

Preplanned Studies

Effectiveness of Frequent Viral Load Testing Plus Additional Interventions to Prevent HIV Transmission in Heterosexual, Serodiscordant Couples — Yunnan Province, China, 2019–2021

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Summary

What is already known about this topic?

HIV transmission among serodiscordant couples remains a persistent issue in China. However, the practice of combining counseling with antiretroviral therapies (ART) to enhance ART adherence is not widely implemented or recommended in Chinese health guidelines.

What is added by this report?

This randomized controlled trial suggests that increased follow-up, counseling, and awareness of HIV risk can enhance ART compliance, thereby maximizing treatment efficacy.

What are the implications for public health practice?

Early testing and counseling of serodiscordant couples, following the identification of a human immunodeficiency virus (HIV) positive spouse, is crucial for initiating ART and reducing the risk of seroconversion in the uninfected partner. Implementing a combination of ART and adjunct counseling in China is advisable.

Approximately two-thirds of newly reported human immunodeficiency virus (HIV) infections in China originate from heterosexual contacts, with one-tenth stemming from a spouse or regular partner (1). It is thus crucial to manage transmission within serodiscordant couples to diminish the incidence of new infections. Although the World Health Organization (WHO) underlines the importance of regular viral load monitoring during the early stages of antiretroviral therapies (ART) (2), Chinese clinical guidelines currently advise conducting such tests only after six months of ART (3). In this study, we enrolled newly diagnosed HIV-1 serodiscordant couples from

15 health centers in Yunnan Province, assigning half to an enhanced prevention strategy. This strategy included a comprehensive reproductive plan, joint spouse testing and consultation, early treatment, frequent viral load monitoring, fertility advice, and ART. The remaining participants received ART alongside the standard care per Chinese guidelines. The enhanced strategy led to a significantly higher number of participants in the intervention group initiating ART within 7 and 14 days of diagnosis compared to the control group. Additionally, the intervention group showed significantly higher rates of viral suppression. None of the spouses in the intervention group were seroconverted (0/100 person-years), while three were in the control group (0.6/100 person-years). These findings support the notion that increased viral load testing frequency and comprehensive counseling can improve treatment outcomes and reduce HIV transmission among serodiscordant couples.

This randomized controlled trial (RCT) recruited HIV-1 serodiscordant couples, all participants being over 18 years of age, from 15 centers in Yunnan Province, China. Inclusion criteria were as follows: the HIV-positive partner was diagnosed during the study period (February 15, 2019 to March 31, 2021); their spouse was confirmed HIV-negative at baseline and maintained this status for three months subsequent to their partner's diagnosis; both partners were aged between 18 and 59 years; the couples consented to participate in the study willingly and were able to provide informed consent; and the couple had engaged in sexual activity at least once in the preceding year. (Data and Sample Collection and HIV-1 Testing refer to the [Supplementary Material](https://weekly.chinacdc.cn/), available at <https://weekly.chinacdc.cn/>)

Trained health professionals monitored newly reported positive cases. Couples who met the inclusion

criteria were randomly assigned in a 1:1 ratio to either the intervention group or the standard-of-care group using sequential random grouping.

In the intervention group, health professionals assessed HIV-positive individuals at an outpatient facility, providing treatment consultations and pre-treatment physical examinations. These individuals began ART within one week and underwent viral load testing at 1, 2, 3, 9, 12, and 18 months post-diagnosis. Health professionals also encouraged the HIV-positive patients to disclose their status to their spouses within a week. Couples received counseling on condom usage, HIV care, treatment adherence, reproductive intentions, and safe pregnancy methods. Those planning to conceive were referred to maternal and child health services for expert fertility advice and strategies to prevent perinatal transmission. Viral load results were shared with both partners. The HIV-negative partners underwent testing for HIV seroconversion at 3, 12, 18, and 24 months after the initial HIV diagnosis of their spouses.

In the standard-of-care group, health professionals initiated contact with the treatment facility to commence ART for HIV-positive partners. These partners underwent viral load testing at 3 and 6 months post-diagnosis for comparative analysis with the intervention group. The HIV-negative spouses received testing for HIV seroconversion 3 months after their partners' diagnosis and again at the study's conclusion.

Cox regression was used to estimate relative risks, which were expressed as hazard ratios with 95% confidence intervals (CI). A *P* value of less than 0.05 was considered statistically significant. Kaplan-Meier estimates were employed to compare cumulative probabilities of seroconversion between participants in the intervention and control groups. All analyses were conducted using SPSS software (version 24.0, IBM, NewYork, USA).

A total of 2,213 HIV-positive individuals, who either were married or in a committed relationship and aged 18–59, were identified between February 15, 2019, and March 31, 2021, across 15 counties in Yunnan province, China (Figure 1). Of these, 1,470 couples were excluded due to not being serodiscordant or unreachable ($n=1,469$), or because one partner died within 3 months of diagnosis ($n=1$). By the close of enrollment on March 31, 2021, 743 couples remained, with 372 assigned to the intervention group and 371 to the control group (Figure 1). Within the intervention group, six couples withdrew due to

divorce. In the control group, adverse outcomes included seroconversion in three couples and withdrawals due to divorce (three couples), death of the HIV-positive spouse (three couples), and one couple's decision to cease participation. The duration of observation for those who withdrew did not significantly differ between the groups ($P=0.808$). (Eligibility flow diagram for HIV serodiscordant couples refer to [Supplementary Figure S1](#), available at <https://weekly.chinacdc.cn/>)

At enrollment, the majority of participants were male farmers with primary school education. The age of the HIV-positive spouse differed significantly between groups, although other demographic characteristics did not (Table 1). The median observation period was 15.3 (10.8–18.8) person-months for the intervention group and 15.8 (10.6–20.5) person-months per couple for the control group. Significantly more participants in the intervention group initiated ART within 7 and 14 days from diagnosis ($P=0.011$ and $P<0.001$, respectively). The median time from diagnosis to initiating ART was 5 days [inter-quartile range (IQR): 2–9] in the intervention group and 6 days (IQR: 2–18) in the control group (Table 2). Rates of achieving a viral load of <1,000 copies/L and <200 copies/L within 3 and 4 months of diagnosis were significantly higher in the intervention group (86.2% and 64.5%; 91.9%, and 75.3%, respectively) compared to the control group (56.3% and 43.1%; 82.8%, and 67.0%). Among those who underwent viral load testing, rates of obtaining viral loads of <1,000 copies/L, <200 copies/L, and <50 copies/L were significantly higher in the intervention group (97.8%, 94.4%, and 90.1%, respectively) compared to the control group (93.9%, 89.0%, and 81.8%) ($P<0.05$) (Figure 1).

Among HIV-negative spouses at baseline, the seroconversion rates were 0.6 per 100 person-years (3/371) in the control group and 0 per 100 person-years in the intervention group ($P=0.081$). (Assessment of Linkage of Seroconversions and Analysis of seroconversion case sequences refer to [Supplementary Figures S2–S3](#), available at <https://weekly.chinacdc.cn/>)

Characteristics of the seroconverted spouses are summarized. (Characteristics of the three seroconverted couples refer to the [Supplementary Table S1](#), available at <https://weekly.chinacdc.cn/>) The time intervals from the diagnosis of HIV-positive status to spouse seroconversion were 117, 121, and

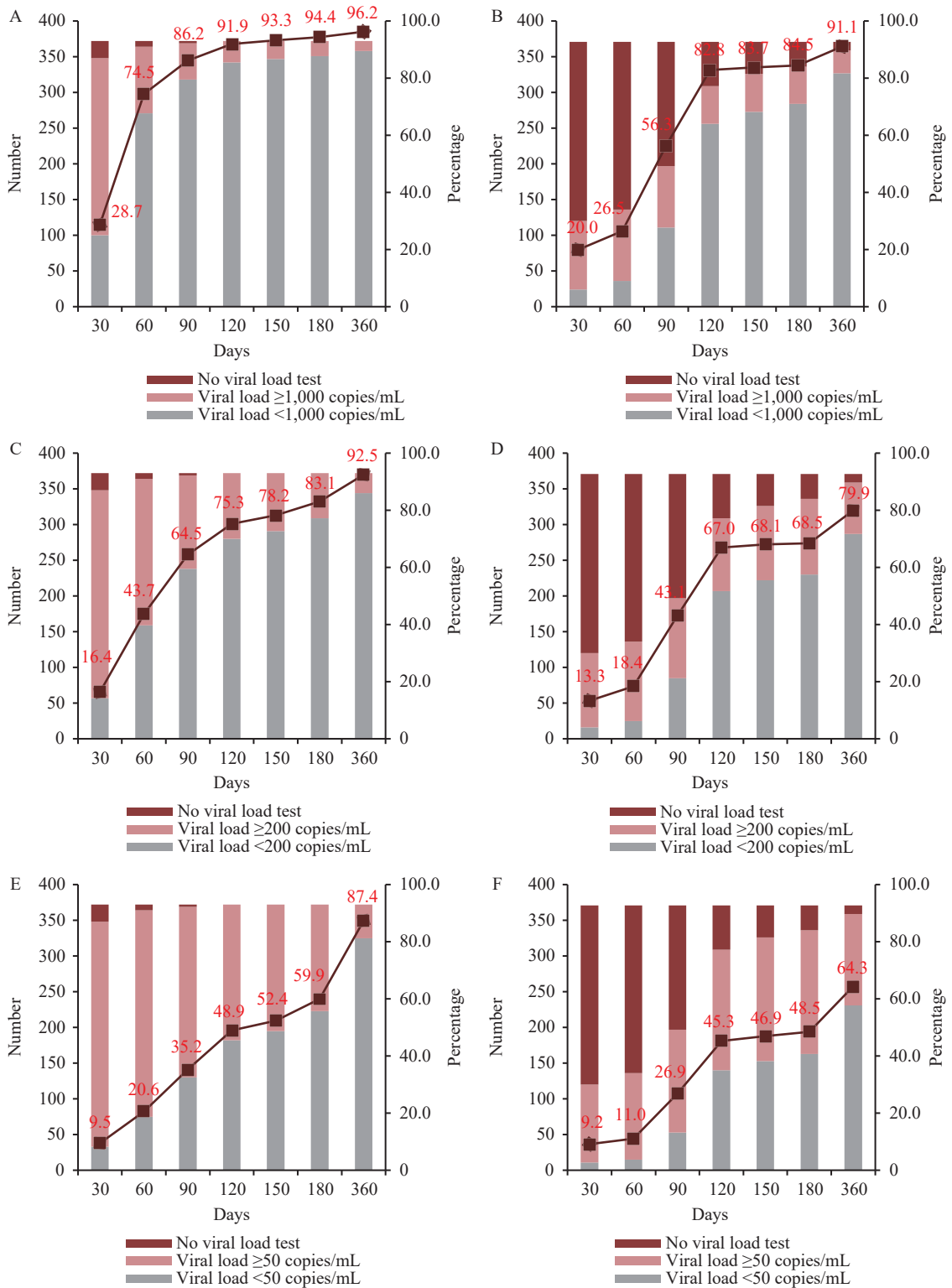


FIGURE 1. Viral suppression in intervention group and standard-of-care group. (A) VL <1000 copies/mL in intervention group; (B) VL <1000 copies/mL in standard-of-care group; (C) VL <200 copies/mL in intervention group; (D) VL <200 copies/mL in standard-of-care group; (E) VL <50 copies/mL in intervention group; (F) VL <50 copies/mL in standard-of-care group.

182 days, respectively. Phylogenetic analysis confirmed that all seroconversion cases were linked to their HIV-positive partners (refer to [Supplementary Figure S2](#)).

DISCUSSION

After 18 months, seroconversion was observed in

TABLE 1. Baseline characteristics of participants in the intervention and control groups.

Variables	Control group participants, N (%)	Intervention group participants, N (%)	P value*
Overall	371 (100.0)	372 (100.0)	
Age, years			
18–39	132 (35.6)	163 (43.8)	0.022
40–59	239 (64.4)	209 (56.2)	
Sex			
Male	250 (67.4)	266 (71.5)	0.223
Female	121 (32.6)	106 (28.5)	
Education			
Illiterate	47 (12.7)	54 (14.5)	0.598
Primary school	175 (47.2)	176 (47.3)	
Junior high school	107 (28.8)	110 (29.6)	
≥Senior high school	42 (11.3)	32 (8.6)	
Occupation			
Farmer	299 (80.6)	311 (83.6)	0.301
Labourer	31 (8.4)	34 (9.1)	
Homemaker/unemployed	26 (7.0)	15 (4.0)	
Others	15 (4.0)	12 (3.2)	
CD4 ⁺ T cell count at diagnosis			
≤200	113 (31.1)	119 (32.8)	0.703
201–499	200 (55.1)	189 (52.1)	
≥500	50 (13.8)	55 (15.2)	
Sexual behavior in the past month			
Had sexual behavior			
No	94 (25.3)	67 (18.0)	0.015
Yes	277 (74.7)	305 (82.0)	
Frequency of sexual behaviors			
≤3	162 (58.5)	166 (54.4)	0.324
≥4	115 (41.5)	139 (45.6)	
Frequency of condom use			
Every time	50 (12.5)	24 (8.7)	0.516
Sometimes	131 (32.7)	68 (24.5)	
Never	401 (68.9)	185 (66.8)	

* Categorical variables and continuous variables were compared between the intervention group and the control group using the Chi-square test and T-test, respectively.

three couples from the standard-of-care group, whereas no seroconversions occurred in the intervention group; however, this difference was not statistically significant. Nevertheless, the reduced viral load titers observed in the intervention group suggest that integrating ART with viral load monitoring and non-medical preventative strategies may enhance ART adherence and reduce the likelihood of HIV transmission between spouses.

All three spouses seroconverted within 6 months of initiating ART, aligning with previous findings that this period represents the highest risk for serodiscordant couples starting ART (4). This increased vulnerability is attributed to the initial inability of ART to sufficiently suppress viral load, coupled with the persistence of unprotected sexual activities that may facilitate HIV transmission between spouses (5–6). Consequently, enhancing provider-

TABLE 2. Follow-up characteristics of participants in the intervention and control groups.

Variables	Control group participants, N (%) / median (IQR)	Intervention group participants N (%) / median (IQR)	P value*
Overall, N (%)	371 (100.0)	372 (100.0)	
Seroconversion			
N/100PY	3/486.7 (0)	0/470.8 (0.6)	0.081
Observation time, person-month			
Median (IQR)	15.8 (10.6–20.5)	15.3 (10.8–18.8)	0.227
Days from diagnosis to spouse test			
Median (IQR)	0 (0–4)	0 (0–3)	0.376
Days from diagnosis to ART initiation			
Median (IQR)	6 (2–18)	5 (2–9)	<0.001
Frequency of viral load test			
Median (IQR)	2 (2–3)	7 (5–8)	<0.001
Initiated ART within 7 days from diagnosis, N (%)			
No	150 (40.4)	117 (31.5)	0.011
Yes	221 (59.6)	255 (68.5)	
Initiated ART within 14 days from diagnosis, N (%)			
No	106 (28.6)	54 (14.5)	<0.001
Yes	265 (71.4)	318 (85.5)	
Sexual behavior in the past month at last recorded follow-up			
Had sexual behavior			
No	129 (34.8)	119 (31.9)	0.421
Yes	242 (65.2)	253 (68.0)	
Frequency of sexual behaviors			
≤3	157 (64.9)	161 (63.6)	0.774
≥4	85 (35.1)	92 (36.4)	
Frequency of condom use during sexual activity			
Sometimes/never	19 (7.9)	10 (4.0)	0.065
Every time	223 (92.1)	243 (96.0)	
Within 3 months after diagnosis			
Viral load test, N (%)			
No	174 (46.9)	3 (0.8)	<0.001
Yes	197 (53.1)	369 (99.2)	
Viral load <1,000 copies/L, N (%)			
No	86 (43.7)	51 (13.8)	<0.001
Yes	111 (56.3)	318 (86.2)	
Viral load <200 copies/L, N (%)			
No	112 (56.9)	131 (35.5)	<0.001
Yes	85 (43.1)	238 (64.5)	
Viral load <50 copies/L, N (%)			
No	144 (73.1)	239 (64.8)	0.044
Yes	53 (26.9)	130 (35.2)	

Continued

Variables	Control group participants, N (%) / median (IQR)	Intervention group participants N (%) / median (IQR)	P value*
Within 4 months after diagnosis			
Viral load test, N (%)			
No	62 (16.7)	0 (0)	<0.001
Yes	309 (83.3)	372 (100.0)	
Viral load <1,000 copies/L, N (%)			
No	53 (17.2)	30 (8.1)	0.001
Yes	256 (82.8)	342 (91.9)	
Viral load <200 copies/L, N (%)			
No	102 (33.0)	92 (24.7)	0.017
Yes	207 (67.0)	280 (75.3)	
Viral load <50 copies/L, N (%)			
No	169 (54.7)	190 (51.1)	0.347
Yes	140 (45.3)	182 (48.9)	
Viral load at last recorded follow-up			
Viral load test, N (%)			
No	8 (2.2)	0 (0)	0.004
Yes	363 (97.8)	372 (100.0)	
Viral load <1,000 copies/L, N (%)			
No	22 (6.1)	8 (2.2)	0.007
Yes	341 (93.9)	364 (97.8)	
Viral load <200 copies/L, N (%)			
No	40 (11.0)	21 (5.6)	0.008
Yes	323 (89.0)	351 (94.4)	
Viral load <50 copies/L, N (%)			
No	66 (18.2)	37 (9.9)	0.001
Yes	297 (81.8)	335 (90.1)	

Abbreviation: IQR=inter-quartile range.

* Categorical variables and continuous variables were compared between the intervention group and the control group using the Chi-square test and T-test, respectively.

patient communication regarding the high likelihood of seroconversion during the initial six months of ART and emphasizing the necessity of adherence could serve as crucial strategies. These measures are essential not only for educating on safer sexual practices but also for achieving viral suppression as swiftly as possible (7).

Although no statistical differences were identified between groups, seroconversion rates were notably lower compared to similar populations documented in other Chinese studies from Yunnan (8–9). For the intervention groups, the low incidence of seroconversion could be attributed to stringent testing and counseling. However, the awareness associated with enrolling in an unblinded RCT might introduce

selection bias and increase motivation among both groups to engage in health-promoting behaviors (10).

Although this study offers insights into the impact of supplementary preventative strategies on HIV seroconversion, several limitations must be acknowledged. Given the non-blinded nature of this study, participants in the standard-of-care group were aware that they were not receiving additional interventions. This awareness may have engendered feelings of disadvantage, potentially prompting more cautious behaviors that could reduce seroconversion rates and introduce bias; however, this effect is presumed to be minimal. The absence of statistical significance between groups suggests that both

intensive and non-intensive preventative strategies are effective, provided that ART is recommended and adhered to. Nevertheless, the sample size and follow-up durations were suboptimal compared to other studies, implying a need for optimization to confidently and safely conclude that extensive viral load monitoring and additional preventative measures do not further reduce the risk of seroconversion. Additionally, the study's restriction to a single province in China might limit its generalizability to other regions with different cultural and demographic profiles, which could affect adherence to and acceptance of HIV and ART protocols. Despite a solid foundation in HIV/Acquired Immune Deficiency Syndrome (AIDS) prevention and control efforts in Yunnan Province, the lack of interventions in the control group might still influence the outcomes due to overarching prevention activities. Future studies that employ blinding, larger cohorts, and extended follow-up durations may yield more definitive conclusions on whether the standard-of-care guidelines in China are as effective as multiple intervention strategies.

Ethics approval and consent to participate: The protocol was reviewed approved by the Institutional Review Board of National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention (Project No: X190111538) and was registered on Chinese Clinical Trial Register (RegistrationNo: ChiCTR1900021192). All participants signed informed consent before enrolling in the study.

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SUPPLEMENTARY MATERIAL

Assessment of Linkage of Seroconversions

When the HIV-negative partner tested positive, an initial questionnaire survey was conducted to determine if the infection originated from the HIV-positive spouse. Samples from both spouses were collected and analyzed at a laboratory within the Yunnan Province Center for Disease Control and Prevention (CDC) to genetically assess whether seroconversion occurred due to the spouse. HIV-1 gag gene sequences were generated through population sequencing for the study-partner pairs, as well as for 10 additional HIV-infected local control subjects from the same county site.

RNA extraction, Reverse Transcription Polymerase Chain Reaction (RT-PCR) amplification, and nucleotide sequencing were conducted in physically distinct laboratories. Viral RNA was extracted utilizing the Viral RNA Extraction Mini Kit (QIAGEN, Hilden, Germany). The extracted RNA was then reverse-transcribed into cDNA, serving as the template for PCR amplification of the HIV-1 pol gene via nested PCR (Takara Biotechnology, China). Subsequently, the PCR products were sequenced using the ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, USA).

Phylogenetic methods were employed to analyze the sequences. MEGA software version 11.0 facilitated phylogenetic and molecular evolutionary analyses. The likelihood of linkage was evaluated using Bayes' theorem, comparing the genetic similarity of HIV-1 among partner pairs to that among local control subjects. Phylogenetic dendrograms were generated through the neighbor-joining method, employing Kimura two-parameter modeling, and node reliability was assessed by bootstrapping with 1,000 replicates.

Data and Sample Collection

At each baseline and follow-up visit, couples participating in the study were interviewed face-to-face by a trained public health professional using a standardized questionnaire. During the baseline assessment, HIV-positive spouses provided information about their demographic characteristics, routes of HIV transmission, sexual practices with their HIV-negative partners over the past month, condom usage frequency, and fertility intentions. Subsequent follow-up visits included additional queries about medication adherence and any instances of missed doses for the HIV-positive spouses. To monitor health indicators, 7 mL and 5 mL of venous blood were collected from HIV-positive individuals for viral load testing and CD4⁺ T cell count analysis, respectively. For HIV-negative spouses, 5 mL of blood was drawn for HIV testing. In cases of seroconversion, genetic and phylogenetic analyses were conducted using blood samples from both spouses.

HIV-1, CD4 and Viral Load Testing

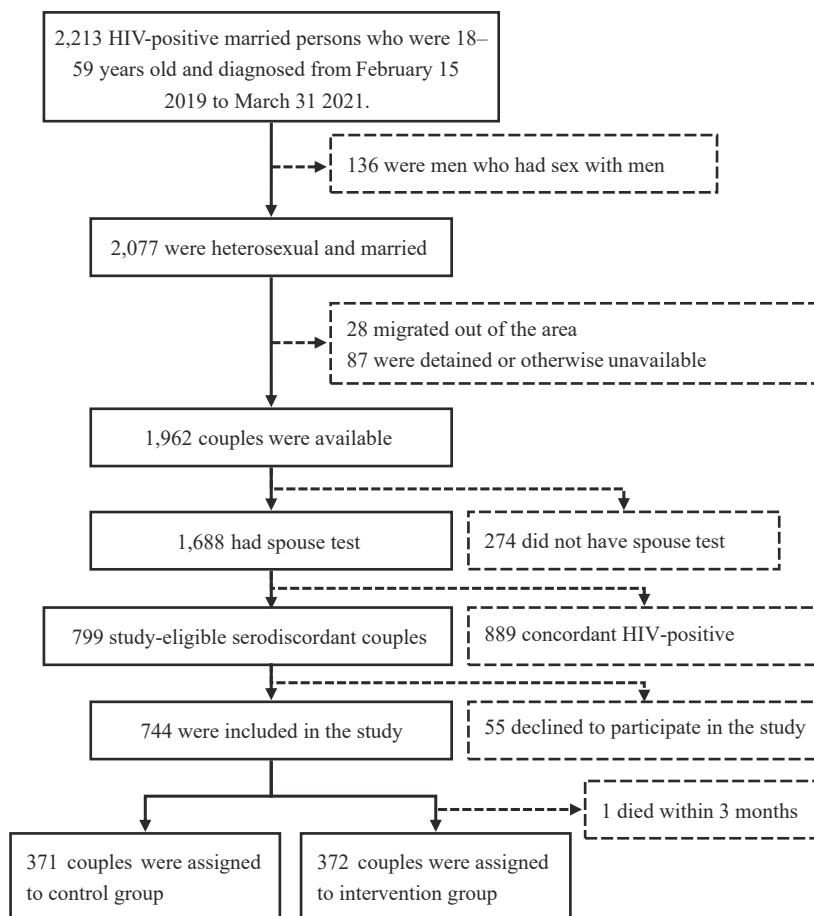
HIV testing was conducted using either a commercially available ELISA or a rapid test. Samples that tested positive were subsequently confirmed through an alternative strategy employing four rapid tests. CD4⁺ T cell counts were quantified using flow cytometers from Agilent, USA. HIV viral load was determined with the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0 (Branchburg, USA), which features a lower detection limit (LDL) of 20 copies/mL. All procedures were carried out following the manufacturer's instructions.

SUPPLEMENTARY TABLE S1. Characteristics of the three seroconverted couples

Characteristics	Subject 1	Subject 2	Subject 3
Variables of seroconverted spouse			
Age (years)	41	50	53
Gender	Male	Male	Female
Education	Illiterate	Illiterate	Primary school
Days from last HIV negative test to HIV positive test	117	112	81
Days from HIV positive spouse diagnosis to seroconversion test	117	121	172
Unprotected sexual behavior with spouse within 3 months before seroconversion testing	13	10	2
First CD4 ⁺ T cell count	364	495	343
Variables of HIV-positive spouse (index cases)			
Age (years)	41	56	43
Gender	Female	Female	Male
Education	Illiterate	Illiterate	Primary school
Viral load at enrollment	-	1,170,000	-
Viral load at 3 months of diagnosis	632	2,070	<20
Days from diagnosis to ART initiation	7	21	13
First CD4 ⁺ T cell count	337	228	169

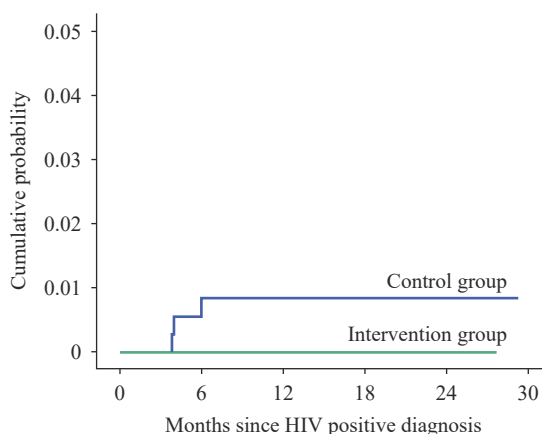
Abbreviation: HIV=human immunodeficiency virus.

"-" means did not receive viral load test at enrollment.

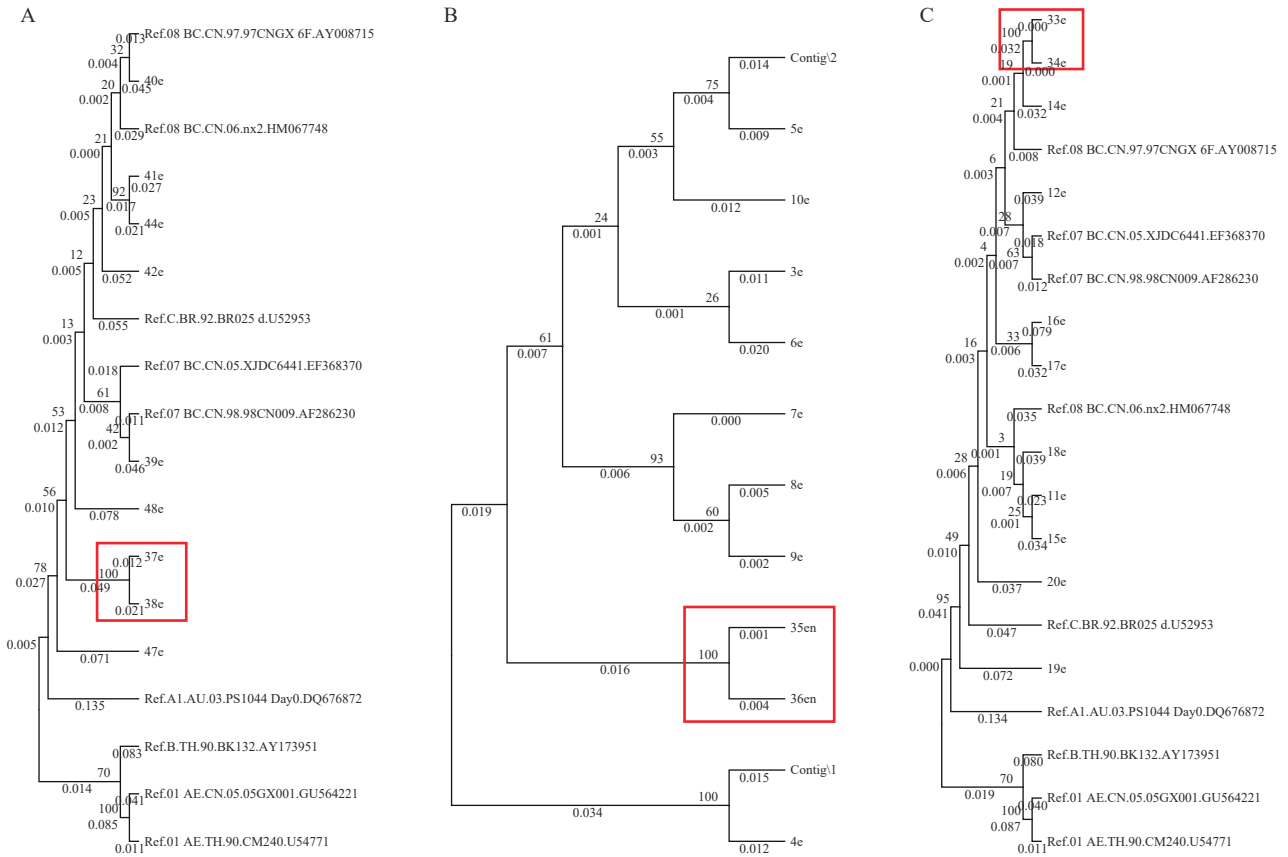


SUPPLEMENTARY FIGURE S1. Eligibility flow diagram for HIV serodiscordant couples.

Note: During the study period, a total of 2,213 participants diagnosed as HIV-positive were initially considered. However, 1,469 couples were excluded due to either not being serodiscordant or being unreachable, and one HIV-positive participant passed away from AIDS-related diseases within three months of diagnosis. Consequently, at the end of the enrollment phase, the study included 743 couples, with 372 couples assigned to the intervention group and 371 to the control group. Abbreviation: HIV=human immunodeficiency virus.



SUPPLEMENTARY FIGURE S2. Kaplan-Meier estimates for the cumulative probabilities of seroconversion among participants in the intervention and control groups. Abbreviation: HIV=human immunodeficiency virus.



SUPPLEMENTARY FIGURE S3. Analysis of seroconversion case sequences. (A) Seroconversion couple 37e and 38e HIV DNA sequence analysis; (B) Seroconversion couple 35en and 36en HIV DNA sequence analysis; (C) Seroconversion couple 33e and 34e HIV DNA sequence analysis.