnicity, n (%)	White	221 (83%)	305 (91%)	170 (62%)	229 (85%)	217 (82%)	301 (89%)	<.0001	<.0001
	Black	24 (9%)	3 (1%)	63 (23%)	34 (13%)	19 (7%)	3 (1%)		
	Asian	4 (2%)	9 (3%)	27 (10%)	1 (0%)	7 (3%)	9 (3%)		
	Other	17 (6%)	18 (5%)	14 (5%)	7 (3%)	22 (8%)	24 (7%)		
	Missing	3	5	5	8	4	3		
Education title, n (%)	High school or less	139 (52%)	76 (23%)	116 (43%)	84 (31%)	119 (46%)	64 (19%)	<.0001	<.0001
	Vocational education	75 (28%)	103 (31%)	76 (28%)	92 (34%)	73 (28%)	88 (26%)		
	College or university	51 (19%)	156 (47%)	77 (29%)	93 (35%)	69 (26%)	183 (55%)		
	Missing	4	5	10	10	8	5		
HIV Acquisition route, n (%)	Heterosexual	97 (37%)	0 (0%)	188 (69%)				<.0001	-
	Homosexual	22 (8%)	324 (97%)	1 (0%)					
	Shared needles or other injection equipment	82 (31%)	0 (0%)	15 (6%)					
	Other	64 (24%)	10 (3%)	67 (25%)					
	Missing	4	6	8					
Years of CL sex, median(IQR)		3.2 (0.7 - 11.4)	1.5 (0.5 - 4.1)	2.9 (0.8 - 7.8)	2.8 (0.6 - 7.5)	3.6 (0.7 - 11.4)	1.4 (0.5 - 3.5)	<.0001	<.0001
	Missing	26	21	26	25	32	23		
Years on ART, median(IQR)		10.6 (4.3 - 15.6)	4.8 (1.9 - 11.4)	7.5 (3.3 - 14.2)				<.0001	-
	Missing	31	16	24	25	32	23		
Self-reported ART adherence >90%, n (%)	Yes	242 (93%)	319 (97%)	235 (94%)				0.1048	-
	Missing	10	11	29					
Informed their partner if	No	23 (9%)	13 (4%)	17 (6%)				0.0016	-

they missed doses of ART, n (%)	Yes	133 (51%)	133 (40%)	123 (45%)					
	Did not miss doses	107 (41%)	190 (57%)	132 (49%)					
	Missing	6	4	7					
Self-reported undetectable HIV load, n (%)	Yes	220 (84%)	309 (94%)	231(87%)				0.0003	-
	Missing	8	12	12					
CD4 count >350 mm^3, n (%)	Yes	229 (85%)	309 (91%)	249 (89%)				0.0787	-

CL=condomless; IQR= interquartile range; M = men; W = women;*Kruskal Wallis test for continuous variables, Chi-Square test for categorical variable;

Unless indicated there are no missing values; The percentages are calculated out of all the participants in that group who contributed to eligible couple-years-of-follow-up and provided a response to that question (missing data are excluded/ignored).

The comparisons are between all of the HIV positive groups and then between all of the HIV negative groups

Table 2. Characteristics during follow-up of HIV-positive and negative partners eligible for the primary analysis (n=888)

	HIV+ heterosexual M (n=269)	HIV+ heterosexual W (n=279)	HIV+ MSM (n=340)	HIV- heterosexual M (n=279)	HIV- heterosexual W (n=269)	HIV- MSM (n=340)	p-value* (HIV+)	p-value* (HIV-)
Years in the study, median (IQR)	1.9 (1.1 - 2.4)	1.8 (1.1 - 2.4)	1.4 (0.8 - 2.1)	1.8 (1.1 - 2.4)	1.9 (1.1 - 2.4)	1.4 (0.8 - 2.1)	<.0001	<.0001
STI, n (%)**	16 (6%)	16 (6%)	59 (18%)	16 (6%)	17 (6%)	56 (17%)	<.0001	<.0001
Missing***	5	3	11	4	6	10		
Gonorrhoea, n (%)	1 (<1%)	0 (0%)	20 (6%)	0 (0%)	0 (0%)	0 (0%)	<.0001	-
Warts, n (%)	2 (1%)	5 (2%)	8 (2%)	8 (3%)	0 (0%)	4 (1%)	0.2957	
other STI, n (%)	2 (1%)	12 (4%)	0 (0%)	0 (0%)	2 (1%)	0 (0%)	<.0001	0.0916
Not specified, n (%)	12 (5%)	1 (<1%)	32 (10%)	8 (3%)	15 (6%)	52 (16%)	<.0001	<.0001
CL sex with other partners, n (%)	-	-	-	11 (4%)	10 (4%)	108 (33%)	-	<.0001
Missing***	-	-	-	7	7	12		
CL sex with other positive partners, n (%)~	-	-	-	9 (3%)	0 (0%)	103 (31%)	-	<.0001
CL sex acts^/year, median (IQR)	28.2 (10.5 - 61.3)	30.1 (11.8 - 60.6)	33.0 (13.0 - 64.8)	34.6 (13.7 - 68.3)	35.6 (13.2 - 70.7)	41.7 (17.6 - 74.8)	0.2368	0.0522
Estimated total number CL sex acts^	15,543	16,945	19,685	18,431	17.509	22,273	-	-
Having missed ART for more than 4 consecutive days, n (%)	15 (6%)	21 (8%)	11 (3%)				0.0675	-
Missing	6	11	3					
Having injected non-prescription drugs, n (%)	7 (3%)	10 (4%)	18 (5%)	5 (2%)	2 (1%)	10 (3%)	0.2082	0.1404
Missing	5	11	7	5	12	14		
CYFU with reported CL sex of, n (%)~~:								
Less than once a month	90 (24%)	76 (17%)	87 (21%)	97 (23%)	72 (19%)	68 (15%)	0.2355	0.2757
1-2 times a month	59 (16%)	63 (14%)	65 (15%)	70 (17%)	64 (17%)	70 (16%)		

	3-4 times a month	54 (14%)	80 (18%)	85 (20%)	76 (18%)	72 (19%)	88 (20%)		
	5-8 times a month	93 (24%)	103 (24%)	95 (23%)	105 (25%)	93 (24%)	121 (27%)		
	More than 8 times a month	47 (12%)	66 (15%)	47 (11%)	53 (13%)	57 (15%)	73 (17%)		
	Not reported/Missing	37 (10%)	50 (11%)	39 (9%)	18 (4%)	23 (6%)	18 (4%)		

ART: antiretroviral therapy; CL: condomless; CYFU: couples years of follow up. IQR= interquartile range; M = men; W = women; STI: sexually transmitted infection;

*Kruskal Wallis test for continuous variables, Chi-Square test for categorical variable;

** *Participants who reported an STI since last visit were asked whether it was Syphilis, Gonorrhea, Chlamydia, Acute Genital Herpes, Chronic Genital Herpes, LGV, Bacterial Vaginosis or Other. None reported being diagnosed with Acute Genital Herpes or Syphilis. The following STIs did not have a frequency above 5 and so were grouped together as "Other STI": Chlamydia, Chronic Genital Herpes, LGV, Bacterial vaginosis and "Other". Participants that replied "Yes" to the question "Since your last visit, have you had a STI" but did not reply to the question "If yes, which STI?" were categorized as "Not specified".*

***never replied to this question during follow-up; ~ Only people that reported CL sex with other partners were asked this question. For this variable missing is treated as "No" and the denominator to calculate the percentages is the number of participants who reported whether they had "CL sex with other partners".

^only sex acts within couples are included;

~~The denominator is the total group-specific eligible couple years of follow-up. ;

Unless indicated there are no missing values; The percentages are calculated out of all the participants in that group who contributed to eligible couple-years-of-follow-up and provided a response to that question (missing data are excluded/ignored). The comparisons are between all of the HIV positive groups and then between all of the HIV negative groups.

Table 3: Condomless sex acts during follow-up according to number of condomless sex acts at baseline.

Number of couple-years of follow-up		**Number of condomless sex acts in the past 4 months reported at baseline by the HIV negative partner**						Total couple years of follow-up
		Once (n=41)	2-10 times (n=291)	11-20 times (n=178)	21-40 times (n=163)	>40 times (n=199)	Not reported (n=16)	
Number of condomless sex acts per 4 months - follow-up	Less than once	12 (23%)	39 (10%)	13 (5%)	7 (3%)	10 (4%)	2 (9%)	84
	Once	1 (2%)	9 (2%)	1 (<1%)	2 (1%)	0 (0%)	0 (0%)	13
	2-10 times	25 (48%)	223 (55%)	101 (41%)	70 (29%)	38 (14%)	9 (41%)	466
	11-20 times	4 (8%)	54 (13%)	52 (21%)	57 (23%)	51 (19%)	3 (14%)	222
	21-40 times	3 (6%)	32 (8%)	44 (18%)	78 (32%)	109 (40%)	3 (14%)	269
	>40 times	1 (2%)	3 (1%)	6 (2%)	13 (5%)	35 (13%)	0 (0%)	58
	Not reported	6 (12%)	41 (10%)	29 (12%)	17 (7%)	29 (11%)	4 (18%)	126
Total couple years of follow-up		52	402	245	245	272	22	1238

The table contains total number eligible couple years of follow-up (one of the main requirement being that condoms are not used))by frequency of condomless sex acts reported at baseline and during follow-up. In brackets we report the number of couples reporting a certain frequency at baseline.
The number of couple-years of follow-up have been rounded to the closest integer and this is the reason why some rows and column do not sum up exactly to the total of the column/row.

Figure 1: Rate (and upper 95% confidence limit (CL)) of HIV transmission according to sexual behaviour reported by the negative partner

[figure uploaded separately]

HTW: heterosexual women, HTM: heterosexual men; MSM: men having sex with men; CL: confidence limit; CYFU: couple year of follow-up; %: percentage of HIV negative members of the eligible couples ever reporting that specific sexual act (the denominator is the group-specific number of HIV negative participants who contributed eligible couple-years-of-follow-up). The upper limit of the 95% confidence interval was estimated using the exact Poisson method.

Figure 2: Phylogenetic tree of *pol* sequences from nine couples with subtype B infection. Bayesian Markov Chain Monte-Carlo (MCMC) inference (012212+I+G+F model). Branch length is proportional to the genetic distance and line weight is proportional to the posterior probability. Partners' sequences are in blue; "N" indicates the initially HIV-negative partner whereas "P" indicates the initially HIV-positive partner. Control sequences comprised the 10 closest sequences identified through BLAST searches of GenBank. Positive control sequences comprised replicate sequences from study partners (in red) and sequences from confirmed transmission pairs obtained in a separate study (in orange) [19]. *Sequences 9N2 and 9N3 were obtained from the same sample in two separate experiments.

[figure uploaded separately]