BMJ Open

Trends and risk factors of HIV, HCV, and syphilis seroconversion among drug users in methadone maintenance treatment program in China: a seven-year retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2015-008162
Article Type:	Research
Date Submitted by the Author:	11-Mar-2015
Complete List of Authors:	Zou, Xia; Sun Yat-sen University, School of Public Health; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy Ling, Li; Sun Yat-sen University, School of Public Health; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy Zhang, Lei; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Addiction, Epidemiology, Infectious diseases
Keywords:	Infection control < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

Trends and risk factors of HIV, HCV, and syphilis seroconversion among drug users in methadone maintenance treatment program in China: a seven-year retrospective cohort study

Xia Zou[1, 2], Li Ling[1, 2]*, Lei Zhang[2, 3]*

[1] Faculty of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, #74, Zhongshan Road II, Guangzhou, 510080, P.R. China

[2] Sun Yat-sen Centre for Migrant Health Policy, Sun Yat-sen University, #74, Zhongshan Road II, Guangzhou, 510080, P.R. China

[3] The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

***Corresponding authors**

Li Ling
E-mail: lingli@mail.sysu.edu.cn;
Phone: (+86)02087333319

Lei Zhang
E-mail: lzhang@kirby.unsw.edu.au;
Phone: (+612) 9385-0869

E-mail address:

Xia Zou: zouxia@mail3.sysu.edu.cn;
Li Ling: lingli@mail.sysu.edu.cn;
Lei Zhang: lzhang@kirby.unsw.edu.au;

Keywords: seroconversion, human immunodeficiency virus, hepatitis C, syphilis, methadone maintenance treatment

Words count:

Full-text: 2,484
Abstract: 255

Abstract

Objective

This study explores the trends and associated factors of HIV, HCV and syphilis seroconversion among Chinese methadone maintenance treatment (MMT) clients over a follow-up period of up to seven years.

Design: Drug users from fourteen MMT clinics in Guangdong province were recruited during 2006-2014. Participants were seronegative with at least one of HIV, HCV and syphilis infections at baseline and had completed at least one follow-up test during the study period. We estimated HIV, HCV and syphilis seroconversion rates in follow-up years and identified the underlying predictors using a multivariate Cox regression model.

Results: Among 9,240 participants, the overall HIV seroconversion rate was 0.20 (0.13-0.28)/100 person-years (pys), 20.54 (18.62-22.46)/100 pys for HCV, and 0.77 (0.62-0.93)/100 pys for syphilis over the study period. HIV seroconversion rate showed a moderate but non-significant annual decline of 13.34% (-42.48-30.56%) (Chi-2 trend test: p=0.369), whereas the decline of HCV seroconversion was 16.12% (5.53-25.52%) per annum (p<0.001). Syphilis seroconversion rate remained stable (p=0.540). Urine results positive for opioid predicted HIV seroconversion (⩾60% versus <60%: *HR*=3.40, 1.07-10.85), being unmarried (*HR*=1.59, 1.15-2.20), injection drug use in the past 30 days (*HR*=2.17, 1.42-3.32), having sexual intercourse in the past 3 months (*HR*=1.74, 1.22-2.47) and higher daily dosage of methadone (⩾60ml versus <60ml: *HR*=1.40, 1.01-1.94) predicted HCV seroconversion. Being female (*HR*=3.56, 2.25-5.64) and infected with HCV at baseline (*HR*=2.40, 1.38-8.36) were associated with subsequent syphilis seroconversion.

Conclusion: MMT in China has demonstrated moderate to good effectiveness in reducing HIV and HCV incidence but not syphilis infection among participating drug users.

Strengths and limitations of this study

1. This study tested 4,536, 1,077, and 3,910 samples for HIV, HCV, and syphilis, respectively, and identified 27, 440, 98 person-times seroconversions. But national data with a larger sample size is needed.
2. The participants in this cohort study were followed for seven years, thus we were able to explore the trends of HIV, HCV, and syphilis seroconversion rate by follow-up years.
3. This study identified significant treatment-related factors that predicted HIV (posotive rate for urine tests) and HCV (average dosage) seroconversions

INTRODUCTION

The rapid spread of blood-borne and sexually transmitted infections represents a major public health concern in many parts of the world. In particular, the transmission of HIV, HCV, and syphilis infections has contributed to high levels of disease burden and mortality worldwide. Sharing of injection equipment for illicit drug use has fuelled the transmission of HIV and HCV in many developing country settings[1-3]. Syphilis infection is commonly reported among drug users as illicit drug use also facilitates risk sexual behaviour[4]. Opioid substitution therapy (OST) has been widely accepted as an effective harm reduction strategy for drug users. Accumulating evidence has shown that OST can effectively reduce risky drug-use behaviours[5] and hence the risk of acquiring HIV and HCV among drug users.

Methadone maintenance treatment (MMT), a key component of OST, was initiated in China since 2004. As of 2013, cumulatively 761 MMT clinics have been established nationwide, linking over 2.47 million opioid drug users to the program in China[6]. The program has shown to be effective in reducing the risk behaviour among participants among Chinese drug users[7]. We established our study sites in Guangdong Province, an open and economically developed southern Chinese province with 370,000 registered drug users. HIV, HCV, and syphilis prevalence among drug users in Guangdong were reportedly as high as 6.3%, 78.7% and 12.6%. MMT in Guangdong was initiated in 2005. By the end of 2013, 60 MMT clinics have been established and cumulatively 30,410 drug users have received MMT.

Previous studies have shown lower HIV and HCV incidence rates among MMT clients than community drug users[8 9]. Participants who retained longer in MMT have also demonstrated lower risk in acquiring HIV, HCV, and syphilis infections[11, 12]. Numerous studies have reported the prevalence of HIV, HCV and syphilis in various subgroups of MMT clients and at the different treatment stages[10-13]. By linking infection status to clients' demographic, biological, behavioural and treatment-related indicators, these studies provided important insights into the contributing factors of these infections at a certain time[14-16]. However, infection prevalence is a good indication of disease burden but not the trend of disease progression. Similarly, factors associated with disease prevalence explains the potential causes of infections at one fixed time but may not reflect the potential changes in risk factors due to treatment. Built on the previous findings, this study further explores the trends of HIV, HCV, and syphilis seroconversion among MMT clients during the course of treatment and identifies associated factors of new infections based on a retrospective cohort approach.

METHODS

Study sites and participants

Our study was conducted in 14 MMT clinics located in nine cities in Guangdong Province, China. This retrospective cohort study recruited 9,412 MMT clients between July 2006 and March 2014. Participants were eligible if he/she was: (1) diagnosed opioid dependence according to the International Classification of Diseases-10, (2) age 20 years or above, (3) a local resident who settled in the catchment areas of the clinics. Written informed consent was obtained at treatment enrolment. We excluded participants who were (1) not tested for any of HIV, HCV, and syphilis, (2) seropositive for HIV, HCV or syphilis at enrolment in their respective cohorts, but clients infected with a different infection (e.g. HCV) were allowed to enrol into other cohorts (e.g. HIV and syphilis cohorts), and (3) those did not complete at least one follow-up test. Three retrospective cohorts were created at baseline with regard to the negative infection status of HIV, HCV, and syphilis, respectively (Figure 1).

Data collection

Data was routinely collected and stored in the national unified MMT Management System. At enrolment, demographic information was collected. Drug use related behaviours and sexual behaviours were surveyed at both enrolment and subsequent half-yearly follow-ups. Blood samples of clients were also collected to assess HIV, hepatitis C and syphilis status at enrolment and follow-ups. Urine-opiate tests were performed at a random day each month. Methadone was dispensed to patients daily. All information was entered into the MMT management system by the clinics staff. For this study, data of 14 studied clinics was extracted from the MMT management system, with personal identified information removed.

Laboratory tests

Blood specimens were tested for HIV-1/2, HCV, and syphilis. The presence of HIV antibodies was screened using colloidal gold method (Aibo Biotech Company, China) in MMT clinics. Positive blood specimens were sent to the provincial Center for Disease Control and Prevention for confirmatory tests. HIV infection was determined by two positive enzyme-linked immunosorbent assays (ELISA-1, Beijing BGI-GBI Biotech Company, Ltd., China; ELISA-2, the 4th generation ELISA, bioMerieux bv, Netherland) and western blot (Abbott, MPBiomedicals, LLC, Singapore). HCV testing used ELISAs (Aibo Biotech Company, China). Toluidine red unheated serum test (TRUST, Rongsheng Biotech Company, China) was performed on all serum samples. Confirmation of positive results was done with a Treponema Palladium Particle Agglutination (TPPA, Lizhu Reagent Company, China) test.

Ethical statement

The Sun Yat-sen University Ethics Committee granted ethical approval ([2013] Ref. No. 26) for the study. MMT clients who were diagnosed with HIV, HCV and syphilis were notified and referred to designated public hospitals for further treatment.

Statistical analysis

Rate of positive urine tests is defined as the proportion of the urine samples tested positive for opioid among the total urine samples submitted. Average MMT dosage is calculated by dividing the total dosage consumed by an individual by the number of days of attendance. Two-sample *t*-test, Mann-Whitney test and χ^2 test were used for normally distributed continuous data, non-parametric data and frequencies, respectively.

The event of interest in this study was HIV, HCV, and syphilis seroconversion during the course of MMT. For syphilis, an occasion of seroconversion was regarded as a case with regard to the curability of the disease. The duration of follow-up was determined as the time between the date of MMT enrolment and the date of seroconversion or the end of study. The date of seroconversion was approximated as the midpoint between the last negative and the first positive test for all infections. Seroconversion rate with 95% confidence intervals (CIs) was calculated as numbers of events over person-years and its temporal trend significance was determined with a Chi-2 trend test. Univariate and multivariate Cox proportional hazard regression analyses were conducted to identify predictors of HIV, HCV, and syphilis seroconversion. Variables with a bivariate p-value<0.20 were included in subsequent multivariate regression. The last behavioural record before seroconversion or endpoint of the study was used for the regression analysis. Multivariate Cox regression analyses were adjusted for both sociodemographic and behavioural variables. Regression models were built using stepwise techniques. P-value <0.05 was considered as statistically significant. Statistical analyses were performed using SAS v9.2 and Joinpoint v3.4.

RESULTS

Among the 9,412 enrolled MMT clients, 9240 had at least one test for any of HIV, HCV and syphilis infections at enrolment and were recruited. Most of participants included were 30 years or older (82.12%), male (92.50%) and unemployed (59.39%). About half of the participants were unmarried (53.92%). The majority of them had low education level (junior high or blow: 84.10%). The prevalence of HIV, HCV, and syphilis at baseline were 8.68 % (768/8851), 75.78% (6721/8869), and 3.11% (207/6665), respectively (Table S1). Co-infections of HIV/HCV, HIV/syphilis and HCV/syphilis were found among 5.31% (452/8512), 0.06% (4/6667) and 2.37% (156/6582) study participants, and 0.12% (8/6667) was reportedly infected with all three diseases (Figure S1). We identified 4,536, 1,077 and 3,910 individuals who were HIV, HCV and syphilis seronegative at enrolment with at least one follow-up test record. Of these, 27, 440 and 98 HIV, HCV and syphilis incidence cases with total follow-up durations of 13,360 person-years (pys), 2,142 pys, and 12,727 pys were reported during the study period (Figure 1). In contrast, 3,547, 2,148, and 2,548 HIV, HCV and syphilis-negative participants dropped-out of MMT after first visit, respectively. Compared with participants included in the analyses, they were more likely to be male, less-educated, employed, HIV-positive but HCV-negative and had a shorter history of drug use and less sexual activities in the past 3 months (Table S1).

The overall seroconversion rate of HIV, HCV, and syphilis was 0.20 (0.13-0.28)/100 pys, 20.54 (18.62-22.46)/100 pys, and 0.77 (0.62-0.93)/100 pys over the course of MMT, respectively. HIV seroconversion rate declined from 0.17/100 pys at baseline to 0/100 pys among those who remained at treatment after seven years, corresponding to a moderate but non-significant annual decline of 13.34% (-42.48-30.56%) (*Chi-2* trend test: p=0.369). In comparison, HCV seroconversion rate declined from 24.66/100 pys to 0/100 pys over the same follow-up period, corresponding to a significant annual reduction of 16.12% (5.53-25.52%, p<0.001). Seroconversion rate of syphilis infection (0.77/100 pys) remained stable over the course of treatment (p=0.540) (Figure 2a).

Positive rate for urine tests was found to be the only predictor for HIV seroconversion (⩾60% versus <60%: *HRs*=3.40, 1.07-10.85). Being unmarried (*HRs*=1.59, 1.15-2.20), injecting drug use in the past 30 days (*HRs*=2.17, 1.42-3.32), having sexual intercourse in the past 3 months (*HRs*=1.74, 1.22-2.47), and taking an average MMT dosage over 60 ml (*HRs*=1.40, 1.01-1.94) predicted higher risk of HCV seroconversion. Participants who were female (*HRs*=3.56, 2.25-5.64) and infected with HCV at baseline (*HRs*=2.40, 1.38-8.36) demonstrated a significantly higher seroconversion rate of syphilis (Table S2, Figure 2b).

DISCUSSION

Our results demonstrated moderate to sharp declines in HIV and HCV seroconversion rates among MMT clients over the 7-year follow-up period, while seroconversion rate of syphilis remained stable. During the course of treatment, seroconversion rates of all three targeted infections (HIV: 0.20/100 pys; syphilis: 0.77/100 pys; HCV: 20.54/100 pys) have been substantially lower than the corresponding rates among community drug users in China (HIV: 0.48-8.01/100 pys; syphilis: 2.41-5.84/100 pys; HCV: 21.24-39.35/100 pys[17-21]).

Despite high efficacy in reducing new HCV incidence, MMT is less effective in reducing HIV and syphilis incidence. This is consistent with previous findings[22]. A plausible explanation may be the disparities in transmission routes and the impacts of MMT on high-risk behaviour. HIV can be transmitted by both blood exchange and sexual contacts. In contrast, HCV is readily blood-borne but transmits much less efficiently via sexual intercourse[23], whereas syphilis predominately transmits sexually. As a harm reduction intervention, MMT is much more effective in reducing injecting behaviours than unsafe sexual practice[24]. Previous studies have indicated that the percentage of injecting drug use among Chinese MMT clients dropped dramatically from 81.9% to 8.1% within the first two years of treatment, whereas the frequency of sexual contacts and proportion of condom use remained largely unvaried[7]. Biologically, HCV can remain infectious *ex vivo* much longer than HIV[25], and therefore reducing injection practices expectedly results in greater observable decline in seroconversion of HCV than HIV among MMT clients.

Our findings suggest that frequent positive urine test, injecting drug use and high MMT dosage predict higher risks of HIV and HCV seroconversion. Urine test is a routine monitoring technique for opioid use among MMT clients. Higher proportion of positive urine test results infers more frequent drug use[26]. Injecting drug use often leads to sharing of injection equipment among drug users and is a well-documented risk factor for HIV and HCV transmission[10 15]. Further, clients with a higher daily methadone dosage may represent drug users with a prolonged drug use and addiction[27 28]. This may imply a subgroup of drug users with high risk behavioural patterns and treatment difficulties and hence is more prone to the infections[29]. Notably, recent evidence have suggested that HCV can also transmit through sexual contacts[30], particularly when co-infected with sexually transmitted diseases such as syphilis. Participants who are already married have relatively stable family relationship. In contrast, participants who were unmarried and have sexual intercourses in the past 30 days have more likelihood of engaging in risky sexual behaviours, thus increasing the risks for HCV infection.

Females are more susceptible to syphilis infection than males anatomically[31 32]. In China, a high proportion of female drug users are reportedly involved in commercial sex[14]. Among them, multiple sexual partnerships and unprotected sex activities are common[33]. These risky sexual behaviours increase their risks of syphilis infection. Besides, HCV

infection is known to synergize syphilis transmission among drug users[34] , which contributes a greater likelihood of syphilis acquisition.

The present study has several limitations. First, frequency for HIV, HCV, and syphilis testing was every six-month. Similar to other studies, the estimated timing of seroconversion relied on the consecutive measurements of negative and positive testing results, rather than a biological test for recent infection. Second, individuals with certain characteristics, especially those with high-risk behaviour, are more likely to drop-out from MMT, exclusion of this subgroup leads to underestimate of the actual seroconversion rates. Third, the percentage of drug use may be underreported as study participants may tend to provide socially desirable responses to surveys. Fourth, due to the scarce number of HIV seroconverted cases, only one significant independent variable has been found to predict HIV seroconversion.

The finding that MMT effectively reduces seroconversion of HIV and HCV highlights the importance in improving program coverage and client retention in MMT in China. Integration of effective HIV intervention strategies, such as needle and syringe exchange and condom distribution programs, should be promoted to reduce high-risk behaviours at the early stage of treatment. Although MMT clinics provide free condoms to clients, the proportion of condom use among MMT clients is reportedly low in China[35]. Low perception of risks for blood-born and sexually transmitted infections is major barrier for health service utilization[36]. Condom programs should be integrated as one of the auxiliary services in MMT clinics. Health education and counselling services about disease prevention and treatment targeting clients, particularly those at high risk, should also be routinely provided in MMT clinics in China.Antiretroviral treatment is provided freely to eligible HIV-positive individuals, but treatment for HCV and syphilis may incur substantial financial burdens for drug users. Although MMT clinics offer treatment referral for positive HCV and syphilis diagnoses, few clients can afford the medical cost for treatment, resulting in delay and missed opportunities for timely treatment[37]. Linking antiretroviral treatment to MMT may reduce mortality among drug users living with HIV[38]. With the new development of HCV cure[39], expansion of HCV treatment for MMT clients may be considered as a priority.

ACKNOWLEDGEMENTS

The authors acknowledge all the staff of the MMT clinics in Guangdong Province, China.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: all authors had financial support from the National Natural Science Foundation of China for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

FUNDING

This work was supported by the National Natural Science Foundation of China (grant 30972552, grant 71173245, grant 81473065), the China Medical Board (grant 12-111).

CONTRIBUTORS

LL, LZ, XZ conceived the study. LL contributed to the study coordination. XZ participated in data collection, analysis and drafted the manuscript. LZ and LL finalized the manuscript. All authors read and approved the final manuscript.

REFERENCE:

1. WHO. Injecting drug use. World Health Organization, 2014.
2. (UNODC) UNOoDaC. World Drug Report 2014. New York, 2014.
3. Nelson PK, Mathers BM, Cowie B, et al. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet 2011;**378**(9791):571-83 doi: 10.1016/s0140-6736(11)61097-0[published Online First: Epub Date]|.
4. Todd CS, Nasir A, Raza Stanekzai M, et al. Prevalence and correlates of syphilis and condom use among male injection drug users in four Afghan cities. Sexually transmitted diseases 2010;**37**(11):719-25 doi: 10.1097/OLQ.0b013e3181e2c76a[published Online First: Epub Date]|.
5. Shi J, Zhao LY, Epstein DH, et al. The effect of methadone maintenance on illicit opioid use, human immunodeficiency virus and hepatitis C virus infection, health status, employment, and criminal activity among heroin abusers during 6 months of treatment in china. Journal of addiction medicine 2007;**1**(4):186-90 doi: 10.1097/ADM.0b013e318156cc19[published Online First: Epub Date]|.
6. Security NCBotMoP. Annual report on drug control in China 2014. Beijing, 2014.
7. Zhou Y, Li X, Zhang C, et al. Rates of HIV, syphilis, and HCV infections among different demographic groups of female sex workers in Guangxi China: Evidence from 2010 national sentinel surveillance data. AIDS Care 2013 doi: 10.1080/09540121.2013.772282[published Online First: Epub Date]|.
8. Duan S, Yang YC, Han J, et al. [Study on incidence of HIV infection among heroin addicts receiving methadone maintenance treatment in Dehong prefecture, Yunnan province]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2011;**32**(12):1227-31
9. Peles E, Schreiber S, Rados V, et al. Low risk for hepatitis C seroconversion in methadone maintenance treatment. Journal of addiction medicine 2011;**5**(3):214-20 doi: 10.1097/ADM.0b013e31820e13dd[published Online First: Epub Date]|.
10. Xiaoli W, Lirong W, Xueliang W, et al. Risk Factors of Hepatitis C Virus Infection in Drug Users From Eleven Methadone Maintenance Treatment Clinics in Xi'an, China. Hepatitis monthly 2014;**14**(11):e19601 doi: 10.5812/hepatmon.19601[published Online First: Epub Date]|.
11. Tsui JI, Evans JL, Lum PJ, et al. ASsociation of opioid agonist therapy with lower incidence of hepatitis c virus infection in young adult injection drug users. JAMA Internal Medicine 2014;**174**(12):1974-81 doi: 10.1001/jamainternmed.2014.5416[published Online First: Epub Date]|.
12. Zhuang X, Liang Y, Chow EP, et al. HIV and HCV prevalence among entrants to methadone maintenance treatment clinics in China: a systematic review and meta-analysis. BMC infectious diseases 2012;**12**:130 doi: 10.1186/1471-2334-12-130[published Online First: Epub Date]|.
13. Broers B, Junet C, Bourquin M, et al. Prevalence and incidence rate of HIV, hepatitis B and C among drug users on methadone maintenance treatment in Geneva between 1988 and 1995. AIDS (London, England) 1998;**12**(15):2059-66
14. Gourevitch MN, Hartel D, Schoenbaum EE, et al. A prospective study of syphilis and HIV infection among injection drug users receiving methadone in the Bronx, NY. American journal of public health 1996;**86**(8):1112-5
15. Zhuang X, Wang Y, Chow EP, et al. Risk factors associated with HIV/HCV infection among entrants

in methadone maintenance treatment clinics in China: a systematic review and meta-analysis. Drug and alcohol dependence 2012;**126**(3):286-95 doi: 10.1016/j.drugalcdep.2012.05.028[published Online First: Epub Date]|.

16. Zou X, Chen W, Ling L. Influencing factors of HIV and syphilis seroconversion among patients with methadone maintenance treatment. Chinese Journal of Public Health 2013;**29**(8):1107-11
17. Li X. A research of the incidence of HIV/HCV/HBV/syphilis and risk behaviors among injecting drug users in Xichang County of Sichuan Province. Suzhou University, 2011.
18. Bruneau J, Roy E, Arruda N, et al. The rising prevalence of prescription opioid injection and its association with hepatitis C incidence among street-drug users. Addiction 2012;**107**(7):1318-27 doi: 10.1111/j.1360-0443.2012.03803.x[published Online First: Epub Date]|.
19. van den Berg CH, Smit C, Bakker M, et al. Major decline of hepatitis C virus incidence rate over two decades in a cohort of drug users. European journal of epidemiology 2007;**22**(3):183-93 doi: 10.1007/s10654-006-9089-7[published Online First: Epub Date]|.
20. Duan S, Han J, Tang RH, et al. [Study on the incidence and risk factors of HCV infection among heroin addicts who were on methadone maintenance treatment in Dehong prefecture,Yunnan province]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2013;**34**(6):552-6
21. Sarna A, Saraswati LR, Sebastian M, et al. High HIV incidence in a cohort of male injection drug users in Delhi, India. Drug Alcohol Depend 2014 doi: 10.1016/j.drugalcdep.2014.03.021[published Online First: Epub Date]|.
22. Ruan Y, Liang S, Zhu J, et al. Evaluation of harm reduction programs on seroincidence of HIV, hepatitis B and C, and syphilis among intravenous drug users in southwest China. Sexually transmitted diseases 2013;**40**(4):323-8 doi: 10.1097/OLQ.0b013e31827fd4d4[published Online First: Epub Date]|.
23. Garfein RS, Vlahov D, Galai N, et al. Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. American journal of public health 1996;**86**(5):655-61
24. Marsch LA. The efficacy of methadone maintenance interventions in reducing illicit opiate use, HIV risk behavior and criminality: a meta-analysis. Addiction (Abingdon, England) 1998;**93**(4):515-32
25. Crofts N, G. D, Locarnini S. *Hepatitis C: an Australian perspective*. Melbourne, 2001.
26. Pergolizzi J, Pappagallo M, Stauffer J, et al. The role of urine drug testing for patients on opioid therapy. Pain practice : the official journal of World Institute of Pain 2010;**10**(6):497-507 doi: 10.1111/j.1533-2500.2010.00375.x[published Online First: Epub Date]|.
27. Li X, Tan H, Sun Z, et al. [Change and influence factors of craving for patients after 6 month methadone maintenance treatment]. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences 2009;**34**(8):718-22
28. Baumeister M, Vogel M, Dürsteler-MacFarland K, et al. Association between methadone dose and concomitant cocaine use in methadone maintenance treatment: a register-based study. Subst Abuse Treat Prev Policy 2014;**9**(1):1-8 doi: 10.1186/1747-597X-9-46[published Online First: Epub Date]|.
29. Xia X, Luo J, Bai J, et al. Epidemiology of hepatitis C virus infection among injection drug users in China: systematic review and meta-analysis. Public health 2008;**122**(10):990-1003 doi:

10.1016/j.puhe.2008.01.014[published Online First: Epub Date]|.
30. Terrault NA, Dodge JL, Murphy EL, et al. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: The HCV partners study. Hepatology 2013;**57**(3):881-89 doi: 10.1002/hep.26164[published Online First: Epub Date]|.
31. Ruan Y, Liang S, Zhu J, et al. Gender and ethnic disparities of HIV and syphilis seroconversions in a 4-year cohort of injection drug users. The Southeast Asian journal of tropical medicine and public health 2013;**44**(5):842-53
32. Coffin LS, Newberry A, Hagan H, et al. Syphilis in drug users in low and middle income countries. The International journal on drug policy 2010;**21**(1):20-7 doi: 10.1016/j.drugpo.2009.02.008[published Online First: Epub Date]|.
33. Folch C, Casabona J, Espelt A, et al. Gender differences in HIV risk behaviours among intravenous drug users in Catalonia, Spain. Gaceta sanitaria / SESPAS 2013;**27**(4):338-43 doi: 10.1016/j.gaceta.2013.02.006[published Online First: Epub Date]|.
34. Liao M, Kang D, Tao X, et al. Syndemics of syphilis, HCV infection, and methamphetamine use along the east coast of China. BMC public health 2014;**14**:172 doi: 10.1186/1471-2458-14-172[published Online First: Epub Date]|.
35. Xue H, Duo L, Lei Y, et al. Cohort analysis of change in high risk behavior among people received methadone maintenance treatment. Chinese Journal of Drug Dependence 2013;**22**(6):460-63
36. Du J, Wang Z, Xie B, et al. Hepatitis C knowledge and alcohol consumption among patients receiving methadone maintenance treatment in Shanghai, China. The American journal of drug and alcohol abuse 2012;**38**(3):228-32 doi: 10.3109/00952990.2011.643974[published Online First: Epub Date]|.
37. Zhao Y, Shi CX, McGoogan JM, et al. Predictors of accessing antiretroviral therapy among HIV-positive drug users in China's National Methadone Maintenance Treatment Programme. Addiction 2015;**110 Suppl 1**:40-50 doi: 10.1111/add.12782[published Online First: Epub Date]|.
38. Cao X, Wu Z, Li L, et al. Mortality among methadone maintenance clients in China: a six-year cohort study. PloS one 2013;**8**(12):e82476 doi: 10.1371/journal.pone.0082476[published Online First: Epub Date]|.
39. Manns M, Pol S, Jacobson IM, et al. All-oral daclatasvir plus asunaprevir for hepatitis C virus genotype 1b: a multinational, phase 3, multicohort study. Lancet 2014 doi: 10.1016/s0140-6736(14)61059-x[published Online First: Epub Date]|.

Figures

- **Figure 1. Eligibility and screening procedures for study participants**
- **Figure 2a. HIV, HCV, and syphilis serovonversion rate among drug users over the course of methadone maintenance treatment.**
- **Figure 2b. Risk factors associated with HIV, HCV, and syphilis seroconversions during the course of MMT treatment, based on multivariate Cox regression.**

Additional files

- **Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients**
- **Figure S1 Infection status of HIV, HCV syphilis and co-infections among drug users at first MMT enrolment.**
- **Table S2a Univariate and multivariate Cox regression for HIV seroconversion**
- **Table S2b Univariate and multivariate Cox regression for syphilis seroconversion**
- **Table S2c Univariate and multivariate Cox regression for syphilis seroconversion**

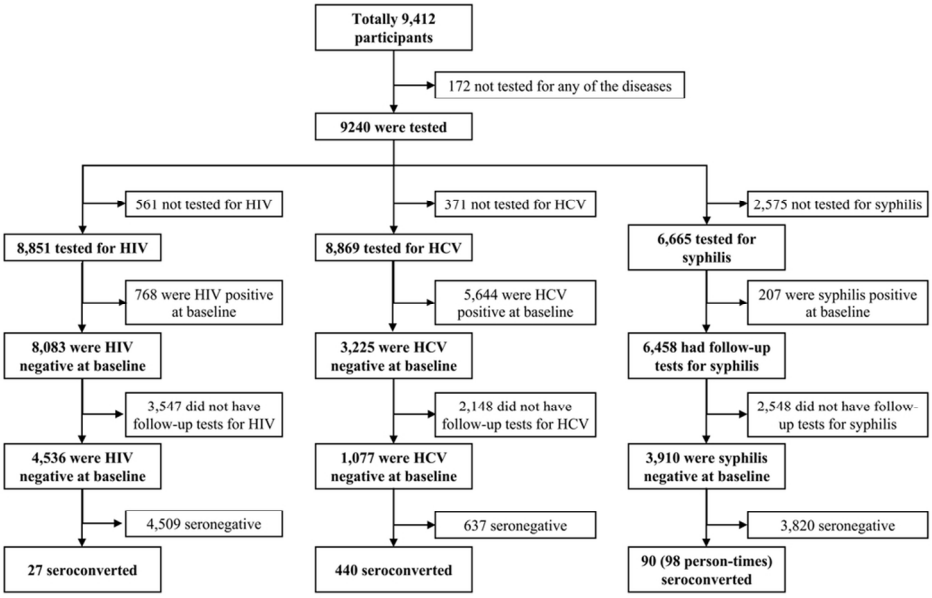

Figure 1 Eligibility and screening procedures for study participants
47x29mm (600 x 600 DPI)

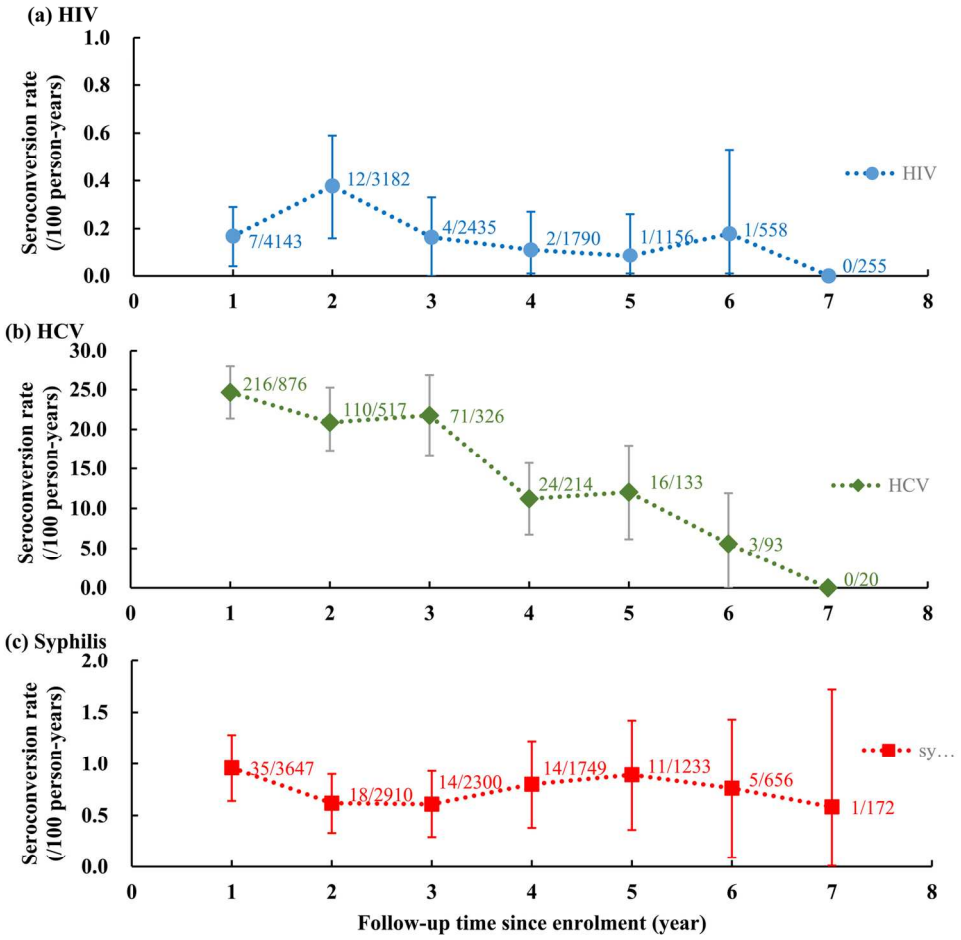

Figure 2a. HIV, HCV, and syphilis serovonversion rate among drug users over the course of methadone maintenance treatment
75x75mm (600 x 600 DPI)

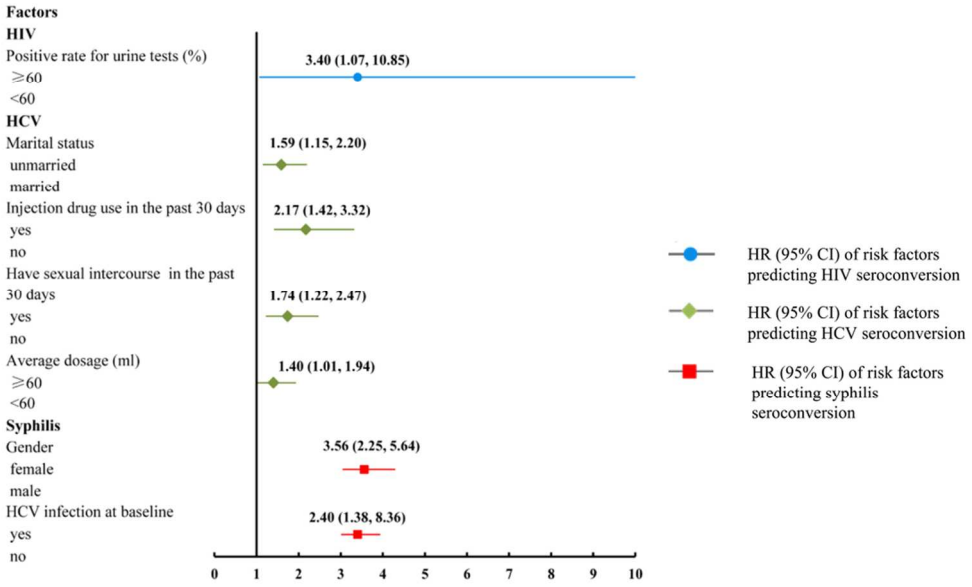

Figure 2b. Risk factors associated with HIV, HCV, and syphilis seroconversions during the course of MMT treatment, based on multivariate Cox regression.
47x29mm (600 x 600 DPI)

Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients

Factors	follow-up N=5186 (%)	drop-out N=4054 (%)	Total N=9240 (%)	t/χ^2	P
Age					
≥30	4279 (82.5)	3309 (81.6)	7588 (82.1)	1.22	0.269
<30	907 (17.5)	745 (18.4)	1652 (17.9)		
Missing#	0 (0.0)	0 (0.0)	0 (0.0)		
Gender					
female	459 (8.9)	234 (5.8)	693 (7.5)	31.09	<0.001
male	4727 (91.1)	3820 (94.2)	8547 (92.5)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Employment					
unemployed	3201 (61.7)	2287 (56.4)	5488 (59.4)	26.61	<0.001
employed	1985 (38.3)	1767 (43.6)	3752 (40.6)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Marriage					
unmarried	2794 (53.9)	2188 (54.0)	4982 (53.9)	0.01	0.927
married	2392 (46.1)	1866 (46.0)	4258 (46.1)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Education					
junior high or below	4311 (83.1)	3460 (85.3)	7771 (84.1)	8.39	0.004
senior high or above	875 (16.9)	594 (14.7)	1469 (15.9)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Years of drug use					
<5	609 (11.7)	555 (13.7)	1164 (12.6)	9.55	0.008
5-	2964 (57.2)	2315 (57.1)	5279 (57.1)		
15-	1613 (31.1)	1184 (29.2)	2797 (30.3)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
HIV infections at baseline					
yes	402 (7.8)	366 (9.9)	768 (8.7)	12.70	<0.001
no	4767 (92.2)	3316 (90.1)	8083 (91.3)		
missing	22 (4.2)	372 (9.2)	371 (4.0)		
HCV infections at baseline					
yes	4000 (77.4)	2721 (73.5)	6721 (75.8)	18.65	<0.001
no	1165 (22.6)	983 (26.5)	2148 (24.2)		
missing	21 (0.4)	350 (8.6)	371 (4.0)		
Syphilis infections at baseline					
yes	154 (3.2)	53 (2.9)	207 (3.1)	0.50	0.479
no	4660 (96.8)	1798 (97.1)	6458 (96.9)		
missing	372 (7.2)	2203 (54.3)	2575 (27.3)		
Inject drug use in the past 30 days					
yes	3453 (80.3)	2380 (79.0)	5833 (79.8)	2.00	0.158
no	845 (19.7)	633 (21.0)	1478 (20.2)		
missing	888 (17.1)	1041 (25.7)	1929 (20.9)		
Needle sharing in the past 30 days					
yes	286 (8.3)	224 (9.4)	510 (8.8)	2.29	0.130
no	3162 (91.7)	2150 (90.6)	5312 (91.2)		
missing	1738 (33.5)	1680 (41.4)	3418 (37.0)		
Had sexual intercourse in the past 3 months					
yes	2984 (69.7)	1931 (64.7)	4915 (67.7)	20.02	<0.001
no	1297 (30.3)	1053 (35.3)	2350 (32.3)		
missing	905 (17.5)	1070 (26.4)	1975 (21.0)		
Had multiple sexual partners in the past 3 months					
yes	336 (11.3)	255 (13.2)	591 (12.1)	4.25	0.039
no	2642 (88.7)	1670 (86.8)	4312 (87.9)		
missing	2208 (42.6)	2129 (52.5)	4337 (46.9)		

Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients (Continue)

Condom use in the last sexual episode					
yes	1315 (43.2)	857 (41.8)	2172 (42.7)	0.97	0.326
no	1728 (56.8)	1192 (58.2)	2920 (57.3)		
missing	2143 (41.3)	2005 (49.5)	4148 (44.9)		

*all eligible participants used drugs in the past 30 days before enrolled in MMT

#the proportion of each category was calculated using the number of the category dividing the number of participants who had records; the proportion of the missing data was calculated using the number of missing data dividing the number of participants who had records.

Figure S1 Infection status of HIV, HCV syphilis and co-infections among drug users at first MMT enrolment.

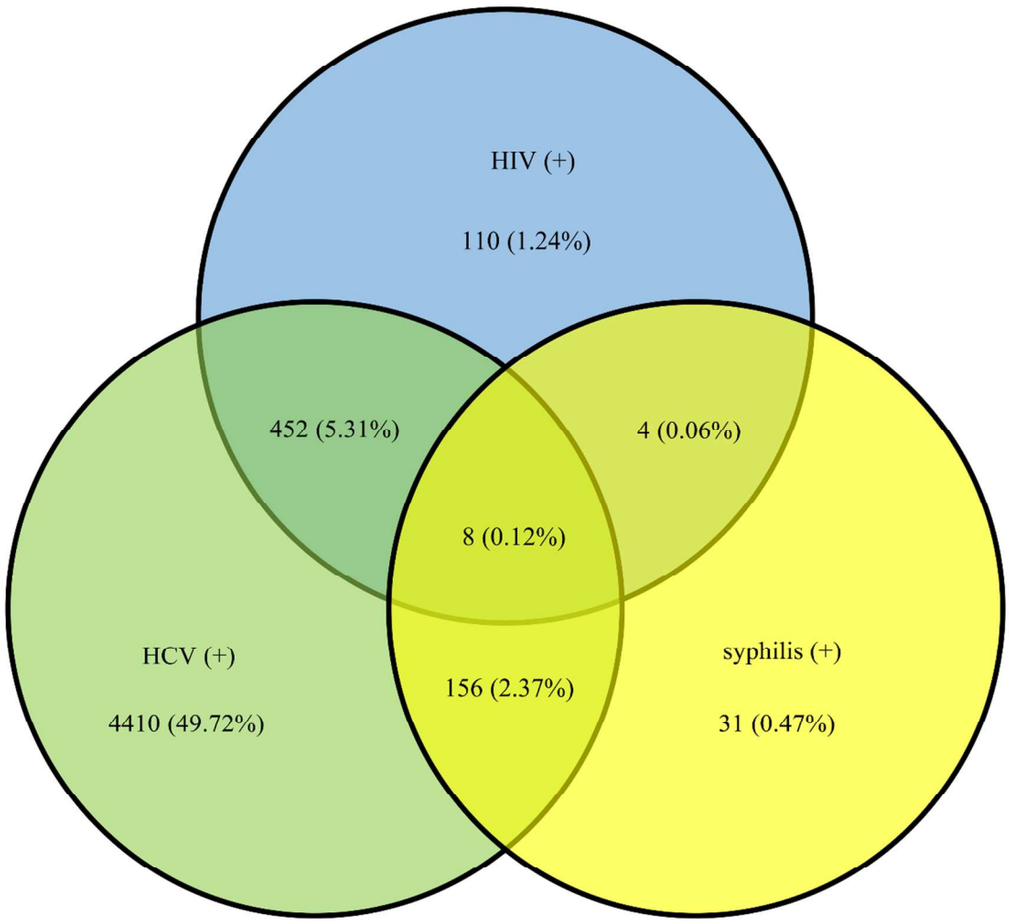

Table S2a Univariate and multivariate Cox regression for HIV seroconversion

Factor	Univariate				Multivariate	
	seroconversion rate (95% CI)	HR (95% CI)	χ^2	*P*	aHR (95% CI)	*P*
Age (year)						
≥30	0.27 (0.07-0.46)	1.47 (0.62-3.47)	0.76	0.384	--	--
<30	0.19 (0.10-0.27)	1.00 (Reference)	--	--	--	--
Gender						
female	0.33 (0.01-0.64)	1.69 (0.59-4.90)	0.94	0.331	--	--
male	0.19 (0.11-0.27)	1.00 (Reference)	--	--	--	--
Employment						
unemployed	0.21 (0.11-0.31)	1.07 (0.49-2.35)	0.03	0.857	--	--
employed	0.19 (0.07-0.31)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	0.18 (0.08-0.28)	0.82 (0.39-1.74)	0.27	0.605	--	--
married	0.22 (0.11-0.34)	1.00 (Reference)	--	--	--	--
Education						
junior high and below	0.21 (0.12-0.30)	1.28 (0.44-3.71)	0.21	0.644	--	--
senior high and above	0.16 (0.00-0.32)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	0.22 (0.07-0.37)	3.16 (0.40-25.31)	1.18	0.278	--	--
5-	0.22 (0.12-0.32)	3.23 (0.43-24.18)	1.30	0.254	--	--
15-	0.07 (0.00-0.20)	1.00 (Reference)	--	--	--	--
HIV infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
HCV infection at baseline						
yes	0.24 (0.15-0.33)	--	--	--	--	--
no	0.00 (0.00-0.00)	--	--	--	--	--
Syphilis infection at baseline						
yes	0.50 (0.00-1.20)	2.55 (0.60-10.78)	1.63	0.202	--	--
no	0.20 (0.12-0.28)	1.00 (Reference)	--	--	--	--
Drug use in the past 30 days						
yes	0.41 (0.22-0.60)	3.77 (1.70-8.40)	10.58	0.001	--	--
no	0.11 (0.04-0.18)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	0.42 (0.20-0.63)	1.08 (0.35-3.27)	0.02	0.899	--	--
no	0.38 (0.01-0.76)	1.00 (Reference)	--	--	--	--
Needle sharing in the past 30						
yes	0.78 (0.10-1.47)	2.23 (0.75-6.66)	2.07	0.150	--	--
no	0.33 (0.11-0.55)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	0.23 (0.13-0.34)	1.35 (0.61-3.00)	0.54	0.464	--	--
no	0.17 (0.06-0.29)	1.00 (Reference)	--	--	--	--
Multiple sexual partners in the						
yes	0.32 (0.00-0.77)	1.39 (0.32-6.04)	0.19	0.661	--	--
no	0.23 (0.12-0.34)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	0.17 (0.04-0.30)	0.56 (0.22-1.45)	1.44	0.231	--	--
no	0.31 (0.13-0.49)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	0.53 (0.23-0.82)	2.33 (1.08-5.03)	4.63	0.031	3.40 (1.07, 0.85)	0.039
<60	0.28 (0.06-0.51)	1.00 (Reference)	--	--	1.00 (Reference)	
Average dosage (ml)						
≥60	0.56 (0.15-0.98)	1.82 (0.85-3.87)	2.41	0.121	--	--
<60	0.35 (0.14-0.55)	1.00 (Reference)	--	--	--	--

Table S2b Univariate and multivariate Cox regression for HCV seroconversion

Factor	Univariate				Multivariate	
	Seroconversion rate (95% CI)	HR(95% CI)	χ^2	*P*	aHR (95% CI)	*P*
Age (year)						
≥30	20.97 (18.75-23.19)	1.06 (0.85-1.33)	0.27	0.603	--	--
<30	19.16 (15.34-22.97)	1.00 (Reference)	--	--	--	--
Gender						
female	31.96 (23.66-40.26)	1.60 (1.21-2.11)	10.95	0.001	--	--
male	19.50 (17.55-21.46)	1.00 (Reference)	--	--	--	--
Employment						
unemployed	21.88 (19.25-24.52)	1.17 (0.97-1.42)	2.66	0.103	--	--
employed	18.79 (16.01-21.58)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	24.48 (21.26-27.69)	1.36 (1.13-1.64)	10.41	0.001	1.59 (1.15, 2.20)	0.005
married	17.63 (15.28-19.97)	1.00 (Reference)	--	--	1.00 (Reference)	--
Education						
junior high and below	22.07 (19.75-24.39)	1.31 (1.05-1.65)	5.46	0.02	--	--
senior high and above	16.32 (13.00-19.63)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	15.92 (12.26-19.57)	0.71 (0.53-0.96)	4.84	0.028	--	--
5-	21.37 (18.82-23.92)	0.96 (0.76-1.21)	0.14	0.712	--	--
15-	23.09 (18.50-27.70)	1.00 (Reference)	--	--	--	--
HIV infection at baseline						
yes	29.89 (21.06-38.72)	1.44 (1.05-1.96)	5.18	0.023	--	--
no	19.88 (17.92-21.84)	1.00 (Reference)	--	--	--	--
HCV infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
Syphilis infection at baseline						
yes	20.21 (18.26-22.16)	1.55 (0.97-2.48)	3.3	0.069	--	--
no	32.21 (17.33-47.10)	1.00 (Reference)	--	--	--	--
Drug use in the past 30 days						
yes	24.61 (20.78-28.43)	1.36 (1.11-1.66)	8.61	0.003	--	--
no	17.30 (15.06-19.54)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	30.82 (25.57-36.08)	2.42 (1.60-3.66)	17.43	<0.00	2.17 (1.42, 3.32)	<0.001
no	12.58 (7.83-17.32)	1.00 (Reference)	--	--	1.00 (Reference)	--
Needle sharing in the past 30						
yes	34.12 (18.36-49.88)	1.07 (0.65-1.76)	0.07	0.787	--	--
no	30.36 (24.79-35.94)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	20.99 (18.42-23.55)	1.19 (0.96-1.47)	2.59	0.108	1.74 (1.22, 2.47)	0.002
no	17.73 (14.70-20.75)	1.00 (Reference)	--	--	1.00 (Reference)	--
Multiple sexual partners in the						
yes	18.97 (8.66-29.29)	0.86 (0.49-1.48)	0.3	0.582	--	--
no	21.01 (18.37-23.66)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	19.92 (16.57-23.26)	0.92 (0.72-1.17)	0.47	0.495	--	--
no	22.34 (18.36-26.32)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	20.75 (17.80-23.70)	0.96 (0.78-1.18)	0.16	0.687	--	--
<60	21.71 (16.51-26.91)	1.00 (Reference)	--	--	--	--
Average dosage (ml)						
≥60	23.82 (19.03-28.61)	1.39 (1.15-1.68)	11.21	0.001	1.40 (1.01, 1.94)	0.044
<60	19.62 (16.60-22.64)	1.00 (Reference)	--	--	1.00 (Reference)	--

Table S2c Univariate and multivariate Cox regression for syphilis seroconversion

Factor	Univariate				Multivariate	
	Seroconversion rate (95% CI)	HR (95% CI)	χ^2	*P*	aHR (95% CI)	*P*
Age (year)						
≥30	0.83 (0.65-1.00)	1.45 (0.83-2.56)	1.67	0.196	--	--
<30	0.56 (0.27-0.85)	1.00 (Reference)	--	--	--	--
Gender						
female	2.24 (1.34-3.13)	3.51 (2.22-5.57)	28.58	<0.00	3.56 (2.25, 5.64)	<0.001
male	0.64 (0.49-0.78)	1.00 (Reference)	--	--	1.00 (Reference)	--
Employment						
unemployed	0.88 (0.67-1.09)	1.48 (0.96-2.28)	3.10	0.078	--	--
employed	0.60 (0.38-0.82)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	0.80 (0.59-1.01)	1.08 (0.73-1.61)	0.15	0.697	--	--
married	0.74 (0.52-0.96)	1.00 (Reference)	--	--	--	--
Education						
junior high and below	0.77 (0.60-0.93)	0.95 (0.58-1.57)	0.04	0.835	--	--
senior high and above	0.80 (0.44-1.17)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	0.90 (0.60-1.21)	1.07 (0.54-2.12)	0.04	0.847	--	--
5-	0.70 (0.51-0.89)	0.84 (0.44-1.61)	0.28	0.599	--	--
15-	0.84 (0.34-1.39)	1.00 (Reference)	--	--		
HIV infection at baseline						
yes	0.55 (0.14-0.96)	0.70 (0.32-1.51)	0.84	0.361	--	--
no	0.80 (0.63-0.96)	1.00 (Reference)	--	--	--	--
HCV infection at baseline						
yes	0.87 (0.69-1.04)	3.32 (1.35-8.16)	6.82	0.009	2.40 (1.38, 8.36)	0.008
no	0.26 (0.03-0.44)	1.00 (Reference)	--	--	1.00 (Reference)	--
Syphilis infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
Drug use in the past 30 days						
yes	0.78 (0.50-1.05)	1.04 (0.67-1.59)	0.02	0.876	--	--
no	0.75 (0.56-0.94)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	0.86 (0.26-1.45)	0.88 (0.39-1.97)	0.10	0.754	--	--
no	0.75 (0.44-1.06)	1.00 (Reference)	--	--	--	--
Needle sharing in the past 30						
yes	1.15 (0.23-2.07)	1.64 (0.65-4.15)	1.08	0.300	--	--
no	0.69 (0.35-0.99)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	0.82 (0.61-1.03)	1.24 (0.81-1.89)	0.99	0.319	--	--
no	0.67 (0.44-0.89)	1.00 (Reference)	--	--	--	--
Multiple sexual partners in the						
yes	1.02 (0.13-1.91)	1.24 (0.49-3.09)	0.2	0.652	--	--
no	0.81 (0.60-1.03)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	0.96 (0.65-1.26)	1.41 (0.83-2.39)	1.63	0.201	--	--
no	0.67 (0.39-0.96)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	0.84 (0.49-1.19)	1.25 (0.79-1.95)	0.91	0.339	--	--
<60	0.74 (0.57-0.91)	1.00 (Reference)	--	--	--	--
Average dosage (ml)						
≥60	0.75 (0.50-1.00)	0.96 (0.64-1.45)	0.04	0.849	--	--
<60	0.76 (0.56-0.96)	1.00 (Reference)	--	--	--	--

Footnote: seroconversion rate was estimated using number of cases divided by years of follow-up (/100 person-years); Hazard ratio (HR) was estimated by using Cox regression method. aHR: adjusted hazard ratio

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	Figure S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table S1
		(b) Indicate number of participants with missing data for each variable of interest	Table S1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Trends and risk factors of HIV, HCV, and syphilis seroconversion among drug users in methadone maintenance treatment program in China: a seven-year retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2015-008162.R1
Article Type:	Research
Date Submitted by the Author:	16-Jun-2015
Complete List of Authors:	Zou, Xia; Sun Yat-sen University, School of Public Health; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy Ling, Li; Sun Yat-sen University, School of Public Health; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy Zhang, Lei; Sun Yat-sen University, Sun Yat-sen Center for Migrant Health Policy
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Addiction, Epidemiology, Infectious diseases
Keywords:	Infection control < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

Trends and risk factors of HIV, HCV, and syphilis seroconversion among drug users in methadone maintenance treatment program in China: a seven-year retrospective cohort study

Xia Zou[1, 2], Li Ling[1, 2]*, Lei Zhang[2, 3]*

[1] Faculty of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, #74, Zhongshan Road II, Guangzhou, 510080, P.R. China

[2] Sun Yat-sen Centre for Migrant Health Policy, Sun Yat-sen University, #74, Zhongshan Road II, Guangzhou, 510080, P.R. China

[3] The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

***Corresponding authors**

Li Ling
E-mail: lingli@mail.sysu.edu.cn;
Phone: (+86)02087333319

Lei Zhang
E-mail: lzhang@kirby.unsw.edu.au;
Phone: (+612) 9385-0869

E-mail address:

Xia Zou: zouxia@mail3.sysu.edu.cn;
Li Ling: lingli@mail.sysu.edu.cn;
Lei Zhang: lzhang@kirby.unsw.edu.au;

Keywords: seroconversion, human immunodeficiency virus, hepatitis C, syphilis, methadone maintenance treatment

Words count:

Full-text: 2,656
Abstract: 255

Abstract

Objective

This study explores the trends and associated factors of HIV, HCV and syphilis seroconversion among Chinese methadone maintenance treatment (MMT) clients over a follow-up period of up to seven years.

Design: Drug users from fourteen MMT clinics in Guangdong province were recruited during 2006-2014. Participants were seronegative with at least one of HIV, HCV and syphilis infections at baseline and had completed at least one follow-up test during the study period. We estimated HIV, HCV and syphilis seroconversion rates in follow-up years and identified the underlying predictors using a multivariate Cox regression model.

Results: Among 9,240 participants, the overall HIV seroconversion rate was 0.20 (0.13-0.28)/100 person-years (pys), 20.54 (18.62-22.46)/100 pys for HCV, and 0.77 (0.62-0.93)/100 pys for syphilis over the study period. HIV seroconversion rate showed a moderate but non-significant annual decline of 13.34% (-42.48-30.56%) (Chi-2 trend test: p=0.369), whereas the decline of HCV seroconversion was 16.12% (5.53-25.52%) per annum (p<0.001). Syphilis seroconversion rate remained stable (p=0.540). Urine results positive for opioid predicted HIV seroconversion (≥60% versus <60%: *HR*=3.40, 1.07-10.85), being unmarried (*HR*=1.59, 1.15-2.20), injection drug use in the past 30 days (*HR*=2.17, 1.42-3.32), having sexual intercourse in the past 3 months (*HR*=1.74, 1.22-2.47) and higher daily dosage of methadone (≥60ml versus <60ml: *HR*=1.40, 1.01-1.94) predicted HCV seroconversion. Being female (*HR*=3.56, 2.25-5.64) and infected with HCV at baseline (*HR*=2.40, 1.38-8.36) were associated with subsequent syphilis seroconversion.

Conclusion: MMT in China has demonstrated moderate to good effectiveness in reducing HIV and HCV incidence but not syphilis infection among participating drug users.

Strengths and limitations of this study

1. This study tested 4,536, 1,077, and 3,910 samples for HIV, HCV, and syphilis, respectively, and identified 27, 440, 98 person-times seroconversions. But national data with a larger sample size is needed.
2. The participants in this cohort study were followed for seven years, thus we were able to explore the trends of HIV, HCV, and syphilis seroconversion rate by follow-up years.
3. This study identified significant treatment-related factors that predicted HIV (posotive rate for urine tests) and HCV (average dosage) seroconversions

INTRODUCTION

The rapid spread of blood-borne and sexually transmitted infections represents a major public health concern in many parts of the world. In particular, the transmission of HIV, HCV, and syphilis infections has contributed to high levels of disease burden and mortality worldwide. Sharing of injection equipment for illicit drug use has fuelled the transmission of HIV and HCV in many developing country settings[1-3]. Syphilis infection is commonly reported among drug users as illicit drug use also facilitates risk sexual behaviour[4]. Opioid substitution therapy (OST) has been widely accepted as an effective harm reduction strategy for drug users. Accumulating evidence has shown that OST can effectively reduce risky drug-use behaviours[5] and hence the risk of acquiring HIV and HCV among drug users.

Methadone maintenance treatment (MMT), a key component of OST, was initiated in China since 2004. As of 2013, cumulatively 761 MMT clinics have been established nationwide, linking over 2.47 million opioid drug users to the program in China[6]. The program has shown to be effective in reducing the risk behaviour among participants among Chinese drug users[7]. We established our study sites in Guangdong Province, an open and economically developed southern Chinese province with 370,000 registered drug users. HIV, HCV, and syphilis prevalence among drug users in Guangdong were reportedly as high as 6.3%, 78.7% and 12.6%. MMT in Guangdong was initiated in 2005. By the end of 2013, 60 MMT clinics have been established and cumulatively 30,410 drug users have received MMT.

Previous studies have shown lower HIV and HCV incidence rates among MMT clients than community drug users[8 9]. Participants who retained longer in MMT have also demonstrated lower risk in acquiring HIV, HCV, and syphilis infections[11, 12]. Numerous studies have reported the prevalence of HIV, HCV and syphilis in various subgroups of MMT clients and at the different treatment stages[10-13]. By linking infection status to clients' demographic, biological, behavioural and treatment-related indicators, these studies provided important insights into the contributing factors of these infections at a certain time[14-16]. However, infection prevalence is a good indication of disease burden but not the trend of disease progression. Similarly, factors associated with disease prevalence explains the potential causes of infections at one fixed time but may not reflect the potential changes in risk factors due to treatment. Built on the previous findings, this study further explores the trends of HIV, HCV, and syphilis seroconversion among MMT clients during the course of treatment and identifies associated factors of new infections based on a retrospective cohort approach.

METHODS

Study sites and participants

Our study was conducted in 14 MMT clinics located in nine cities in Guangdong Province, China. This retrospective cohort study recruited 9,412 MMT clients between July 2006 and March 2014. Participants were eligible if he/she was: (1) diagnosed opioid dependence according to the International Classification of Diseases-10, (2) age 20 years or above, (3) a local resident who settled in the catchment areas of the clinics. Written informed consent was obtained at treatment enrolment. We excluded participants who were (1) not tested for any of HIV, HCV, and syphilis, (2) seropositive for HIV, HCV or syphilis at enrolment in their respective cohorts, but clients infected with a different infection (e.g. HCV) were allowed to enrol into other cohorts (e.g. HIV and syphilis cohorts), and (3) those did not complete at least one follow-up test. Three retrospective cohorts were created at baseline with regard to the negative infection status of HIV, HCV, and syphilis, respectively (Figure 1).

Data collection

Data was routinely collected and stored in the national unified MMT Management System. At enrolment, demographic information was collected. Drug use related behaviours and sexual behaviours were surveyed at both enrolment and subsequent half-yearly follow-ups. Blood samples of clients were also collected to assess HIV, hepatitis C and syphilis status at enrolment and follow-ups. Urine-opiate tests were performed at a random day each month. Methadone was dispensed to patients daily. All information was entered into the MMT management system by the clinics staff. For this study, data of 14 studied clinics was extracted from the MMT management system, with personal identified information removed.

Laboratory tests

Blood specimens were tested for HIV-1/2, HCV, and syphilis. The presence of HIV antibodies was screened using colloidal gold method (Aibo Biotech Company, China) in MMT clinics. Positive blood specimens were sent to the provincial Center for Disease Control and Prevention for confirmatory tests. HIV infection was determined by two positive enzyme-linked immunosorbent assays (ELISA-1, Beijing BGI-GBI Biotech Company, Ltd., China; ELISA-2, the 4th generation ELISA, bioMerieux bv, Netherland) and western blot (Abbott, MPBiomedicals, LLC, Singapore). HCV testing used ELISAs (Aibo Biotech Company, China). Toluidine red unheated serum test (TRUST, Rongsheng Biotech Company, China) was performed on all serum samples. Confirmation of positive results was done with a Treponema Palladium Particle Agglutination (TPPA, Lizhu Reagent Company, China) test.

Ethical statement

The Sun Yat-sen University Ethics Committee granted ethical approval ([2013] Ref. No. 26) for the study. MMT clients who were diagnosed with HIV, HCV and syphilis were notified and referred to designated public hospitals for further treatment.

Statistical analysis

Rate of positive urine tests is defined as the proportion of the urine samples tested positive for opioid among the total urine samples submitted. Average MMT dosage is calculated by dividing the total dosage consumed by an individual by the number of days of attendance. Two-sample *t*-test, Mann-Whitney test and χ^2 test were used for normally distributed continuous data, non-parametric data and frequencies, respectively.

The event of interest in this study was HIV, HCV, and syphilis seroconversion during the course of MMT. For syphilis, an occasion of seroconversion was regarded as a case with regard to the curability of the disease. The duration of follow-up was determined as the time between the date of MMT enrolment and the date of seroconversion or the end of study. The date of seroconversion was approximated as the midpoint between the last negative and the first positive test for all infections. Seroconversion rate with 95% confidence intervals (CIs) was calculated as numbers of events over person-years and its temporal trend significance was determined with a Joinpoint regression model [17]. Univariate and multivariate Cox proportional hazard regression analyses were conducted to identify predictors of HIV, HCV, and syphilis seroconversion. Variables with a bivariate p-value<0.20 were included in subsequent multivariate regression. The last behavioural record before seroconversion or endpoint of the study was used for the regression analysis. Multivariate Cox regression analyses were adjusted for both sociodemographic and behavioural variables. Regression models were built using stepwise techniques. P-value <0.05 was considered as statistically significant. Statistical analyses were performed using SAS v9.2 and Joinpoint v3.4.

RESULTS

Among the 9,412 enrolled MMT clients, 9,240 had at least one test for any of HIV, HCV and syphilis infections at enrolment and were recruited. Most of participants included were 30 years or older (82.12%), male (92.50%) and unemployed (59.39%). About half of the participants were unmarried (53.92%). The majority of them had low education level (junior high or blow: 84.10%). The prevalence of HIV, HCV, and syphilis at baseline were 8.68 % (768/8851), 75.78% (6721/8869), and 3.11% (207/6665), respectively (Table S1). Co-infections of HIV/HCV, HIV/syphilis and HCV/syphilis were found among 5.31% (452/8512), 0.06% (4/6667) and 2.37% (156/6582) study participants, and 0.12% (8/6667) was reportedly infected with all three diseases (Figure S1). We identified 4,536, 1,077 and 3,910 individuals who were HIV, HCV and syphilis seronegative at enrolment with at least one follow-up test record. Of these, 27, 440 and 98 HIV, HCV and syphilis incidence cases with total follow-up durations of 13,360 person-years (pys), 2,142 pys, and 12,727 pys were reported during the study period (Figure 1). In contrast, 3,547, 2,148, and 2,548 HIV, HCV and syphilis-negative participants dropped-out of MMT after first visit, respectively. Compared with participants included in the analyses, they were more likely to be male, less-educated, employed, HIV-positive but HCV-negative and had a shorter history of drug use and less sexual activities in the past 3 months (Table S1).

Among the seronegative participants at baseline, follow-up rate at each visit were 51.26%, 39.37%, 30.12%, 22.15%, 14.30%, 6.90%, 3.15% for HIV, 81.33%, 48.00%, 30.27%, 19.87%, 12.35%, 8.64%, 1.86% for HCV, and 93.27%, 74.42%, 58.82%, 44.73%, 31.53%, 16.78%, 4.40% for syphilis, respectively. The overall seroconversion rate of HIV, HCV, and syphilis was 0.20 (0.13-0.28)/100 pys, 20.54 (18.62-22.46)/100 pys, and 0.77 (0.62-0.93)/100 pys over the course of MMT, respectively. HIV seroconversion rate declined from 0.17/100 pys at baseline to 0/100 pys among those who remained at treatment after seven years, corresponding to a moderate but non-significant annual decline of 13.34% (-42.48-30.56%) (*joinpoint regression model*: p=0.369). In comparison, HCV seroconversion rate declined from 24.66/100 pys to 0/100 pys over the same follow-up period, corresponding to a significant annual reduction of 16.12% (5.53-25.52%, p<0.001). Seroconversion rate of syphilis infection (0.77/100 pys) remained stable over the course of treatment (p=0.540) (Figure 2a).

Positive rate for urine tests was found to be the only predictor for HIV seroconversion (≥60% versus <60%: *HRs*=3.40, 1.07-10.85). Being unmarried (*HRs*=1.59, 1.15-2.20), injecting drug use in the past 30 days (*HRs*=2.17, 1.42-3.32), having sexual intercourse in the past 3 months (*HRs*=1.74, 1.22-2.47), and taking an average MMT dosage over 60 ml (*HRs*=1.40, 1.01-1.94) predicted higher risk of HCV seroconversion. Participants who were female (*HRs*=3.56, 2.25-5.64) and infected with HCV at baseline (*HRs*=2.40, 1.38-8.36) demonstrated a significantly higher seroconversion rate of syphilis (Table S2, Figure 2b).

DISCUSSION

Our results demonstrated moderate to sharp declines in HIV and HCV seroconversion rates among MMT clients over the 7-year follow-up period, while seroconversion rate of syphilis remained stable. During the course of treatment, seroconversion rates of all three targeted infections (HIV: 0.20/100 pys; syphilis: 0.77/100 pys; HCV: 20.54/100 pys) have been substantially lower than the corresponding rates among community drug users in China (HIV: 0.48-8.01/100 pys; syphilis: 2.41-5.84/100 pys; HCV: 21.24-39.35/100 pys[18-22]). The seroconversion rates of HIV, HCV and syphilis among MMT participants in our study were similar to those in other studies in China [23-25].

Despite high efficacy in reducing new HCV incidence, MMT is less effective in reducing HIV and syphilis incidence. This is consistent with previous findings[26]. A plausible explanation may be the disparities in transmission routes and the impacts of MMT on high-risk behaviour. HIV can be transmitted by both blood exchange and sexual contacts. In contrast, HCV is readily blood-borne but transmits much less efficiently via sexual intercourse[27], whereas syphilis predominately transmits sexually. As a harm reduction intervention, MMT is much more effective in reducing injecting behaviours than unsafe sexual practice[28]. Previous studies have indicated that the percentage of injecting drug use among Chinese MMT clients dropped dramatically from 81.9% to 8.1% within the first two years of treatment, whereas the frequency of sexual contacts and proportion of condom use remained largely unvaried[7]. Biologically, HCV can remain infectious *ex vivo* much longer than HIV[29], and therefore reducing injection practices expectedly results in greater observable decline in seroconversion of HCV than HIV among MMT clients. Given the low seroconversion rate of HIV among MMT participants, our findings suggested that HCV seroconversion rate could be a more sensible biological indicator for assessing the effectiveness of MMT in reducing blood-born infectious diseases.

Our findings suggest that frequent positive urine test, injecting drug use and high MMT dosage predict higher risks of HIV and HCV seroconversion. Urine test is a routine monitoring technique for opioid use among MMT clients. Higher proportion of positive urine test results infers more frequent drug use[30]. Injecting drug use often leads to sharing of injection equipment among drug users and is a well-documented risk factor for HIV and HCV transmission[10 15]. Further, clients with a higher daily methadone dosage may represent drug users with a prolonged drug use and addiction[31 32]. This may imply a subgroup of drug users with high risk behavioural patterns and treatment difficulties and hence is more prone to the infections[33]. Notably, recent evidence have suggested that HCV can also transmit through sexual contacts[34], particularly when co-infected with sexually transmitted diseases such as syphilis. Participants who are already married have relatively stable family relationship. In contrast, participants who were unmarried and have sexual intercourses in the past 30 days have more likelihood of engaging in risky sexual behaviours, thus increasing the risks for HCV infection.

Females are more susceptible to syphilis infection than males anatomically[35 36]. In China, a high proportion of female drug users are reportedly involved in commercial sex[14]. Among them, multiple sexual partnerships and unprotected sex activities are common[37]. These risky sexual behaviours increase their risks of syphilis infection. Besides, HCV infection is known to synergize syphilis transmission among drug users[38] , which contributes a greater likelihood of syphilis acquisition.

The present study has several limitations. First, frequency for HIV, HCV, and syphilis testing was every six-month. Similar to other studies, the estimated timing of seroconversion relied on the consecutive measurements of negative and positive testing results, rather than a biological test for recent infection. Second, individuals with certain characteristics, especially those with high-risk behaviour, are more likely to drop-out from MMT, exclusion of this subgroup leads to underestimate of the actual seroconversion rates. Third, the percentage of drug use may be underreported as study participants may tend to provide socially desirable responses to surveys. Fourth, due to the scarce number of HIV seroconverted cases, only one significant independent variable has been found to predict HIV seroconversion.

The finding that MMT effectively reduces seroconversion of HIV and HCV highlights the importance in improving program coverage and client retention in MMT in China. Integration of effective HIV intervention strategies, such as needle and syringe exchange and condom distribution programs, should be promoted to reduce high-risk behaviours at the early stage of treatment. Although MMT clinics provide free condoms to clients, the proportion of condom use among MMT clients is reportedly low in China[40]. Low perception of risks for blood-born and sexually transmitted infections is major barrier for health service utilization[41]. Condom programs should be integrated as one of the auxiliary services in MMT clinics. Health education and counselling services about disease prevention and treatment targeting clients, particularly those at high risk, should also be routinely provided in MMT clinics in China. China has actively responded to the HIV epidemic and substantial achievements have been made in the past decade. Antiretroviral treatment is provided freely to eligible HIV-positive individuals, but treatment for HCV and syphilis may incur substantial financial burdens for drug users. Given the currently high cost of direct-acting antiviral agents for HCV infection, its treatment is unlikely to be provided freely for the needed in the near future. Although MMT clinics offer treatment referral for positive HCV and syphilis diagnoses, few clients can afford the medical cost for treatment, resulting in delay and missed opportunities for timely treatment[42]. In the absence of a national treatment program, the MMT program could thus be a feasible alternative for preventing HCV transmission among drug users. Further, linking antiretroviral treatment to MMT may reduce mortality among drug users living with HIV[43]. With the new development of HCV cure[44], expansion of HCV treatment for MMT clients may be considered as a priority.

ACKNOWLEDGEMENTS

The authors acknowledge all the staff of the MMT clinics in Guangdong Province, China.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: all authors had financial support from the National Natural Science Foundation of China for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

FUNDING

This work was supported by the National Natural Science Foundation of China (grant 30972552, grant 71173245, grant 81473065), the China Medical Board (grant 12-111).

CONTRIBUTORS

LL, LZ, XZ conceived the study. LL contributed to the study coordination. XZ participated in data collection, analysis and drafted the manuscript. LZ and LL finalized the manuscript. All authors read and approved the final manuscript.

REFERENCE:

1. WHO. Injecting drug use. World Health Organization, 2014.
2. (UNODC) UNOoDaC. World Drug Report 2014. New York, 2014.
3. Nelson PK, Mathers BM, Cowie B, et al. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet 2011;**378**(9791):571-83 doi: 10.1016/s0140-6736(11)61097-0[published Online First: Epub Date]|.
4. Todd CS, Nasir A, Raza Stanekzai M, et al. Prevalence and correlates of syphilis and condom use among male injection drug users in four Afghan cities. Sexually transmitted diseases 2010;**37**(11):719-25 doi: 10.1097/OLQ.0b013e3181e2c76a[published Online First: Epub Date]|.
5. Shi J, Zhao LY, Epstein DH, et al. The effect of methadone maintenance on illicit opioid use, human immunodeficiency virus and hepatitis C virus infection, health status, employment, and criminal activity among heroin abusers during 6 months of treatment in china. Journal of addiction medicine 2007;**1**(4):186-90 doi: 10.1097/ADM.0b013e318156cc19[published Online First: Epub Date]|.
6. Security NCBotMoP. Annual report on drug control in China 2014. Beijing, 2014.
7. Zhou Y, Li X, Zhang C, et al. Rates of HIV, syphilis, and HCV infections among different demographic groups of female sex workers in Guangxi China: Evidence from 2010 national sentinel surveillance data. AIDS Care 2013 doi: 10.1080/09540121.2013.772282[published Online First: Epub Date]|.
8. Duan S, Yang YC, Han J, et al. [Study on incidence of HIV infection among heroin addicts receiving methadone maintenance treatment in Dehong prefecture, Yunnan province]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2011;**32**(12):1227-31
9. Peles E, Schreiber S, Rados V, et al. Low risk for hepatitis C seroconversion in methadone maintenance treatment. Journal of addiction medicine 2011;**5**(3):214-20 doi: 10.1097/ADM.0b013e31820e13dd[published Online First: Epub Date]|.
10. Xiaoli W, Lirong W, Xueliang W, et al. Risk Factors of Hepatitis C Virus Infection in Drug Users From Eleven Methadone Maintenance Treatment Clinics in Xi'an, China. Hepatitis monthly 2014;**14**(11):e19601 doi: 10.5812/hepatmon.19601[published Online First: Epub Date]|.
11. Tsui JI, Evans JL, Lum PJ, et al. ASsociation of opioid agonist therapy with lower incidence of hepatitis c virus infection in young adult injection drug users. JAMA Internal Medicine 2014;**174**(12):1974-81 doi: 10.1001/jamainternmed.2014.5416[published Online First: Epub Date]|.
12. Zhuang X, Liang Y, Chow EP, et al. HIV and HCV prevalence among entrants to methadone maintenance treatment clinics in China: a systematic review and meta-analysis. BMC infectious diseases 2012;**12**:130 doi: 10.1186/1471-2334-12-130[published Online First: Epub Date]|.
13. Broers B, Junet C, Bourquin M, et al. Prevalence and incidence rate of HIV, hepatitis B and C among drug users on methadone maintenance treatment in Geneva between 1988 and 1995. AIDS (London, England) 1998;**12**(15):2059-66
14. Gourevitch MN, Hartel D, Schoenbaum EE, et al. A prospective study of syphilis and HIV infection among injection drug users receiving methadone in the Bronx, NY. American journal of public health 1996;**86**(8):1112-5
15. Zhuang X, Wang Y, Chow EP, et al. Risk factors associated with HIV/HCV infection among

entrants in methadone maintenance treatment clinics in China: a systematic review and meta-analysis. Drug and alcohol dependence 2012;**126**(3):286-95 doi: 10.1016/j.drugalcdep.2012.05.028[published Online First: Epub Date]|.

16. Zou X, Chen W, Ling L. Influencing factors of HIV and syphilis seroconversion among patients with methadone maintenance treatment. Chinese Journal of Public Health 2013;**29**(8):1107-11
17. Yu B, Barrett MJ, Kim H-J, et al. Estimating joinpoints in continuous time scale for multiple change-point models. Computational Statistics & Data Analysis 2007;**51**(5):2420-27 doi: http://dx.doi.org/10.1016/j.csda.2006.07.044[published Online First: Epub Date]|.
18. Li X. A research of the incidence of HIV/HCV/HBV/syphilis and risk behaviors among injecting drug users in Xichang County of Sichuan Province. Suzhou University, 2011.
19. Bruneau J, Roy E, Arruda N, et al. The rising prevalence of prescription opioid injection and its association with hepatitis C incidence among street-drug users. Addiction 2012;**107**(7):1318-27 doi: 10.1111/j.1360-0443.2012.03803.x[published Online First: Epub Date]|.
20. van den Berg CH, Smit C, Bakker M, et al. Major decline of hepatitis C virus incidence rate over two decades in a cohort of drug users. European journal of epidemiology 2007;**22**(3):183-93 doi: 10.1007/s10654-006-9089-7[published Online First: Epub Date]|.
21. Duan S, Han J, Tang RH, et al. [Study on the incidence and risk factors of HCV infection among heroin addicts who were on methadone maintenance treatment in Dehong prefecture,Yunnan province]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2013;**34**(6):552-6
22. Sarna A, Saraswati LR, Sebastian M, et al. High HIV incidence in a cohort of male injection drug users in Delhi, India. Drug and alcohol dependence 2014 doi: 10.1016/j.drugalcdep.2014.03.021[published Online First: Epub Date]|.
23. Zhou W, Wang X, Zhou S, et al. Hepatitis C seroconversion in methadone maintenance treatment programs in Wuhan, China. Addiction 2015;**110**(5):796-802 doi: 10.1111/add.12836[published Online First: Epub Date]|.
24. Y-P. Chang, L. Duo, A. M. V. Kumar, et al. Retention and HIV seroconversion among drug users on methadone maintenance treatment in Yunnan, China. Public Health Action 2014;**4**(1):28-34
25. Xia Zou WC, Li Ling. Influencing factors of HIV and syphilis seroconversion among patients with methadone maintenance treatment. Chinese Journal of Public Health 2013;**29**(8):1107-11
26. Ruan Y, Liang S, Zhu J, et al. Evaluation of harm reduction programs on seroincidence of HIV, hepatitis B and C, and syphilis among intravenous drug users in southwest China. Sexually transmitted diseases 2013;**40**(4):323-8 doi: 10.1097/OLQ.0b013e31827fd4d4[published Online First: Epub Date]|.
27. Garfein RS, Vlahov D, Galai N, et al. Viral infections in short-term injection drug users: the prevalence of the hepatitis C, hepatitis B, human immunodeficiency, and human T-lymphotropic viruses. American journal of public health 1996;**86**(5):655-61
28. Marsch LA. The efficacy of methadone maintenance interventions in reducing illicit opiate use, HIV risk behavior and criminality: a meta-analysis. Addiction 1998;**93**(4):515-32
29. Crofts N, G. D, Locarnini S. *Hepatitis C: an Australian perspective*. Melbourne, 2001.

30. Pergolizzi J, Pappagallo M, Stauffer J, et al. The role of urine drug testing for patients on opioid therapy. Pain practice : the official journal of World Institute of Pain 2010;**10**(6):497-507 doi: 10.1111/j.1533-2500.2010.00375.x[published Online First: Epub Date]|.
31. Li X, Tan H, Sun Z, et al. [Change and influence factors of craving for patients after 6 month methadone maintenance treatment]. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences 2009;**34**(8):718-22
32. Baumeister M, Vogel M, Dürsteler-MacFarland K, et al. Association between methadone dose and concomitant cocaine use in methadone maintenance treatment: a register-based study. Subst Abuse Treat Prev Policy 2014;**9**(1):1-8 doi: 10.1186/1747-597X-9-46[published Online First: Epub Date]|.
33. Xia X, Luo J, Bai J, et al. Epidemiology of hepatitis C virus infection among injection drug users in China: systematic review and meta-analysis. Public health 2008;**122**(10):990-1003 doi: 10.1016/j.puhe.2008.01.014[published Online First: Epub Date]|.
34. Terrault NA, Dodge JL, Murphy EL, et al. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: The HCV partners study. Hepatology 2013;**57**(3):881-89 doi: 10.1002/hep.26164[published Online First: Epub Date]|.
35. Ruan Y, Liang S, Zhu J, et al. Gender and ethnic disparities of HIV and syphilis seroconversions in a 4-year cohort of injection drug users. The Southeast Asian journal of tropical medicine and public health 2013;**44**(5):842-53
36. Coffin LS, Newberry A, Hagan H, et al. Syphilis in drug users in low and middle income countries. The International journal on drug policy 2010;**21**(1):20-7 doi: 10.1016/j.drugpo.2009.02.008[published Online First: Epub Date]|.
37. Folch C, Casabona J, Espelt A, et al. Gender differences in HIV risk behaviours among intravenous drug users in Catalonia, Spain. Gaceta sanitaria / SESPAS 2013;**27**(4):338-43 doi: 10.1016/j.gaceta.2013.02.006[published Online First: Epub Date]|.
38. Liao M, Kang D, Tao X, et al. Syndemics of syphilis, HCV infection, and methamphetamine use along the east coast of China. BMC public health 2014;**14**:172 doi: 10.1186/1471-2458-14-172[published Online First: Epub Date]|.
39. Du J, Wang Z, Xie B, et al. Hepatitis C knowledge and alcohol consumption among patients receiving Methadone Maintenance Treatment in Shanghai, China. The American journal of drug and alcohol abuse 2012;**38**(3):228-32 doi: 10.3109/00952990.2011.643974[published Online First: Epub Date]|.
40. Xue H, Duo L, Lei Y, et al. Cohort analysis of change in high risk behavior among people received methadone maintenance treatment. Chinese Journal of Drug Dependence 2013;**22**(6):460-63
41. Du J, Wang Z, Xie B, et al. Hepatitis C knowledge and alcohol consumption among patients receiving methadone maintenance treatment in Shanghai, China. The American journal of drug and alcohol abuse 2012;**38**(3):228-32 doi: 10.3109/00952990.2011.643974[published Online First: Epub Date]|.
42. Zhao Y, Shi CX, McGoogan JM, et al. Predictors of accessing antiretroviral therapy among HIV-positive drug users in China's National Methadone Maintenance Treatment Programme. Addiction 2015;**110 Suppl 1**:40-50 doi: 10.1111/add.12782[published Online First: Epub Date]|.
43. Cao X, Wu Z, Li L, et al. Mortality among methadone maintenance clients in China: a six-year

cohort study. PloS one 2013;**8**(12):e82476 doi: 10.1371/journal.pone.0082476[published Online First: Epub Date]|.

44. Manns M, Pol S, Jacobson IM, et al. All-oral daclatasvir plus asunaprevir for hepatitis C virus genotype 1b: a multinational, phase 3, multicohort study. Lancet 2014 doi: 10.1016/s0140-6736(14)61059-x[published Online First: Epub Date]|.

Figures

- **Figure 1. Eligibility and screening procedures for study participants**
- **Figure 2a. HIV, HCV, and syphilis serovonversion rate among drug users over the course of methadone maintenance treatment.**
- **Figure 2b. Risk factors associated with HIV, HCV, and syphilis seroconversions during the course of MMT treatment, based on multivariate Cox regression.**

Additional files

- **Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients**
- **Figure S1 Infection status of HIV, HCV syphilis and co-infections among drug users at first MMT enrolment.**
- **Table S2a Univariate and multivariate Cox regression for HIV seroconversion**
- **Table S2b Univariate and multivariate Cox regression for syphilis seroconversion**
- **Table S2c Univariate and multivariate Cox regression for syphilis seroconversion**

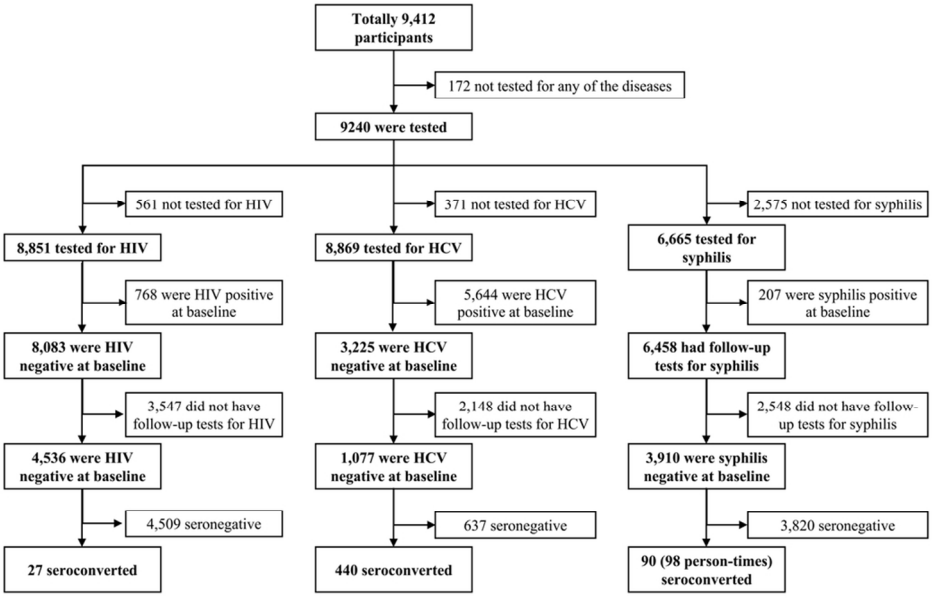

Figure 1 Eligibility and screening procedures for study participants
47x29mm (600 x 600 DPI)

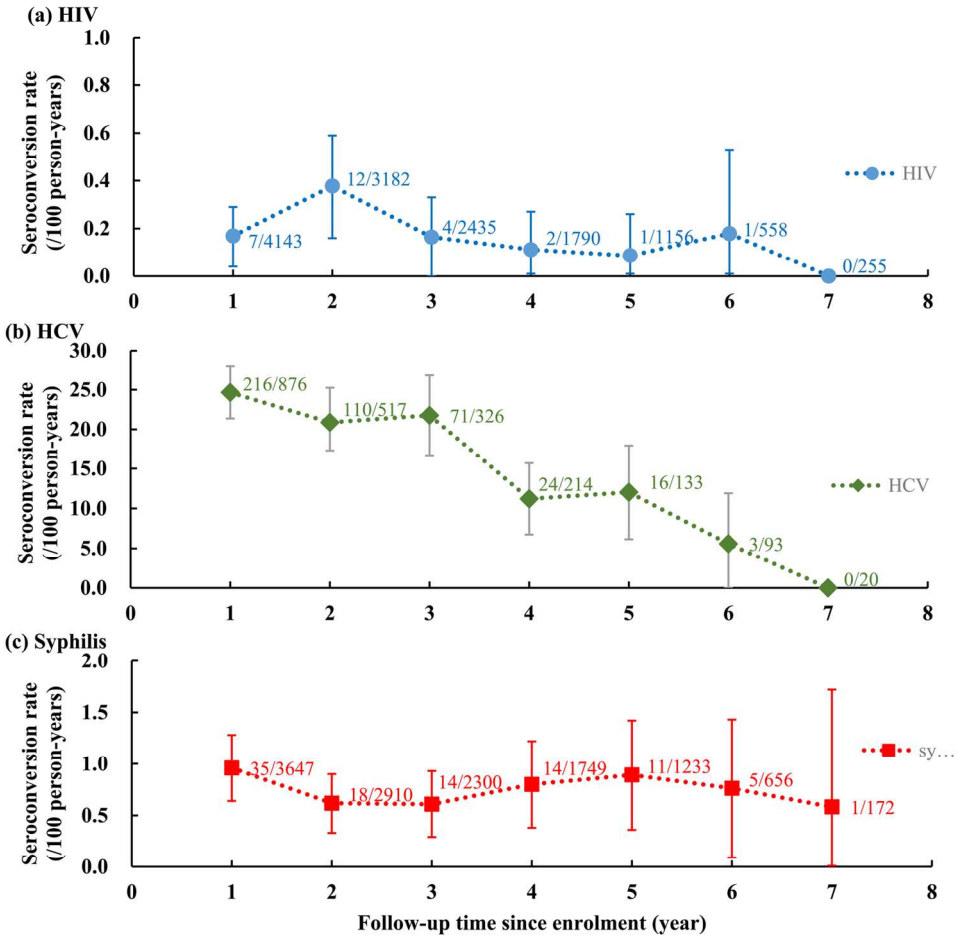

Figure 2a. HIV, HCV, and syphilis serovonversion rate among drug users over the course of methadone maintenance treatment
75x75mm (600 x 600 DPI)

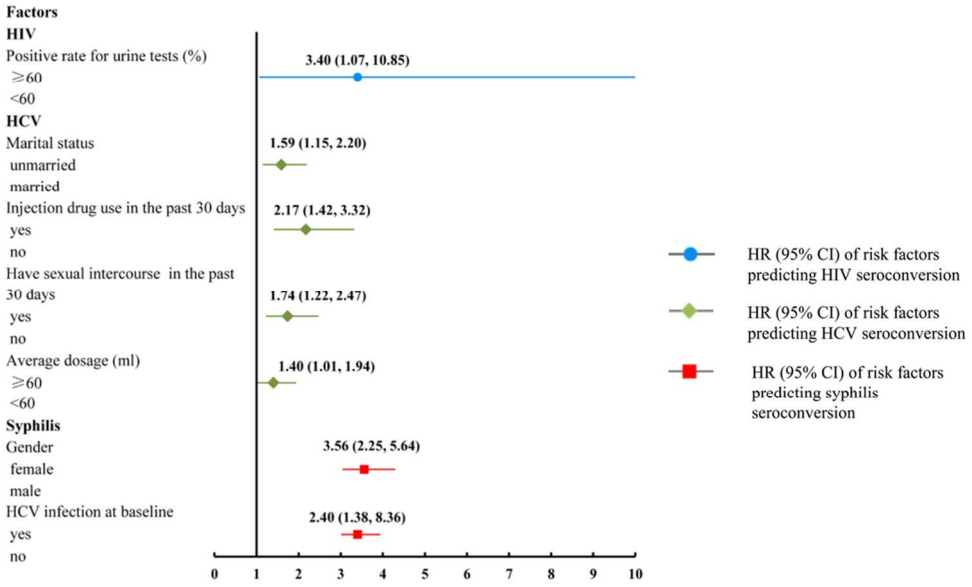

Figure 2b. Risk factors associated with HIV, HCV, and syphilis seroconversions during the course of MMT treatment, based on multivariate Cox regression.
47x29mm (600 x 600 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	Figure S1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table S1
		(b) Indicate number of participants with missing data for each variable of interest	Table S1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients

Factors	follow-up N=5186 (%)	drop-out N=4054 (%)	Total N=9240 (%)	t/χ^2	P
Age					
≥30	4279 (82.5)	3309 (81.6)	7588 (82.1)	1.22	0.269
<30	907 (17.5)	745 (18.4)	1652 (17.9)		
Missing#	0 (0.0)	0 (0.0)	0 (0.0)		
Gender					
female	459 (8.9)	234 (5.8)	693 (7.5)	31.09	<0.001
male	4727 (91.1)	3820 (94.2)	8547 (92.5)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Employment					
unemployed	3201 (61.7)	2287 (56.4)	5488 (59.4)	26.61	<0.001
employed	1985 (38.3)	1767 (43.6)	3752 (40.6)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Marriage					
unmarried	2794 (53.9)	2188 (54.0)	4982 (53.9)	0.01	0.927
married	2392 (46.1)	1866 (46.0)	4258 (46.1)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Education					
junior high or below	4311 (83.1)	3460 (85.3)	7771 (84.1)	8.39	0.004
senior high or above	875 (16.9)	594 (14.7)	1469 (15.9)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
Years of drug use					
<5	609 (11.7)	555 (13.7)	1164 (12.6)	9.55	0.008
5-	2964 (57.2)	2315 (57.1)	5279 (57.1)		
15-	1613 (31.1)	1184 (29.2)	2797 (30.3)		
missing	0 (0.0)	0 (0.0)	0 (0.0)		
HIV infections at baseline					
yes	402 (7.8)	366 (9.9)	768 (8.7)	12.70	<0.001
no	4767 (92.2)	3316 (90.1)	8083 (91.3)		
missing	22 (4.2)	372 (9.2)	371 (4.0)		
HCV infections at baseline					
yes	4000 (77.4)	2721 (73.5)	6721 (75.8)	18.65	<0.001
no	1165 (22.6)	983 (26.5)	2148 (24.2)		
missing	21 (0.4)	350 (8.6)	371 (4.0)		
Syphilis infections at baseline					
yes	154 (3.2)	53 (2.9)	207 (3.1)	0.50	0.479
no	4660 (96.8)	1798 (97.1)	6458 (96.9)		
missing	372 (7.2)	2203 (54.3)	2575 (27.3)		
Inject drug use in the past 30 days					
yes	3453 (80.3)	2380 (79.0)	5833 (79.8)	2.00	0.158
no	845 (19.7)	633 (21.0)	1478 (20.2)		
missing	888 (17.1)	1041 (25.7)	1929 (20.9)		
Needle sharing in the past 30 days					
yes	286 (8.3)	224 (9.4)	510 (8.8)	2.29	0.130
no	3162 (91.7)	2150 (90.6)	5312 (91.2)		
missing	1738 (33.5)	1680 (41.4)	3418 (37.0)		
Had sexual intercourse in the past 3 months					
yes	2984 (69.7)	1931 (64.7)	4915 (67.7)	20.02	<0.001
no	1297 (30.3)	1053 (35.3)	2350 (32.3)		
missing	905 (17.5)	1070 (26.4)	1975 (21.0)		
Had multiple sexual partners in the past 3 months					
yes	336 (11.3)	255 (13.2)	591 (12.1)	4.25	0.039
no	2642 (88.7)	1670 (86.8)	4312 (87.9)		
missing	2208 (42.6)	2129 (52.5)	4337 (46.9)		

Table S1 Comparison of demographic characteristics and risk behaviour among retained and dropped-out MMT clients (Continue)

Condom use in the last sexual episode					
yes	1315 (43.2)	857 (41.8)	2172 (42.7)	0.97	0.326
no	1728 (56.8)	1192 (58.2)	2920 (57.3)		
missing	2143 (41.3)	2005 (49.5)	4148 (44.9)		

*all eligible participants used drugs in the past 30 days before enrolled in MMT

#the proportion of each category was calculated using the number of the category dividing the number of participants who had records; the proportion of the missing data was calculated using the number of missing data dividing the number of participants who had records.

Figure S1 Infection status of HIV, HCV syphilis and co-infections among drug users at first MMT enrolment.

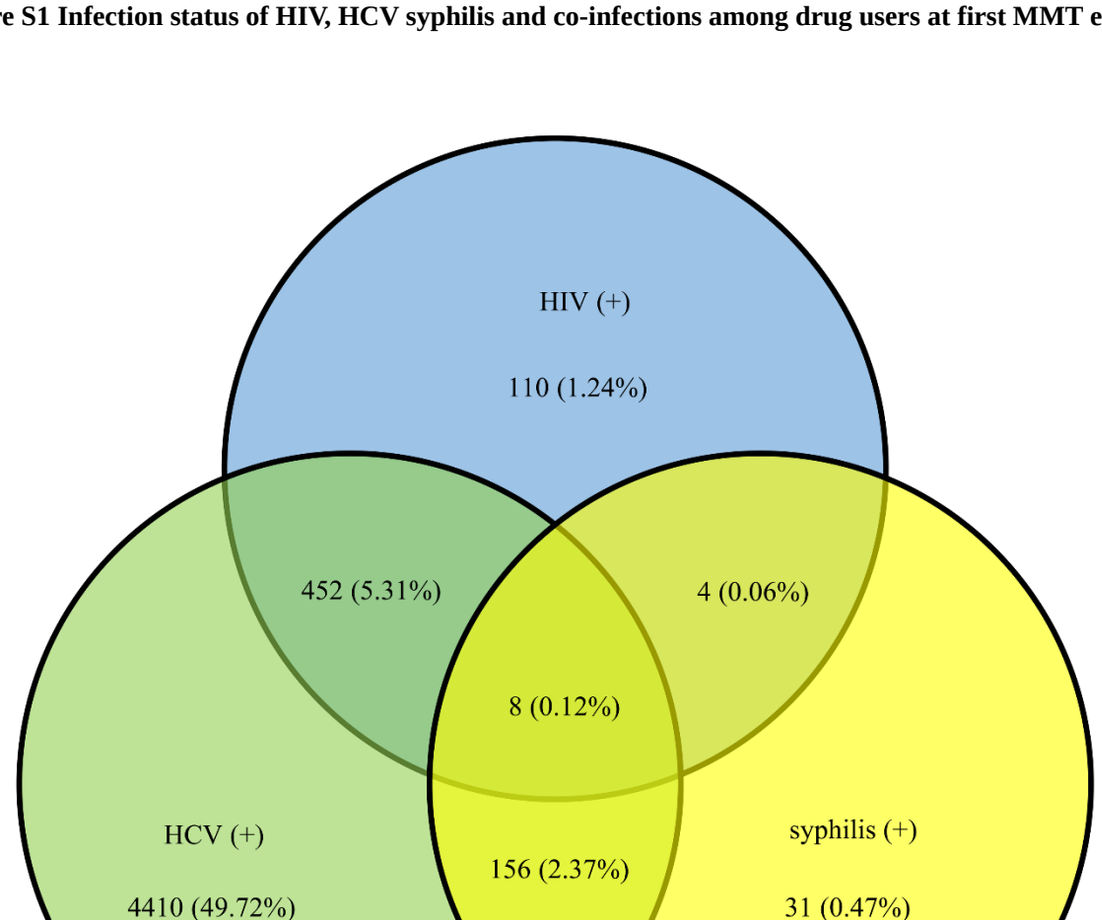

Table S2a Univariate and multivariate Cox regression for HIV seroconversion

Factor	Univariate				Multivariate	
	seroconversion rate (95% CI)	HR (95% CI)	χ^2	*P*	aHR (95% CI)	*P*
Age (year)						
≥30	0.27 (0.07-0.46)	1.47 (0.62-3.47)	0.76	0.384	--	--
<30	0.19 (0.10-0.27)	1.00 (Reference)	--	--	--	--
Gender						
female	0.33 (0.01-0.64)	1.69 (0.59-4.90)	0.94	0.331	--	--
male	0.19 (0.11-0.27)	1.00 (Reference)	--	--	--	--
Employment						
unemployed	0.21 (0.11-0.31)	1.07 (0.49-2.35)	0.03	0.857	--	--
employed	0.19 (0.07-0.31)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	0.18 (0.08-0.28)	0.82 (0.39-1.74)	0.27	0.605	--	--
married	0.22 (0.11-0.34)	1.00 (Reference)	--	--	--	--
Education						
junior high and below	0.21 (0.12-0.30)	1.28 (0.44-3.71)	0.21	0.644	--	--
senior high and above	0.16 (0.00-0.32)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	0.22 (0.07-0.37)	3.16 (0.40-25.31)	1.18	0.278	--	--
5-	0.22 (0.12-0.32)	3.23 (0.43-24.18)	1.30	0.254	--	--
15-	0.07 (0.00-0.20)	1.00 (Reference)	--	--	--	--
HIV infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
HCV infection at baseline						
yes	0.24 (0.15-0.33)	--	--	--	--	--
no	0.00 (0.00-0.00)	--	--	--	--	--
Syphilis infection at baseline						
yes	0.50 (0.00-1.20)	2.55 (0.60-10.78)	1.63	0.202	--	--
no	0.20 (0.12-0.28)	1.00 (Reference)	--	--	--	--
Drug use in the past 30 days						
yes	0.41 (0.22-0.60)	3.77 (1.70-8.40)	10.58	0.001	--	--
no	0.11 (0.04-0.18)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	0.42 (0.20-0.63)	1.08 (0.35-3.27)	0.02	0.899	--	--
no	0.38 (0.01-0.76)	1.00 (Reference)	--	--	--	--
Needle sharing in the past 30						
yes	0.78 (0.10-1.47)	2.23 (0.75-6.66)	2.07	0.150	--	--
no	0.33 (0.11-0.55)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	0.23 (0.13-0.34)	1.35 (0.61-3.00)	0.54	0.464	--	--
no	0.17 (0.06-0.29)	1.00 (Reference)	--	--	--	--
Multiple sexual partners in the						
yes	0.32 (0.00-0.77)	1.39 (0.32-6.04)	0.19	0.661	--	--
no	0.23 (0.12-0.34)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	0.17 (0.04-0.30)	0.56 (0.22-1.45)	1.44	0.231	--	--
no	0.31 (0.13-0.49)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	0.53 (0.23-0.82)	2.33 (1.08-5.03)	4.63	0.031	3.40 (1.07, 0.85)	0.039
<60	0.28 (0.06-0.51)	1.00 (Reference)	--	--	1.00 (Reference)	
Average dosage (ml)						
≥60	0.56 (0.15-0.98)	1.82 (0.85-3.87)	2.41	0.121	--	--
<60	0.35 (0.14-0.55)	1.00 (Reference)	--	--	--	--

Table S2b Univariate and multivariate Cox regression for HCV seroconversion

Factor	Seroconversion rate (95% CI)	Univariate HR(95% CI)	χ^2	*P*	Multivariate aHR (95% CI)	*P*
Age (year)						
≥30	20.97 (18.75-23.19)	1.06 (0.85-1.33)	0.27	0.603	--	--
<30	19.16 (15.34-22.97)	1.00 (Reference)	--	--	--	--
Gender						
female	31.96 (23.66-40.26)	1.60 (1.21-2.11)	10.95	0.001	--	--
male	19.50 (17.55-21.46)	1.00 (Reference)	--	--	--	--
Employment						
unemployed	21.88 (19.25-24.52)	1.17 (0.97-1.42)	2.66	0.103	--	--
employed	18.79 (16.01-21.58)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	24.48 (21.26-27.69)	1.36 (1.13-1.64)	10.41	0.001	1.59 (1.15, 2.20)	0.005
married	17.63 (15.28-19.97)	1.00 (Reference)	--	--	1.00 (Reference)	--
Education						
junior high and below	22.07 (19.75-24.39)	1.31 (1.05-1.65)	5.46	0.02	--	--
senior high and above	16.32 (13.00-19.63)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	15.92 (12.26-19.57)	0.71 (0.53-0.96)	4.84	0.028	--	--
5-	21.37 (18.82-23.92)	0.96 (0.76-1.21)	0.14	0.712	--	--
15-	23.09 (18.50-27.70)	1.00 (Reference)	--	--	--	--
HIV infection at baseline						
yes	29.89 (21.06-38.72)	1.44 (1.05-1.96)	5.18	0.023	--	--
no	19.88 (17.92-21.84)	1.00 (Reference)	--	--	--	--
HCV infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
Syphilis infection at baseline						
yes	20.21 (18.26-22.16)	1.55 (0.97-2.48)	3.3	0.069	--	--
no	32.21 (17.33-47.10)	1.00 (Reference)	--	--	--	--
Drug use in the past 30 days						
yes	24.61 (20.78-28.43)	1.36 (1.11-1.66)	8.61	0.003	--	--
no	17.30 (15.06-19.54)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	30.82 (25.57-36.08)	2.42 (1.60-3.66)	17.43	<0.00	2.17 (1.42, 3.32)	<0.001
no	12.58 (7.83-17.32)	1.00 (Reference)	--	--	1.00 (Reference)	--
Needle sharing in the past 30						
yes	34.12 (18.36-49.88)	1.07 (0.65-1.76)	0.07	0.787	--	--
no	30.36 (24.79-35.94)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	20.99 (18.42-23.55)	1.19 (0.96-1.47)	2.59	0.108	1.74 (1.22, 2.47)	0.002
no	17.73 (14.70-20.75)	1.00 (Reference)	--	--	1.00 (Reference)	--
Multiple sexual partners in the						
yes	18.97 (8.66-29.29)	0.86 (0.49-1.48)	0.3	0.582	--	--
no	21.01 (18.37-23.66)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	19.92 (16.57-23.26)	0.92 (0.72-1.17)	0.47	0.495	--	--
no	22.34 (18.36-26.32)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	20.75 (17.80-23.70)	0.96 (0.78-1.18)	0.16	0.687	--	--
<60	21.71 (16.51-26.91)	1.00 (Reference)	--	--	--	--
Average dosage (ml)						
≥60	23.82 (19.03-28.61)	1.39 (1.15-1.68)	11.21	0.001	1.40 (1.01, 1.94)	0.044
<60	19.62 (16.60-22.64)	1.00 (Reference)	--	--	1.00 (Reference)	--

Table S2c Univariate and multivariate Cox regression for syphilis seroconversion

Factor	Univariate				Multivariate	
	Seroconversion rate (95% CI)	HR (95% CI)	χ^2	*P*	aHR (95% CI)	*P*
Age (year)						
≥30	0.83 (0.65-1.00)	1.45 (0.83-2.56)	1.67	0.196	--	--
<30	0.56 (0.27-0.85)	1.00 (Reference)	--	--	--	--
Gender						
female	2.24 (1.34-3.13)	3.51 (2.22-5.57)	28.58	<0.00	3.56 (2.25, 5.64)	<0.001
male	0.64 (0.49-0.78)	1.00 (Reference)	--	--	1.00 (Reference)	--
Employment						
unemployed	0.88 (0.67-1.09)	1.48 (0.96-2.28)	3.10	0.078	--	--
employed	0.60 (0.38-0.82)	1.00 (Reference)	--	--	--	--
Marital status						
unmarried	0.80 (0.59-1.01)	1.08 (0.73-1.61)	0.15	0.697	--	--
married	0.74 (0.52-0.96)	1.00 (Reference)	--	--	--	--
Education						
junior high and below	0.77 (0.60-0.93)	0.95 (0.58-1.57)	0.04	0.835	--	--
senior high and above	0.80 (0.44-1.17)	1.00 (Reference)	--	--	--	--
Duration of drug use (year)						
<5	0.90 (0.60-1.21)	1.07 (0.54-2.12)	0.04	0.847	--	--
5-	0.70 (0.51-0.89)	0.84 (0.44-1.61)	0.28	0.599	--	--
15-	0.84 (0.34-1.39)	1.00 (Reference)	--	--		
HIV infection at baseline						
yes	0.55 (0.14-0.96)	0.70 (0.32-1.51)	0.84	0.361	--	--
no	0.80 (0.63-0.96)	1.00 (Reference)	--	--	--	--
HCV infection at baseline						
yes	0.87 (0.69-1.04)	3.32 (1.35-8.16)	6.82	0.009	2.40 (1.38, 8.36)	0.008
no	0.26 (0.03-0.44)	1.00 (Reference)	--	--	1.00 (Reference)	--
Syphilis infection at baseline						
yes	--	--	--	--	--	--
no	--	--	--	--	--	--
Drug use in the past 30 days						
yes	0.78 (0.50-1.05)	1.04 (0.67-1.59)	0.02	0.876	--	--
no	0.75 (0.56-0.94)	1.00 (Reference)	--	--	--	--
Injection drug use in the past						
yes	0.86 (0.26-1.45)	0.88 (0.39-1.97)	0.10	0.754	--	--
no	0.75 (0.44-1.06)	1.00 (Reference)	--	--	--	--
Needle sharing in the past 30						
yes	1.15 (0.23-2.07)	1.64 (0.65-4.15)	1.08	0.300	--	--
no	0.69 (0.35-0.99)	1.00 (Reference)	--	--	--	--
Have sexual intercourses in the						
yes	0.82 (0.61-1.03)	1.24 (0.81-1.89)	0.99	0.319	--	--
no	0.67 (0.44-0.89)	1.00 (Reference)	--	--	--	--
Multiple sexual partners in the						
yes	1.02 (0.13-1.91)	1.24 (0.49-3.09)	0.2	0.652	--	--
no	0.81 (0.60-1.03)	1.00 (Reference)	--	--	--	--
Condom use in the past 3						
yes	0.96 (0.65-1.26)	1.41 (0.83-2.39)	1.63	0.201	--	--
no	0.67 (0.39-0.96)	1.00 (Reference)	--	--	--	--
Average urine positive rate (%)						
≥60	0.84 (0.49-1.19)	1.25 (0.79-1.95)	0.91	0.339	--	--
<60	0.74 (0.57-0.91)	1.00 (Reference)	--	--	--	--
Average dosage (ml)						
≥60	0.75 (0.50-1.00)	0.96 (0.64-1.45)	0.04	0.849	--	--
<60	0.76 (0.56-0.96)	1.00 (Reference)	--	--	--	--

Footnote: seroconversion rate was estimated using number of cases divided by years of follow-up (/100 person-years); Hazard ratio (HR) was estimated by using Cox regression method. aHR: adjusted hazard ratio