Appendix 5 (as submitted by the authors): Description of included studies

Randomized controlled trials

*Cohen et al., 2016* (1)
The objective of this randomised, controlled, open-label clinical trial (HIV Prevention Trials Network 052 trial [HPTN 052]) was to determine the effect of combination ART on the risk of sexual transmission of HIV among serodiscordant couples. In order to enrol in this clinical trial, the HIV index partner was required to have a CD4 count between 350 and 550 and to have never used ART to treat their HIV. A total of 1,763 couples (97% were opposite-sex couples) were recruited at sites in Brazil, the USA, India, Thailand, Kenya, Zimbabwe, Malawi, Botswana, and South Africa from June 2007 to May 2010. The study follow-up concluded in May 2015.

HIV-positive participants were randomly selected to receive ART immediately upon enrolment (early arm) or to receive ART when their CD4 count fell below 250 cells/μL or they developed an AIDS-defining condition (delayed arm). The delayed arm was discontinued after the interim study data was analysed in 2011 when it was found that early ART initiation is associated with a substantially lower risk of HIV transmission than delayed ART initiation. After the delayed arm of the study was dissolved, all participants were offered ART and 83% of the index partners in the delayed arm were on ART within one year. Participants attended quarterly study visits where the index partner’s viral load and CD4 count was tested and their HIV-negative partner received HIV testing.

Across the entire study, 78 HIV infections (19 in the early arm and 59 in the delayed arm) were observed over 8,509 person-years of follow-up for an overall incidence of 0.92 per 100 person-years (95%CI: 0.73-1.14). Considering only infections that occurred while the index partner was on ART (regardless of study arm), the incidence was 0.48 per 100 person-years (95%CI: 0.33-0.68) based on 32 infections (19 in early arm and 13 in delayed arm) over 6,620 person-years of follow-up while the index partner was on ART.

Genetic linkage analysis revealed that only eight linked infections occurred between partners when the index partner was on ART (three in the early group and five in the delayed group) for an incidence of 0.12 per 100 person-years (95%CI:0.05-0.24). Three additional infections that occurred while the index partner was on ART were unable to be properly analysed for genetic linkage and, therefore, it is unclear whether the transmission of the infections occurred within the couple or from another source outside of the relationship.

Of the eight linked infections that occurred while the index partner was on ART, none occurred during periods when the index partner was confirmed to have been virally suppressed (<400 copies/mL). Four of the infections were likely to have occurred when the index partner had been on ART for less than three months (and was therefore unlikely to have achieved an undetectable viral load). Three occurred during treatment lapses, and the final transmission was transmitted from an index partner who was intermittently viremic before being lost to follow-up for more than one year (2). Overall, zero confirmed linked transmissions were identified between serodiscordant partners where the index partner was on ART and confirmed to have a viral load <400 copies/mL (see Appendix 6 for characterisation of transmission events).

In interpreting the results of this study, there are important factors to consider. First, condom use was high (at the time of the interim data analysis 96% of those in the early-therapy group...
and 95% of those in the delayed-therapy group had reported 100% condom use. Similarly, adherence was high for those on ART – at interim analysis, 79% of early arm participants and 74% of delayed arm participants on ART had at least 95% adherence based on pill counts. Some limitations of this study include the fact that 97% of couples were female-male, which may limit the generalizability of the findings to groups such as MSM. Also, while the authors went to lengths to determine when linked transmissions occurred relative to ART use and viral suppression, it is not possible to determine the timing of HIV infections among couples with the index on ART with complete certainty. The study was examined for risk of bias using the Quality in Prognostic Studies (QUIPS) Risk of Bias Assessment Instrument for Prognostic Factor Studies and was determined to have an overall low risk of bias.

Various study authors declared receiving support from the NIH, other bodies, and various companies related to, and outside of this study, and the conflict of interest disclosure forms for all authors are publically available.

**Observational studies**

*Grulich et al., 2015* (3)

This poster presents the preliminary results of the Opposites Attract study, a prospective cohort study of serodiscordant couples of MSM in Australia, Thailand, and Brazil. The objective of the study is to determine HIV transmission risk for condomless sex when the index partner has an undetectable viral load on ART. The authors recruited 234 couples from various high HIV caseload clinics, of whom 152 had at least one follow-up visit and were included in this analysis. At baseline, 84.2% of the HIV-positive index partners remaining in the study were on ART and 82.9% had an undetectable viral load (limit of detection varied between 20-150 copies/mL depending on study site). In index partners, the prevalence of other STIs was 11.2% and in the HIV-negative partners it was 6.6%.

Participants followed in this cohort were tested for viral load and CD4 count (index partner) and HIV antibodies (HIV-negative partner) at baseline and at follow-up visits, which occurred at least twice per year.

The 152 couples in this analysis provided 149.96 couple years of follow-up (90.83 during which condomless anal intercourse [CLAI] was reported). Over this period, an estimated 5,905 instances of CLAI (based on self-report) and zero genetically linked transmissions were observed. The overall incidence rate for this study is therefore 0.0 transmissions per 100 person-years (95% CI: 0.00-2.46). Insertive CLAI was reported over 77.87 couple-years of follow-up (approximately 3,569 acts) during which zero transmissions occurred, yielding an incidence of 0.0 transmissions per 100 person-years (95% CI: 0.00-4.74). Similarly, receptive CLAI was reported over 57.08 couple-years of follow-up (approximately 2,337 acts) during which zero transmissions occurred, yielding an incidence of 0.0 transmissions per 100 person-years (95% CI: 0.00-6.46).

The major limitation of this study is its relatively short follow-up period, which may have been too short to view any transmissions, although the authors collected data on a relative large number of condomless sex acts. This study was determined to have a moderate risk of bias in our assessment using the QUIPS tool.
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The authors of the study declared that they have no conflicts of interest. Funding for the study was received from the Australian National Health and Medical Research Council for the Foundation for AIDS Research. No pharmaceutical grants were received in the development of the study.

Rodger et al, 2016 (4)

The aim of the PARTNER study was to assess the risk of HIV transmission in couples that practice condomless sex when the HIV-positive partners had a viral load less than 200 copies/mL. This prospective observational cohort study evaluated risk of transmission from September 2010 to June 2014 in the 548 heterosexual couples, and assessment is continuing for 340 MSM couples. Participants were recruited from 75 clinics in 14 European countries and followed up every 6-12 months for evidence of HIV seroconversion or viral load testing.

There were no phylogenetically linked HIV transmissions over the 1,238 eligible couple-years of follow up. The within-couple rate of HIV transmission was therefore 0.00 transmissions per 100 person-years (upper-bound of 95% CI= 0.30). Eleven HIV-negative partners seroconverted (10 MSM; 1 heterosexual) during the study period, but these transmissions were not phylogenetically linked and eight (73%) of the partners that seroconverted reported condomless sex with a partner other than the one they enrolled with. The study also investigated the risk of HIV transmission according to sexual behaviour. For general condomless vaginal and anal sex, the upper-bounds of the 95% CIs were 0.59 and 0.71 per 100 couple-years of follow-up, respectively. When looking at the different forms of condomless anal sex, the upper-bounds of the 95% CIs were 0.88 and 2.23 per 100 couple-years of follow-up for insertive anal sex with and without ejaculation and receptive anal sex with ejaculation, respectively. When only investigating the incidence of receptive anal sex with ejaculation reported by HIV-negative heterosexual women, the upper-bound of the 95% CI rose to 12.71 per 100 couple-years of follow-up due to the small amount of follow-up.

Important limitations to consider when interpreting the results of this study are the moderate dropout rates and relatively short follow-up time, which reduces the generalizability of the results. Another limitation of the study is that there was not enough information to determine if the rate of HIV transmission changes over the duration of the partnerships and over periods of condomless sex. Our assessment of this study using the QUIPS tool found an overall low risk of bias.

The study was supported by a grant from the National Institutes of Health Research. The PARTNER study-coordinating centre, the Centre of Excellence for Health, Immunity and Infections (CHIP), was also supported by the Danish National Research Foundation. Various study authors declared receiving support from the National Institutes of Health Research and other organisations. Conflict of interest disclosure forms for all authors are publically available.

Mujugira et al., 2016 (5)

Mujugira and colleagues aimed to evaluate the risk of HIV transmission among HIV serodiscordant couples after the index partner started on ART, with particular focus on the first six months of ART use. To achieve this objective, the authors carried out a prospective cohort analysis of HIV serodiscordant couples enrolled in the placebo arm of a HIV pre-exposure prophylaxis randomised clinical trial, the Partners Pre-Exposure Prophylaxis (PrEP) Study (6,7). They followed 1,573 couples recruited from nine research sites in Kenya and Uganda.
Index partners became eligible for ART at CD4 cell count <200 (Kenya) or <250 cells/μL (Uganda) initially, and later at ≤ 350 cells/μL following the revisions of national guidelines in both Kenya and Uganda. Index participants self-reported ART use and sexual behaviour at quarterly follow-up visits. Viral load was quantified in blood plasma (limit of detection of 40 copies/mL) and semen or endocervical swabs every six months. HIV-negative partners were tested for HIV infection monthly.

The 1,573 couples contributed a total of 2,979 couple-years of follow-up, of which 2,644 couple-years did not involve ART use. Among couples with an index partner who was not on ART, 55 infections occurred yielding an incidence of 2.08 transmissions per 100 person-years (95%CI: 1.57-2.71). A total of 168 couple-years were accrued while the index partner was on ART for less than six months. Over this time, three genetically linked transmissions occurred for an incidence of 1.79 transmissions per 100 person-years (95%CI: 0.36-5.22). All three of these transmissions occurred before complete viral suppression was achieved in the index partner. Conversely, zero transmissions occurred over 167 couple-years of follow up in which the index partner had been on ART for more than six months for an incidence of 0.00 transmissions per 100 couple-years (95%CI: 0.00-2.20).

The primary limitation of this study is the relatively short follow-up time while index partners were on ART. In addition, self-reported condomless sex was low (10.5% for <6 months of ART, 9.1% for >6 months of ART) and thus the incidence of HIV transmissions among couples where the index partner was on ART may be confounded by condom use. Determination of ART use and sexual behaviour were both self-reported, which may be subject to bias. Also, viral load measurements were only provided to participants at the end of the study, which may not reflect clinical practice. Assessment of this study using the QUIPS tool found an overall moderate risk of bias.

The authors reported that they have no conflicts of interest. The study was funded by research grants from the Bill and Melinda Gates Foundation, the National Institute of Mental Health of the US National Institutes of Health, and the University of Washington Centers for AIDS Research.

Birungi et al. 2015 (8)

The Highly Active ART as Prevention (HAARP) study aimed to assess the effectiveness of ART to prevent HIV transmission between co-habiting serodiscordant couples. Between June 2009 and 2011, 586 co-habiting couples were recruited for this prospective cohort study from The AIDS Support Organization in Jinja, Uganda. Of these, 544 (92.8%) couples were followed-up past their baseline assessment. A total of 249 index partners (42.5%) started on ART before enrollment, 99 index partners (17%) started on ART during the study, and the remaining index partners (238; 40.6%) did not receive ART during the study. Among those that initiated ART during the study, individuals were not considered to be on ART until they had been on it for three months. Condom use at the last sex act of partners who initiated ART during the study or before enrollment was 69% and 76%, respectively. Despite high reported condom use, herpes simplex virus type 2 (HSV-2) prevalence in these two groups was 87% and 83% respectively.

Follow-up of participants continued until December 2011 (median duration of follow-up: 1.5 years). During this period, 17 infections occurred; nine infections in the partners of people on ART for more than three months and eight in the partners of those not on ART or on ART for three months or less. The group on ART for more than three months was followed for 431 person-years and had a total risk of transmission of 2.09 per 100 person-years (95% CI: 0.95-
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Of the nine infections in this group, five were genetically linked for a risk of 1.16 linked transmissions per 100 person-years (95% CI: 0.37-2.71). A higher risk of transmission was observed in the group not on ART or on ART for three months or less: 2.31 transmissions per 100 person-years (95% CI: 0.99-4.54) overall and 1.73 linked transmissions per 100 person-years (95% CI: 0.63-3.76) over 347 person-years. The risk of linked transmissions is a result of six genetically linked cases. It was also found that three of the HIV positive individuals who were on ART whose partners seroconverted had viral load measurements >1,000 copies/mL.

An important limitation was that, due to the lower than expected HIV incidence, the study was not sufficiently powered to detect the true preventative effect of ART use by the index partner on the risk of HIV transmission. Another limitation was that viral load testing was only conducted every six months; therefore, it is possible that an episode of high viremia was missed. This bias is compounded by the fact that the study does not report adherence to ART. A final limitation is missing data; three transmissions were missing linkage data and one participant was missing viral load results. This study was determined to have an overall moderate risk of bias in our assessment using the QUIPS tool.

The authors of the study declare no conflicts of interest. Funding for the study was received from the Canadian Institutes of Health Research and the Canadian Foundation of AIDS Research.

He et al., 2013 (9)
The aim of this prospective, longitudinal cohort study was to determine whether China’s current HIV treatment program averts HIV infections among heterosexual discordant couples in rural China. Between June 2009 and March 2011, 813 “previously identified HIV-discordant couples” completed a baseline survey and one follow-up visit within 21 months of the baseline survey. An estimated 30.0% of HIV-negative partners and 36.4% of HIV-positive index partners had herpes simplex virus type 2 at baseline. Consistent condom use over the past 12 months was by 74.7% of HIV-negative people with a partner using ART at baseline and 76.4% at follow-up. For the analyses, couples with an index partner that was on ART at baseline or started on ART before the midpoint of the study were considered to be using ART.

Among couples where the index partner was on ART, there were five transmissions in 622 person-years for a risk of 0.8 transmissions per 100 person-years (95% CI: 0.26-1.88). Among couples using ART, but not consistently using condoms, there was a risk of transmission of six per 100 person-years (95% CI: 1.94-14.06; five transmissions in 83 person-years). When condoms were used consistently, this rate decreased to zero transmissions per 100 person-years (95% CI: 0-0.78; corresponding to zero transmissions in 468 person-years). Conversely, 2.4 transmissions per 100 person-years were observed when ART was not used (95% CI: 1.23-4.15; 12 transmissions in 505 person-years). The study authors state that all transmissions were phylogenetically linked based on HIV subtype and viral sequence clustering in phylogenetic tree analysis; however, a high history of injection drug use (39.2% in index partners; 0.6% in HIV-negative partners) may confound these findings.

An estimated 82.6% of the HIV-positive people using ART had a viral load <400 copies/mL at the baseline and 85.0% of this population had the same viral load at follow-up visit. Nonetheless, all of the individuals who seroconverted while their HIV-positive partner was using ART (n=5) had a viral load ≥11,000 copies/mL at the baseline or follow-up visit.
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The study authors acknowledged that only conducting one follow-up visit and the inconsistencies in the follow-up period make it difficult to observe changes in HIV transmission risk over time and in the long-term. The authors also state that "stable, heterosexual" couples in rural China may not be representative of the general HIV-discordant population. Moreover, it should be acknowledged that information on ART adherence or duration of ART use at baseline was not reported so accurate conclusions about the effects of ART on HIV transmission risk cannot be made from this study. This study was found to have an overall moderate risk of bias using the QUIPS tool.

Funding for this study was obtained from the Chinese Ministry of Health through the National Science and Technology Major Project on Prevention and Treatment of Major Infectious Diseases including AIDS and Viral Hepatitis. The authors declared that they have no conflicts of interest.

Del Romero et al., 2015 (10)
The purpose of this longitudinal cohort study was to evaluate the impact of combination ART on the risk of HIV transmission within heterosexual HIV serodiscordant couples. For this study, 469 of 716 couples recruited from an STI clinic in Madrid, Spain were followed for a median of 2.2 years (IQR: 0.8-4.3) between 1989 and December 31, 2010. Couples were provided with free ART in line with international and local guidelines at the time (i.e. options of no treatment, mono/dual therapy, or combination ART as appropriate).

In couples where the index partner was on ART, 93% of those tested for viral load (n=128) were undetectable. There were zero transmissions in 514 couple-years (362 couple-years with unprotected sexual acts or condom breakage/slippage). This corresponds to a rate of 0.0 transmissions per 100 couple-years (95% CI: 0-0.71) or 0.0 transmission per 100 couple-years with some unprotected sex acts (95% CI: 0-1.01). An estimated 7,600 risky exposures (no condom or condom slippage/breakage) were recorded, to provide a risk of transmission per 1,000 risky exposures of 0.0 (85%CI: 0.0-0.5).

In couples where the index partner was not on HAART, there were five seroconversions in 886 couple-years (464 with unprotected sexual acts or condom breakage/slippage). This corresponds to a rate of 0.6 transmissions per 100 couple-years (95% CI: 0.18-1.32) or 1.08 per 100 couple-years with unprotected sex acts (95% CI: 0.35-2.52).

A limitation of this study is the fact that sexual behaviour was self-reported since the previous follow-up. Also, there was insufficient data to determine the risk of HIV transmission in the context of STIs while virally suppressed. It is important to recognise that there were fewer incidences of condom failure in couples on highly active ART (HAART) than couples not on treatment (30.30% vs. 46.23%). The authors were unable to conduct phylogenetic linkage because the technology was not available when the five HIV transmissions occurred. They also point out that the index partners not on HAART had no indication for treatment and had relatively low viral loads, which may make them unrepresentative of the general population of people living with HIV who are not on HAART. Despite these limitations, the study was found to have an overall low risk of bias in our assessment using the QUIPS tool.

Funding for this study was obtained from the I Fellowship Programme, Gilead Spain, and the Ministry of Economy and Competitiveness. The authors declare that they have no conflicts of interest.
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Studies included in Supervie et al. (11) and Loutfy et al. (12)

The following four studies were identified in the reviews by Supervie et al. (11) and Loutfy et al. (12). These studies were not captured in the search for our update of Supervie et al. (11) and Loutfy et al. (12) because they were published before November 1, 2012.

Reynolds et al., 2011 (13)

Reynolds and colleagues aimed to evaluate the impact of ART initiation on rates of HIV-1 transmission among married couples from the Rakai Community Cohort study, an open population-based cohort of people aged 15-49 in the Rakai district of Uganda. This analysis included HIV discordant male-female couples from monogamous or polygamous partnerships identified between 2004 and 2009. Participants were offered ART when their CD4 cell count was ≤ 250 cells/µL or they had WHO stage IV disease.

In the 459.4 person-years where couples were not on ART, there were 42 HIV transmissions corresponding to a transmission rate of 9.2 transmissions per 100 person-years (95% CI: 6.59-12.36). In the 53.6 person-years where 32 couples were on ART, there were no transmissions corresponding to a transmission rate of zero transmissions per 100 person-years (95% CI: 0-6.7). This occurred in combination with a reduction in viral load as 71.4% of couples on ART for six months had a viral load <400 copies/mL.

The authors note that limited couples started using ART during the study, which weakened the study’s power. Moreover, as condom use was higher in couples on ART than those who were not, it is difficult to distinguish between the effects of condom use and the effects of ART on HIV transmission rates. This study was found to have an overall moderate risk of bias in our assessment using the QUIPS tool.

Funding for this study was obtained from the National Institute of Allergy and Infectious Diseases and the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Donnell et al., 2010 (14)

The study authors conducted a prospective cohort analysis of participants in a randomised controlled trial in order to determine the impact of ART use on the rate of HIV-1 transmission in heterosexual serodiscordant couples, with index partners seropositive for HIV-1 and herpes simplex virus type 2 (HSV-2). The 3,321 couples from the Partners in Prevention HSV/HIV Transmission Study were from seven African countries (Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, and Zambia). Couples were followed for up to 24 months between November 2004 and October 2008. HIV testing was conducted quarterly on all the HIV-1 uninfected partners, CD4 counts were collected every six months, and viral load was determined at baseline; three, six, and 12 months after the baseline visit; and at the final study visit. High rates of HSV-2 were observed in both index and HIV-1 seronegative partners in the study with all index partners and 67.9% of seronegative partners testing positive for the virus.

Overall, there were 0.37 HIV transmissions per 100 person years (95% CI: 0.09-2.04) in couples where the index case had initiated ART (10%, n= 349) corresponding to one phylogenetically linked HIV transmission in 273 person-years of follow-up. This is comparable to the rate of 2.24 transmissions per 100 person-years (95% CI: 1.84-2.71) in couples where the index case had
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not initiated ART (corresponding to 102 linked transmissions in 4,558 person-years of follow-up).

The authors acknowledged that there are several limitations associated with their study. Information on ART initiation was only obtained through self-report; therefore, the ART-exposed follow-up time may have been overestimated. Moreover, while the study aimed to have ART initiated at CD4 counts <200-250 cells/μL (in accordance with national guidelines at the time when the study was conducted), participants may have been reluctant to initiate ART resulting in follow-up time recorded among participants with CD4 counts <200 cells/μL. There was no information available on ART adherence and the follow-up time was too short to draw conclusions about long-term ART use; however, the study did find that 70% of index case participants had an undetectable viral load at a median of seven months after ART initiation. The study authors also note that all HIV-1 infected index cases were seropositive for HSV-2, but the authors state that this does not limit the generalizability of the findings as HSV-2 is common among persons with HIV-1 worldwide (they provide the seroprevalence estimate of 50-90%). This study was determined to have an overall low risk of bias in our assessment using the QUIPS tool.

Funding for the Partners in Prevention HSV/HIV Transmission study was received from the Bill and Melinda Gates Foundation and the US National Institutes of Health. Two authors, James McIntyre and Connie Celum, received research grant support from GlaxoSmithKline. McIntyre also received speaking feeds from Abbott Laboratories.

Apondi et al. 2011 (15)

Apondi and colleagues undertook a prospective cohort study from May 2003 to December 2007 to assess sexual behaviour and predictors of HIV sexual transmission in HIV serodiscordant couples when the index partner is on ART. The analysis took place in Uganda and included 62 cohabitating serodiscordant partners. Seronegative partners were tested annually for HIV antibodies and HIV-positive partners undertook viral load testing every three months. The majority of HIV-positive participants (97.5%) on ART at baseline had viral load levels <1,700 copies/mL within 36 months of ART initiation. The risk of HIV transmission in cohabiting and sexually active partners over the 36 months of follow-up was found to be 0.5 per 100 person-years on ART (95% CI: 0.01-0.3). One transmission occurred over 184 person-years of follow-up, in the first year of the study. Genetic sequencing linked the transmission to the individual’s spouse who took six months to achieve viral suppression.

This study had a number of limitations, such as not assessing STI incidence and circumcision status. These limitations may influence disinhibition effects and the risk of HIV transmission. Our assessment of this study using the QUIPS tool found an overall moderate risk of bias.

The authors of the study declare no conflicts of interest. Funding for the study was received from the US Department of Health and Human Services and Centers for Disease Control and Prevention through the Emergency Plan for AIDS Relief.

Melo et al., 2008 (16)

Melo and colleagues sought to estimate the presence of STIs and the rate of HIV transmission by gender among heterosexual HIV-1 serodiscordant couples, as well as differences in median viral loads between HIV positive partners who transmit HIV and those who do not transmit HIV. For this study, 56 couples were retrospectively enrolled and 37 couples were prospectively
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enrolled from an urban HIV/AIDS referral center and surrounding basic health units in Porto Alegre, Brazil between February 2000 and January 2006. Forty-one of the 93 included couples were treated using HAART (33 were treated because of pregnancy and eight were treated because their CD4 count was <350 cells/μL).

There were zero transmissions in the 41 couples on HAART (zero transmissions per 100 person-years [95% CI: 0-4.1]). Among couples with an index partner who achieved confirmed viral suppression, there were zero transmissions observed over 90.4 person-years (zero transmissions per 100 person-years [95% CI: 0-4.02]). There were six transmissions in the 52 couples not on HAART (5.7 transmissions in 100 person-years [95% CI: 2.6-11.8 transmissions in 100 person-years]. The median viral load of the index partners in couples not on ART where transmission occurred was 24,082 copies/mL (range: 1,479-100,539).

Of the 37 couples enrolled prospectively, 13 of the 13 couples with a male index case and eight of the 24 couples with a female index case report using condoms consistently. The study authors stated that there was no correlation found between condom use and HIV transmission; however, they also noted that condom use may not have been as consistent as reported. This study was found to have an overall moderate risk of bias in our assessment using the QUIPS tool.

There is no information on the sources of funding or potential conflicts of interest available.

References

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