

Dual Infection Contributes to Rapid Disease Progression in Men Who Have Sex With Men in China

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Background: Considerable numbers of HIV-1-infected men who have sex with men (MSM) show a relatively rapid disease progression in China; however, the cause remains elusive. HIV-1 dual infection was reported to occur commonly among the MSM population, and its contribution to clinical prognosis remains controversial. We investigated the occurrence and impact on disease progression of dual infection in a prospective MSM cohort in China.

Methods: Sixty-four HIV-1 early-infected participants were longitudinally followed up for 2 years. Deep sequencing was used as dual-infection screening. CD4⁺ T-cell counts and HIV-1 viral load were compared between coinfection and single-infection participants and pre- versus post-superinfection.

Results: Eight coinfecting participants and 10 superinfected participants were identified, including 9 participants with intersubtype and 9 with intrasubtype dual infections. The prevalence of coinfection was 13.1%, with a superinfection incidence of 15.6%. Coinfection participants showed lower CD4⁺ T-cell counts at 120 days after infection ($P = 0.042$) and a higher viral set point tendency ($P = 0.053$) as compared with single-infection participants. Kaplan–Meier analysis showed that the time for the viral load to increase to above 4 log₁₀ copies per milliliter was shorter in coinfection

participants than in single-infection participants ($P < 0.001$). After superinfection, the median CD4⁺ T-cell count decreased from 635 to 481 cells/μL ($P = 0.027$).

Conclusions: The occurrence of dual infection among Chinese MSM is relatively high, and HIV-1 dual infection might contribute to rapid disease progression seen in the MSM population.

Key Words: HIV-1, dual infection, superinfection, MSM, deep sequencing

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INTRODUCTION

HIV-1 dual infection is defined as one person infected with 2 or more different HIV-1 strains. According to the timing of infection with the second strain, dual infection can be divided into coinfection (acquisition of a second strain either simultaneously or before seroconversion) and superinfection (acquisition of a second strain after seroconversion). Dual infection can also be further categorized into intersubtype and intrasubtype. Intersubtype dual infection occurs when an individual is infected by 2 HIV-1 strains of distinct viral clades, and intrasubtype dual infection occurs when both strains are of the same viral clade. A number of studies have demonstrated that the frequency of HIV-1 dual infection varies between different high-risk populations. The incidence of dual infection may be as high as 60% in injection drug users (IDUs).^{1,2} Among the men who have sex with men (MSM) population, dual-infection incidences are much lower at 14.4%³ in the United States. A large-scale study on the rate of HIV-1 dual infection in a 149-case heterosexual cohort in the general population in Rakai, Uganda, identified 7 dual-infection cases (4.7%).⁴ These discrepancies imply different efficiencies among different transmission routes. Because of the absence of a mucosal barrier, dual infections seem more likely to occur among blood transmission than heterosexual individuals. As a special form of sexual transmission, the virus may transmit through intestinal mucosal damage during sexual contact between MSM, which is substantially similar to blood transmission; thus, dual infection may be more common in this population. On the other hand, dual infection may also be related to high-risk behavior: the more sexual partners the more opportunities for an individual to be infected with additional HIV strains.

The HIV epidemic among MSM in China has increased rapidly in recent years. According to the 2015 China AIDS

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Response Progress Report, the nationwide average prevalence of HIV-1 infection among MSM population reached 7.7% (<http://unaids.org.cn/en/index/index.asp>).⁵ In 2014, the proportion of MSM among newly identified HIV cases reached 25.8%.⁵ Meanwhile, epidemiological studies on the MSM population of China have shown that most MSM engage in high-risk behaviors, such as multiple sexual partners and unprotected anal intercourse,^{6–8} which might increase opportunities for HIV-1 dual infection. As indirect evidence, 2.7% of HIV-1 strains identified in a large cross-sectional study on MSM in 9 cities were unclassified recombinants,⁹ suggesting that the HIV epidemic is complex and the potential of dual or multiple HIV infection occurring in some cases. Until now, however, few studies have focused on HIV-1 dual infection among MSM in China.¹⁰

From a biological standpoint, dual infection plays important roles in viral evolution,^{11,12} the development of drug resistance,^{13,14} host CD4⁺ T-cell immune responses,^{15,16} and neutralizing antibody responses.^{17–20} However, the impact of dual infection on HIV disease progression remains controversial. Some studies have found dual infection to be associated with increased viral load (VL), accelerated decline in CD4⁺ T cells, and shortened time to AIDS.^{21–24} Other researchers have asserted that superinfection has a limited impact on the occurrence of clinical events.²⁵ Conversely, few dual-infection individuals have been reported as long-term nonprogressors and elite controllers.^{26,27}

A prospective cohort study among MSM in Beijing, China, showed that the rates of CD4⁺ T-cell decline and HIV-RNA increase were faster as compared with MSM from Europe.²⁸ Our unpublished data also demonstrated a rapid disease progression among a prospective primary HIV infection cohort among MSM in Liaoning province, northeastern China. The CD4⁺ T-cell counts of 35% of participants decreased below 350 cells/ μ L in the first year after HIV-1 infection, and the percentage increased more than 50% in the second year after infection. Whether HIV-1 dual infection contributes to rapid disease progression among this population remains unclear.

In the present study, 64 early-infected HIV-1 participants, identified from the regular follow-up of a prospective HIV-1 seronegative cohort, were longitudinally followed for up to 2 years. Dual infection (coinfection and superinfection) was screened via deep sequencing. In addition, CD4⁺ T-cell counts and HIV-1 VL were compared between coinfection and single-infection participants and also pre- versus post-superinfection. This study may help elucidate the occurrence of HIV-1 dual infection among Chinese MSM and the impact of HIV-1 dual infection on disease progression in HIV-infected MSM.

MATERIALS AND METHODS

Study Design and Participant Enrollment

An HIV-1 seronegative MSM cohort was recruited from participants having MSM high-risk behavior at the First Affiliated Hospital of China Medical University in Shenyang, Liaoning province, northeastern China, between December 2008 and April 2013. The participants were followed up every 2–3 months to test HIV-1 antibody with third enzyme-

linked immunosorbent assay (ELISA) and collected the epidemiology information. The HIV-1 antibody-positive samples were validated with Western blot assay (WB). The HIV-1-negative samples were pooled and tested for HIV-1 nucleic acid. The HIV early-infected cases identified with above testing strategy were suggested to be enrolled in an HIV early infection cohort. In this study, we included 64 participants among this cohort with laboratory or epidemiology evidence of HIV infection with 90 days. The estimated date of infection (EDI) was determined according to the results of laboratory diagnosis tests following the standard Fiebig staging system²⁹ with minor modifications according to epidemiological information. The details were as follows: 11 participants with HIV-1 RNA+/third ELISA-/WB- were defined as 14 days after infection (Fiebig stage II); 36 participants with third ELISA+/WB indeterminate were defined as 30 days after infection (Fiebig stage IV); and the remaining 17 participants with third ELISA+/WB+/p31- were defined as 49 days after infection (Fiebig stage V). If the exact date of the high-risk behavior was available and consistent with laboratory test results, the time of infection was determined according to this information. At study baseline, the median time from EDI was 33 days [interquartile range (IQR), 26–40 days]. These participants were followed up regularly for disease progression evaluation or treatment. Clinical and epidemiological information was collected at each follow-up examination and CD4⁺ T-cell count and plasma VL. Plasma samples were obtained and stored at -80°C to determine dual infection. At baseline, 64 plasma samples were collected for dual-infection screening; because polymerase chain reaction (PCR) failed, 3 samples did not get valid 454 pyrosequencing results. However, only 33 and 24 samples were available at 1- and 2-year time points, respectively, because of the start of antiretroviral therapy or loss to follow-up. The start of antiretroviral therapy was the endpoint of this study. This study was approved by the Medical Research Ethics Committee of the First Affiliated Hospital of China Medical University, and written informed consent was obtained from all participants.

RNA Extraction, Complementary DNA Synthesis, and PCR Amplification

One-half milliliter of plasma was centrifuged at 23,000g for 1 hour at 4°C . After removing the overlying 360- μ L supernatant, the 140- μ L precipitate was used for RNA extraction. The QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) was used to extract viral RNA according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized with Transcriptor First-Strand cDNA Synthesis Kit (Roche, Indiana, USA) using the envelope region-specific primer ED14 (HXB₂: 7932–7961) (see Table S1, <http://links.lww.com/QAI/B13>) in triplicate to maximize the quantity and diversity of viral RNA genomes per sample. The 2-step reverse transcriptase PCR reaction was performed as described in Supplemental Digital Content, Methods, <http://links.lww.com/QAI/B13>. Three 20- μ L cDNA products from each sample were pooled to obtain 60- μ L cDNA. HIV-1 envelope C2-V4 fragment was amplified by nested PCR with specific primers in triplicate

(see Supplemental Digital Content, Methods, <http://links.lww.com/QAI/B13>). Second round PCR products were pooled and separated with 1% Tris base-acetic acid-EDTA agarose gels under electrophoresis to verify predicted amplification.

454 Pyrosequencing and Data Cleaning Strategy

Amplified PCR products were pooled, and subsequently, a second PCR was performed with primers (Table S2, <http://links.lww.com/QAI/B13>) containing adaptors for 454 pyrosequencing and a multiplex identifier (MID) sequence to identify each sample (for details on MID sequences, see Table S3, <http://links.lww.com/QAI/B13>). PCR products were then purified using AMPure PCR purification beads (Beckman Coulter, California, CA) and quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, California, CA). In addition, an Agilent 2100 bioanalyzer (Agilent Life Science, California, CA) was used to verify the quality of amplicons. After quality controls, PCR amplicons were clonally amplified on capture beads in water-in-oil emulsion microreactors and sequenced in both forward and reverse directions on the Roche 454 GS-Junior platform (Roche, Switzerland) according to the manufacturer's instructions.

Sequence Analysis

Sequence reads were analyzed using GS Amplicon Variant Analyzer software Version 2.5 (Roche). This software assigned each read to the proper sample according to the MID sequence. Sequence reads were aligned with the HXB₂ envelope gene sequence, and sequence alignments were manually edited to correct insertion or deletion errors in homopolymeric regions that would result in a frameshift. All reads were compared, and identical short and long sequences were merged into a single consensus sequence. Consensus sequences with lengths (without adaptor and MID sequences) shorter than 200 bp were excluded. The remaining sequences were used for subsequent phylogenetic analysis. Subtype reference sequences were downloaded from the Los Alamos HIV sequences database (<http://www.hiv.lanl.gov>), and consensus sequences and subtype reference sequences were aligned using HIV Align (<http://www.hiv.lanl.gov/content/sequence/VIRALIGN/viralalign.html>). Phylogenetic trees were generated using the neighbor-joining method based on Kimura 2-parameter model with 1000 bootstrap replicates using MEGA 5.04 (<http://www.megasoftware.net/>). Mean and pairwise genetic distances were calculated using MEGA 5.04. Differences between sequences were visualized with the Highlighter tool (http://www.hiv.lanl.gov/content/sequence/HIGHLIGHT/HIGHLIGHT_XYLOT/highlighter.html).

Sensitivity of 454 Pyrosequencing for Detecting Minor Variants

To assess the detection threshold of minor variants with 454 pyrosequencing, 10 plasmids carrying an HIV-1 envelope insertion were used as templates for C2-V4 region amplification and then sequenced with 454 pyrosequencing in

parallel with Sanger sequencing. Inconsistent nucleotides between these 2 methods were considered 454 pyrosequencing background noises. The mean background noise of 454 pyrosequencing was 1.32% (95% confidence interval, 0.59% to 2.06%) in this study. The maximum of the 95% confidence interval was used as the detection limit of 454 pyrosequencing for detecting minor variants.

Definition of HIV-1 Dual Infection, Coinfection, and Superinfection

HIV-1 dual infection was defined when the phylogenetic tree of a case included 2 or more clusters that were separated with a bootstrap value >80%,³⁰ and the genetic distance between 2 strains was exceeded 5%^{3,31}; then, this case was preliminarily judged as dual infection. Highlighter plots were created to visually inspect nucleotide variation. Longitudinal viral sequences from each case were also assembled and used to build a single phylogenetic tree to further confirm dual infection. To eliminate the new populations caused by contamination, all the consensus sequences were blasted in our local sequences database of HIV-1 envelop sequences from clinical samples and laboratory-developed molecular clones before used for phylogenetic analysis. Based on the timing of infection with the second strain, coinfection was defined as infection with 2 different strains at baseline and superinfection was defined as infection with one viral strain at baseline and then a second strain at a later time point.

Statistical Analyses

Continuous values were summarized as median with IQR. CD4⁺ T-cell counts and VL were compared between the 2 groups using a Mann-Whitney test. Kaplan-Meier plots and log rank tests were used to compare the impact of coinfection on HIV-1 disease progression, and *P* values below 0.05 were considered statistically significant.

RESULTS

Characteristics of the Study Cohort

In this prospective early HIV infection MSM cohort, 64 participants were enrolled between December 2008 and April 2013. Thirty-three participants were followed for 1 year and 24 for 2 years, mainly because of the initiation of antiretroviral therapy and loss to follow-up. At baseline, the median age of the participants was 28 years old (IQR, 24.0–37.0 years). The median infection time was estimated as 33 days (IQR, 26.0–40.0 days) after HIV infection. Clinical status included the following: the median VL was 5.05 log₁₀ copies per milliliter (IQR, 4.46–5.90 log₁₀ copies per milliliter), and the median CD4⁺ T-cell count was 402 cells/μL (IQR, 291–581 cells/μL). Other clinical data and demographic characteristics are summarized in Table S4 (see Supplemental Digital Content, <http://links.lww.com/QAI/B13>) according to dual-infection status. At the 1-year follow-up time point, median VL was 4.32 log₁₀ copies per milliliter (IQR, 3.72–4.65 log₁₀ copies

per milliliter) and the median CD4⁺ T-cell count was 394 cells/μL (IQR, 311–537 cells/μL). At the 2-year follow-up time point, the median VL was 4.25 log₁₀ copies per milliliter (IQR, 4.03–4.70 log₁₀ copies per milliliter) and the median CD4⁺ T-cell count was 377 cells/μL (IQR, 219–519 cells/μL).

The Occurrence of HIV-1 Dual Infection in This MSM Cohort Was Relatively High

In this study, 18 participants were identified as having HIV-1 dual infection with deep sequencing, among which 8 were determined to have HIV-1 coinfection at baseline; the prevalence of coinfection was 13.1%. Five participants acquired HIV-1 superinfection in the first year after EDI and another 5 participants acquired superinfection between the first and second years after EDI, with an overall superinfection incidence of 15.6%. The incidence density of superinfection was 19.6 per 100 person-years. The remaining participants kept single HIV-1 strain infection until the end of this study. The estimated time of dual infections is shown in Table 1.

Both Intersubtype and Intrasubtype Dual Infections Could Be Detected Among This MSM Cohort

According to the phylogenetic tree, the genetic distance value (Table S5, <http://links.lww.com/QAI/B13>), and highlighter analysis, 9 intersubtype and 9 intrasubtype dual infections were identified among the 64 participants. Inter-

subtype dual infections included 1 CRF01_AE/B and 8 CRF01_AE/CRF07_BC participants. For example, participant P1 showed monophyletic CRF07_BC infection at baseline, and he was identified to be infected with a CRF01_AE virus at the 2-year time point (Fig. 1). All intrasubtype participants were infected with 2 CRF01_AE HIV-1 strains. As shown in Figure 2, participant P19 was initially infected with a CRF01_AE strain at baseline, whereas another CRF01_AE strain was detected in his 2-year time point sample.

Dual Infection Is Associated With Accelerated Disease Progression Among Liaoning MSM

To investigate whether the clinical characteristics of coinfection participants differed from those of single-infection participants, we compared CD4⁺ T-cell counts and VLs of the 8 coinfection participants with those of the 18 participants with evidence of single infection during the follow-up period. To exclude the impacts of the CD4⁺ T-cell count and VL fluctuation during the early infection phase, data from 120 days after EDI were used for comparison. We found that CD4⁺ T-cell counts were lower in coinfection participants than in single-infection participants (median, 354 versus 522 cells/μL; *P* = 0.042; Fig. 3A), whereas viral set points showed an increasing trend in coinfection participants (median, 5.07 versus 4.46 log₁₀ copies per milliliter; *P* = 0.053; Fig. 3B).

TABLE 1. Estimated Time of Dual Infection and Subtype Distribution in 18 HIV-1 Dual-Infected Participants From an MSM Cohort*

Participant ID	Estimated Time of Dual Infection (Days Since EDI)†	Estimated Window of Superinfection (Days Since EDI)	Baseline, Subtype		1 yr Follow-up, Subtype		2 yrs Follow-up, Subtype	
P36	30	‡	07_BC	AE2	§		§	
P39	22	‡	07_BC	AE1	§		§	
P47	48	‡	07_BC	AE2	§		§	
P58	40	‡	AE	AE2	§		§	
P64	36	‡	AE	AE2	§		§	
P7	20	‡	AE2	AE2	AE2		AE2	
P8	21	‡	AE2	AE2	AE2		AE2	
P11	49	‡	AE1	AE2	AE1		AE1	
P23	214	182–245	AE2		AE2	07_BC	§	
P26	197	105–288	AE2		AE2	07_BC	§	
P28	137	47–227	AE1		AE1	07_BC	§	
P2	159	96–221	AE1		AE1	07_BC	AE1	07_BC
P12	153	133–172	AE2		AE2	AE2	AE2	AE2
P1	432	392–471	07_BC		07_BC		07_BC	AE1
P3	386	337–435	AE1		AE1		AE1	AE2
P19	518	417–619	AE2		AE2		AE1	AE2
P20	397	383–411	AE2		AE2		AE1	AE2
P24	531	388–673	§		B		B	AE2

*The results of dual-infection strains are shown in bold.

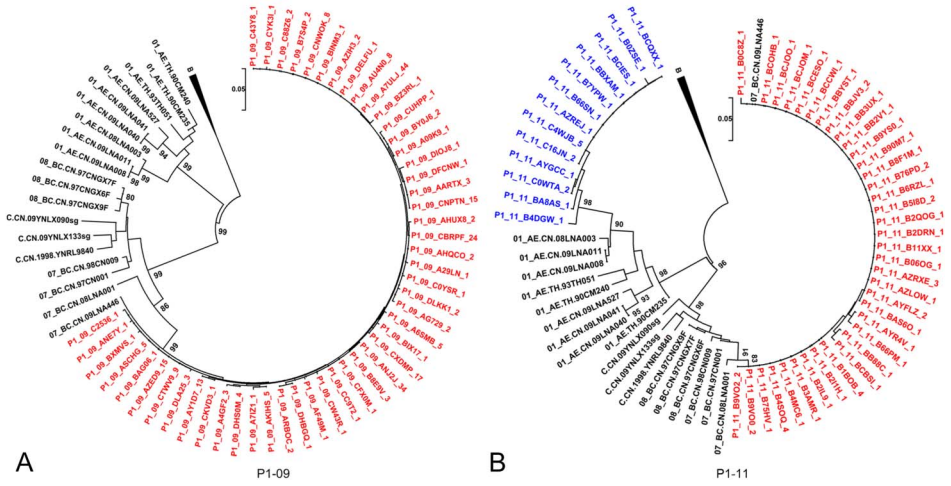
†Midpoint between estimated windows.

‡Not a superinfection case.

§Not 454 pyrosequencing.

07_BC, CRF07_BC; AE, CRF01_AE; AE1, CRF01_AE cluster 1 (33); AE2, CRF01_AE cluster 2 (33).

FIGURE 1. Intersubtype dual-infection participant P1. Neighbor-joining tree analyses of consensus sequences (>200 bp identical reads) from P1 in 2009 (A) and 2011 (B) are shown. Red branch labels represent consensus sequences from initial strains (sequence labels include sample number, year of sampling, sequence number, and the number containing the raw sequence), blue branch labels represent consensus sequences of superinfecting strains, and black branch labels represent reference sequences. Reference sequences were from the Los Alamos database. Bootstrap values >80% are indicated (1000 replicates).



To determine the impact of coinfection on disease progression, Kaplan–Meier plots and log rank tests were used to evaluate the time of disease progression between coinfection and single-infection participants. No significant difference was detected in the time for CD4⁺ T-cell count to decline below 350 cells/μL between the coinfection and single-infection groups (*P* = 0.172; Fig. 3C). By contrast, the time for VL to increase above 4 log₁₀ copies per milliliter was significantly shorter in coinfection than in single-infection participants (*P* < 0.001; Fig. 3D).

To elucidate whether CD4⁺ T-cell count and VL changed with the acquisition of superinfection, we compared CD4⁺ T-cell counts and VLs pre- versus post-superinfection in 10 superinfection participants. As a control group, CD4⁺ T-cell counts and VLs of 12 participants who kept single infection throughout the follow-up period at the corresponding time point were also analyzed. CD4⁺ T-cell counts decreased after superinfection in the 10 superinfection participants (median, 635 versus 481 cells/μL; *P* = 0.027; Fig. 4A), and VL showed an increasing trend (median, 3.96 versus 4.00 log₁₀ copies per milliliter; *P* = 0.058; Fig. 4B). CD4⁺ T-cell counts and VLs of the 12 single-infection

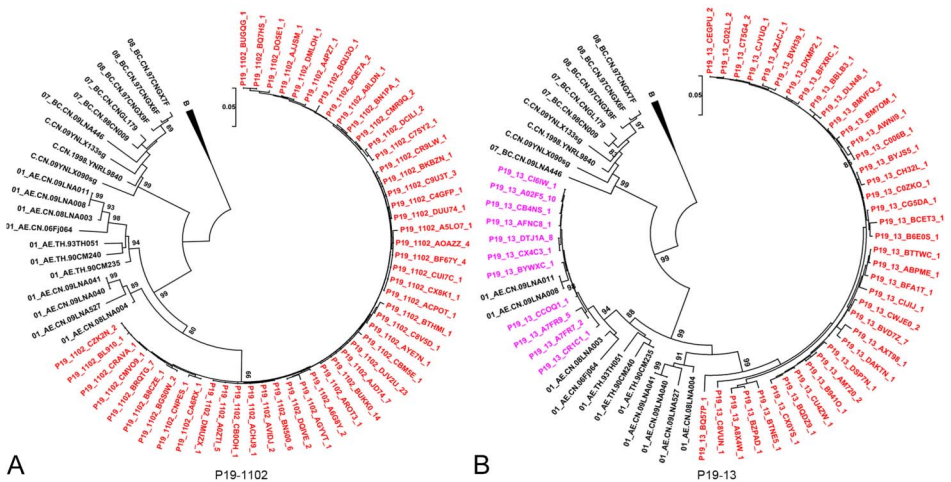
participants were not significantly changed at the corresponding time point (CD4⁺ T-cell counts: median, 377 versus 344 cells/μL; *P* = 0.064; VLs: median, 4.56 versus 4.55 log₁₀ copies per milliliter; *P* = 0.148; Figs. 4C, D).

DISCUSSION

In this study, the occurrence of HIV-1 dual infection was investigated in a prospective MSM cohort for the first time in China. Sequence analyses according to deep sequencing data showed that the occurrence of dual infection in the Chinese MSM population was higher than the occurrence of dual infection in MSM cohorts in both the United States and the Netherlands.^{3,23,32} The higher dual infection implied high-risk behaviors among the Chinese MSM population, which might also contribute to the generation of new recombinant strains and to the complex HIV-1 epidemic.

In the present study, most (77.8%; 14/18) of the dual-infection participants were determined to carry HIV-1 strains belonging to the same CRF01_AE lineage, CRF01_AE cluster 2, which was first identified in the Chinese MSM population and demonstrated to be the one of 2 major HIV-1

FIGURE 2. Intrasubtype dual-infection participant P19. Neighbor-joining tree analyses of consensus sequences (>200 bp identical reads) from P19 in 2011 (A) and 2013 (B) are shown. Red branch labels represent consensus sequences from initial strains of individual P19, blue branch labels represent consensus sequences of superinfecting strains, and black branch labels represent reference sequences. Reference sequences were from the Los Alamos database. Bootstrap values >80% are indicated (1000 replicates).



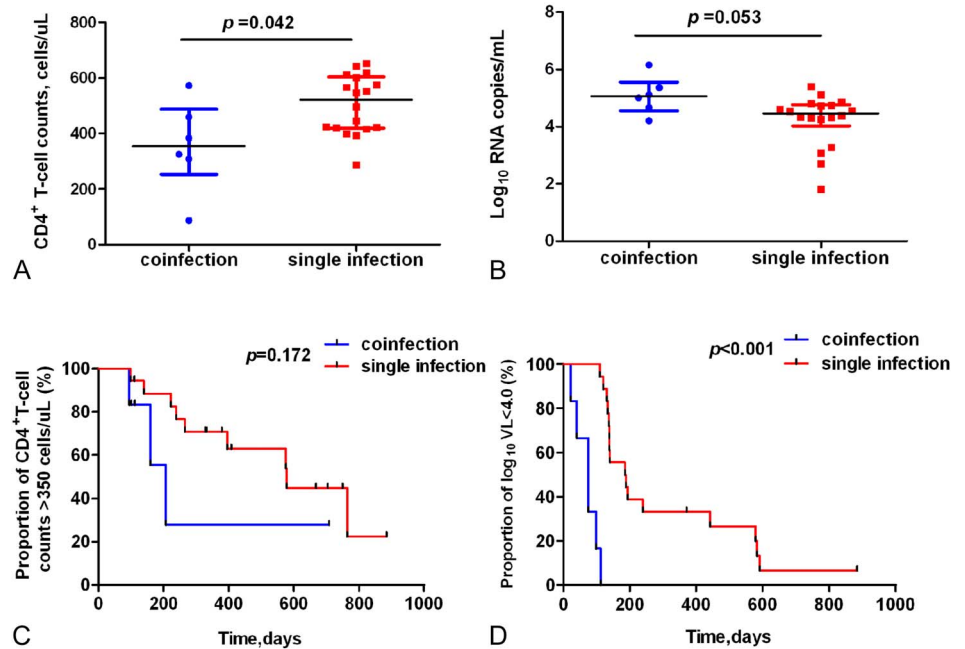


FIGURE 3. Impacts of coinfection on CD4⁺ T-cell count, VL, and disease progression. CD4⁺ T-cell count (A) and VL (B) were compared between coinfection and single-infection participants. Kaplan–Meier curves of the time for CD4⁺ T-cell counts to decline below 350 cells/μL or VL to increase above 4 log₁₀ copies per milliliter in coinfection and single-infection participants; *P* values were calculated using a log rank test (C and D).

lineages among MSM in China.^{9,33} Moreover, this CRF01_AE lineage also showed a great influence in the heterosexually transmitted population in northern China (Unpublished data). Our data suggest that CRF01_AE cluster 2 strains are not only spreading widely among different high-risk populations but also might be associated with more active high-risk behaviors. Further research is in urgent need to elucidate the epidemiological and biological characteristics of dual-infection participants, such as the site of homosexual contact and ways of partner seeking, in addition to sexually

transmitted diseases and VL levels, to provide more effective targeted intervention and aiming at HIV dual infection and new recombinant controlling.

We also found that 44.4% (8/18) of dual-infected participants were infected with CRF07_BC strains. CRF07_BC strains were estimated to originate in 1993 among IDUs in China and then transmitted to MSM around 2004.³⁴ CRF07_BC strains epidemic among MSM show high levels of genetic homogeneity and form a unique lineage distinct from the CRF07_BC strains among IDUs.⁹ In this

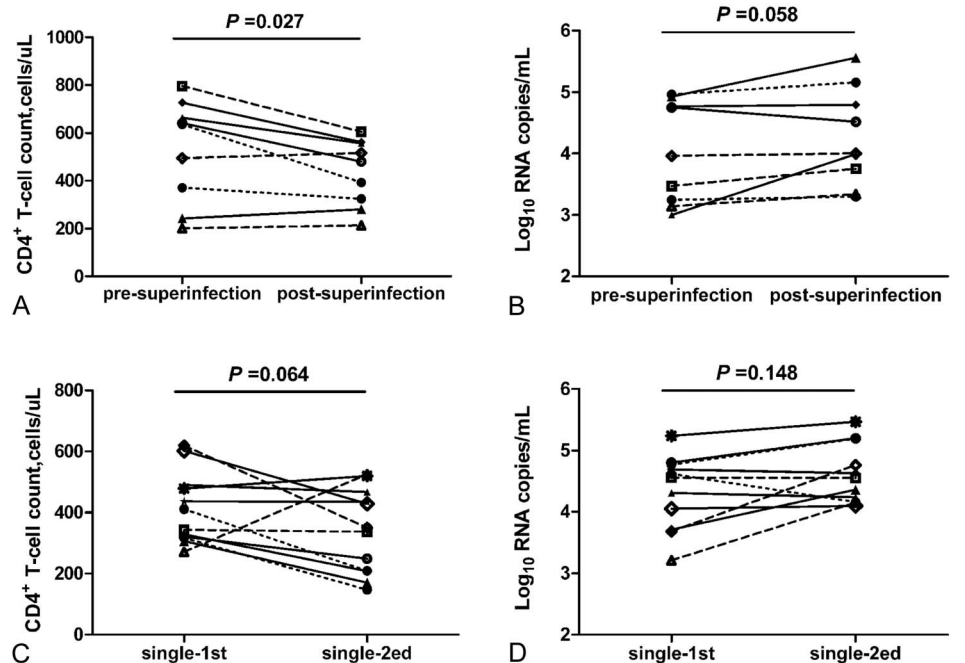


FIGURE 4. Impacts of superinfection on CD4⁺ T-cell count and VL. CD4⁺ T-cell counts (A) and VL (B) were compared pre- versus post-superinfection in 10 superinfected participants. As a control group, changes in CD4⁺ T-cell count and VL of 12 single-infection participants were determined at corresponding time points (C and D).

study, phylogenetic analysis showed a close relationship between CRF07_BC strains in dual-infection participants and the CRF07_BC lineage among MSM, rather than those among IDUs, which implies the origin of CRF07_BC strain for MSM dual infection. In addition, the CRF07_BC genotype accounted for only a small number of participants, 3.7% participants in the local primary HIV-1 infection MSM cohort.⁹ Its disproportionate prevalence of CRF07_BC strain among dual-infection participants calls for detailed behavioral and sociodemographic studies to explore factors associated with CRF07_BC strain involvement in dual infection and to help control HIV dual infection among MSM.

In this study, 3 participants (P7, P8, and P11) were infected with 2 strains at study baseline but only a single strain was detected at the 1-year and 2-year time points. Possible reasons include the following: first, coinfection may have initially occurred in these 3 participants, and one strain was rapidly eliminated or recombined with the other strain because of host immune pressure or internal environment, and only a single strain was detected during the follow-up period. Second, minor variants may have been present at very low levels that were below the detection limit of deep sequencing. This phenomenon suggests that the incidence of dual infection might be substantially underappreciated in cross-sectional studies, even with next generation sequencing techniques.

Regarding HIV-1 disease progression among Chinese MSM, our unpublished data implied that a considerable proportion of HIV-1-infected participants progress to AIDS very quickly. In this study, we observed that coinfection participants had lower CD4⁺ T-cell counts and higher VL trends as compared with single-infection participants. In addition, survival analysis also supported that the time to reach higher VL (>4 log₁₀ copies per milliliter) was significantly shorter in coinfection participants than in single-infection participants, and there was a trend toward a lower CD4 cell count in coinfection participants than those with single infection; yet, the association is not significant maybe because of the limited sample size. It is further supported that CD4⁺ T-cell counts decrease significantly after superinfection. These findings suggest that dual infection is associated with accelerated disease progression. Our findings provide evidence for a relationship between HIV-1 dual infection and disease progression.

Consistent with other studies, our study has several limitations. First, we only examined one genome region to identify dual-infection strain, and therefore, some dual infections might have been missed.³⁵ Second, dual-infection status could not be determined among participants lost to follow-up. Therefore, the occurrence of HIV-1 dual infection among this MSM population might be underestimated in the present study.

In conclusion, this study provided the first prospective evidence to show the occurrence and impacts on disease progression of HIV-1 dual infection among the MSM population in China. We found that the frequency of dual infection among Chinese MSM was relatively high, which might contribute to the rapid disease progression seen in some Chinese MSM. Moreover, the multiplicity of HIV strains

involved in dual infection calls for more effort in epidemiological, behavioral, and biological studies to better control further complications of the HIV-1 epidemic among Chinese MSM.

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