Title

Viral Load Tests among People Living with Human Immunodeficiency Virus on Antiretroviral Therapy in Wenshan Prefecture, Yunnan Province of China

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Viral Load Tests among People Living with Human Immunodeficiency Virus on Antiretroviral Therapy in Wenshan Prefecture, Yunnan Province of China

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Epidemiology

by

Youran Xu

2019

ABSTRACT OF THE DISSERTATION

Viral Load Tests among

People Living with Human Immunodeficiency Virus on Antiretroviral Therapy

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Youran Xu

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2019

Professor Roger Detels, Chair

Background

China has made great achievements in antiretroviral therapy (ART) among people living with HIV (PLWH), however, there is still a gap with the 90-90-90 targets, especially in rural areas. This study investigated three topics about the centralized viral load (VL) testing in Wenshan, China, including the coverage and timeliness of VL tests, the turnaround time (TAT) of VL tests, and factors associated with sustained viral suppression (SVS).

Methods

To address the three topics, three clinic-based studies were conducted respectively. First, a retrospective cohort study was conducted among 815 PLWH who initiated ART from 2015 to 2016. Second, a sequential explanatory mixed-method study was conducted: (1) 2 892 VL tests performed in 2018 were reviewed; (2) in-depth interviews were conducted among 11 healthcare providers. Third, a questionnaire survey was conducted among 264 PLWH.

Results

First, in Wenshan, the cumulative VL testing rates (%) at 12, 18, and 24 months after ART initiation were 58.5%, 78.1%, and 93.0%, respectively. Patients who had healthier baseline status [adjusted hazard ratio (aHR)=1.40, 95% confidence interval (95%CI)=1.18-1.65] were more likely to undergo VL tests timely. On the contrary, patients who lived far away from ART sites (aHR=0.70, 95%CI=0.59-0.83) had delayed VL tests. Second, the median VL testing TAT was 54 days (IQR: 36, 92). Factors associated with prolonged TAT mainly included the annual urban-to-rural labor migration, the shortage of healthcare professionals and lab technicians, limited VL testing instruments, and the immature reagent procurement system and testing results reporting system. Third, 61.0% (n=161) of the PLWH who participated in the survey had achieved SVS. A total of 58.3% (n=154) participants reported they had ever proactively asked about VL testing results and they were more likely to achieve SVS [adjusted odds ratio (aOR)=3.08, 95%CI=1.52-6.26)]. Other factors associated with SVS included baseline CD4 count, VL-related knowledge, support from non-governmental organizations (NGO), age, and time interval from HIV diagnosis to ART initiation.

Conclusions

To achieve the 90-90-90 targets, the VL testing in rural China should be improved by combining the efforts of PLWH, local healthcare providers, NGO staff, and healthcare policymakers at all levels of government.

The dissertation of Youran Xu is approved.

Abdelmonem A. Afifi

Li Li

Zuo-Feng Zhang

Roger Detels, Committee Chair

University of California, Los Angeles

2019

Dedicated to my dearest parents, 徐元明 (Yuanming Xu) and 王苗珍 (Miaozhen Wang)

For their unwavering support and love

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3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
AZT	Zidovudine
bDNA	Branched-chain deoxyribonucleic acid
CDC	Center for Disease Center and Prevention
CI	Confidence interval
EFV	Efavirenz
GDP	Gross domestic product
HIV	Human immunodeficiency virus
HR	Hazard ratio
IDU	Injection drug users
IQR	Interquartile range
IRB	Institutional Review Board
LMIC	Low- and mid-income country
LPV/r	Lopinavir/ritonavir
LTFU	Loss to follow-up
MSM	Men who have sex with men
NASBA	Nucleic acid sequence-based amplification
NAT	Nucleic acids-based test

NCAIDS	National Center for AIDS/STD Control and Prevention
NFATP	National Free ART Program
NGO	Non-governmental organization
NHC	National Health Commission of China
NVP	Nevirapine
OR	Odds ratio
PLWH	People living with HIV
POC	Point-of-care
PY	Person-year
RT-PCR	Reverse transcription-polymerase chain reaction
SES	Socioeconomic status
SVS	Sustained viral suppression
TAT	Turnaround time
TDF	Tenofovir
UNAIDS	Joint United Nations Programme on HIV/AIDS
VL	Viral load
WHO	World Health Organization

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Xu Y, Detels R. Timeliness of viral load test among people living with HIV receiving antiretroviral therapy in rural China. Manuscript in preparation.

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Wu J, Chen Z, Yu F, **Xu Y**, Ma Y, Ji G, Scott SR, Mi G, Wu Z. HIV infection among young men who have sex with men in China: comparison of risks among students and non-students. Manuscript submitted for publication.

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Chapter 1. Introduction

1.1 Global HIV/AIDS epidemic

Acquired Immune Deficiency Syndrome (AIDS) was first recognized as a new disease in the early 1980s and then spread rapidly all over the world[1–4]. The total number of people living with HIV (PLWH) has been continuously increased. By 2018, there had been 38 million PLWH globally, with 68% in sub-Saharan Africa and 16% in Asia and the Pacific[5]. Despite the growing total number of PLWH, the newly diagnosed HIV-infected cases have been steadily decreased year by year since the second half of the 1990s[5,6]. There were 1.7 million new HIV infections in 2018, which had been reduced by 40% since the peak in 1997[5]. In addition, HIV-related mortality has continuously decreased since the implementation and scale-up of antiretroviral therapy (ART)[7–9]. The global estimated number of HIV-related death in 2018 was 770 000, which had decreased by 57% compared with a peak of 1.8 million HIV-related deaths in 2005[5,7].

1.2 Progress towards UNAIDS '90-90-90' targets

To end the AIDS epidemic, ambitious 90-90-90 targets were established by the Joint United Nations Programme on HIV/AIDS (UNAIDS) in 2014. The targets required: (1) 90% of all PLWH will be diagnosed by 2020, (2) 90% of all diagnosed PLWH will receive sustained ART by 2020; (3) 90% of all PLWH receiving ART will have viral suppression by 2020[10]. It is estimated that by achieving the 90-90-90 targets, 28 million total new infections and 21 million AIDS-related deaths will be averted during 2015-2030, and the global AIDS epidemic can be ended by 2030[10–12]. By the end of 2016, remarkable progress had been made towards the 90-90-90 targets: 70% of PLWH were diagnosed, 77% of diagnosed PLWH were receiving ART, and

82% PLWH on ART achieved viral suppression[13]. In Asia and the Pacific, where China is located, the three key numbers of the 90-90-90 targets were 71%, 66% and 83%, respectively[13].

In 2018, there were 23.3 million PLWH receiving ART, and the global ART coverage rate (%) reached over 60% among all PLWH, which increased by 150% compared to that in 2010[14]. In Asia and the Pacific, around 3.2 million PLWH were receiving ART in 2018, making the ART coverage rate reach 54%[14]. An important reason for ART coverage increasing is that the criteria for ART initiation have been modified a few times. As early as 2002, the World Health Organization (WHO) guidelines recommended that only the patients with clinical HIV disease (WHO stage III or stage IV) or CD4<200/μL should initiate ART[15]. In 2010 and 2013, the criteria for ART initiation were adjusted to CD4<350/μL and CD4<500/μL, respectively[16,17]. Recently, important studies[18–20] showed that initiating ART at a CD4 count level higher than 500/μL was significantly associated with better virological outcomes and less AIDS-related events, hence, the WHO guidelines recommended to start ART immediately after HIV diagnosis regardless of CD4 count[21].

After ART initiation, it is necessary to routinely monitor the treatment response. Viral load (VL) is the most important indicator of HIV disease progression and ART response[22–25]. Routine VL testing should be performed at 6 months and 12 months after ART initiation, and then at least every 12 months thereafter, so that the virological failure (two successive VL>1 000 copies/mL within a 3-months interval) can be detected timely[21]. Among the PLWH receiving HIV clinical care and have undergone VL tests, good virological outcomes are observed in low- and mid-income countries (LMIC). A study conducted in rural east Africa[26] reported that 90% of PLWH

treated had viral suppression (VL<500 copies/mL). However, there is still a gap between VL testing demand and VL testing coverage in LMICs. In sub-Saharan Africa, more than 6 million PLWH on ART do not have access to VL testing[27].

1.3 HIV/AIDS epidemic and ART response in China

The HIV virus entered China in the mid-1980s, and the first AIDS outbreak occurred in 1989 among 146 injection drug users (IDU) in Yunnan Province, which bordered Myanmar, Laos, and Vietnam and was near the "Golden Triangle"[28]. The HIV epidemic then spread from Yunnan Province to other parts of China through the drug distribution channels and the IDUs' sexual partners and children[29]. In the mid-1990s, the second AIDS outbreak occurred among the commercial plasma donors in east-central provinces[29,30]. By 1998, HIV had reached all the 31 provinces of mainland China[31]. After entering the 21st century, the total numbers of PLWH and the number of newly diagnosed HIV cases have kept growing year by year (Figure 1.1)[32–38]. In 2017, there were 134 512 newly reported HIV cases and brought the total number of PLWH to 758 610, which increased by 150% and 110% compared to those in 2010, respectively[32,37,38]. The

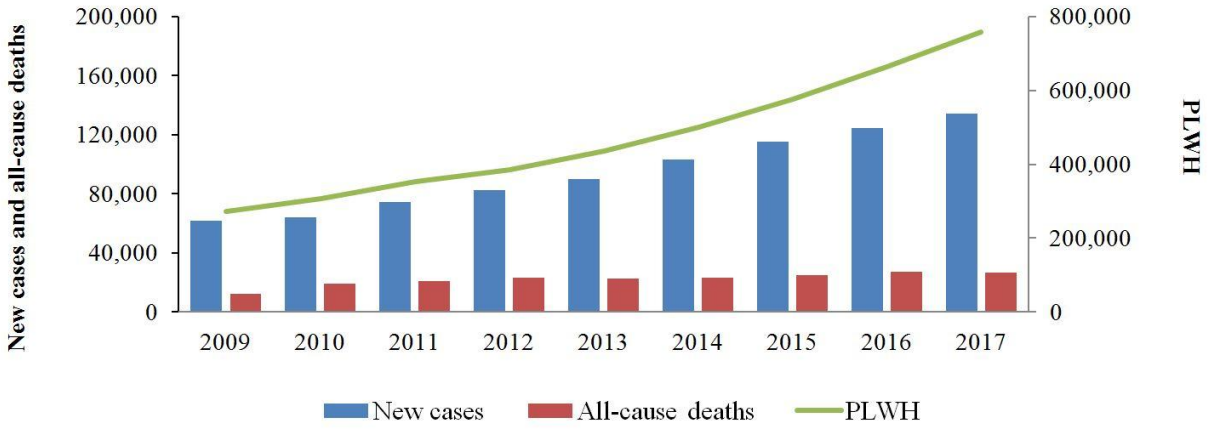

Figure 1.1 Newly diagnosed HIV cases, all-cause deaths and people living with HIV (PLWH) in China, 2009- 2017

number of all-cause death among PLWH in 2017 was 26 787, which only increased by 41% compared to the number in 2010[32,38].

Although the national HIV prevalence remains low (<0.1%), the distribution of PLWH in China is unbalanced. By 2014, three provinces (Yunnan, Sichuan, and Guangxi) had the highest HIV prevalence, and twelve provinces (Yunnan, Sichuan, Guangxi, Henan, Guangdong, Xinjiang, Chongqing, Guizhou, Hunan, Zhejiang, Jiangsu, and Beijing) had contributed more than 83% of the total HIV cases in the country[34]. IDU was once the most serious HIV transmission route before 2006, however, the proportion of sexual transmission had increased fast since 2005 (Figure 1.2)[39]. Currently, over 90% of new HIV cases are transmitted sexually. In 2016, 67.1% (N=83533) of all newly diagnosed HIV cases were transmitted by heterosexual contact and 27.6% (N=34 399) transmitted among men who have sex with men (MSM)[36].

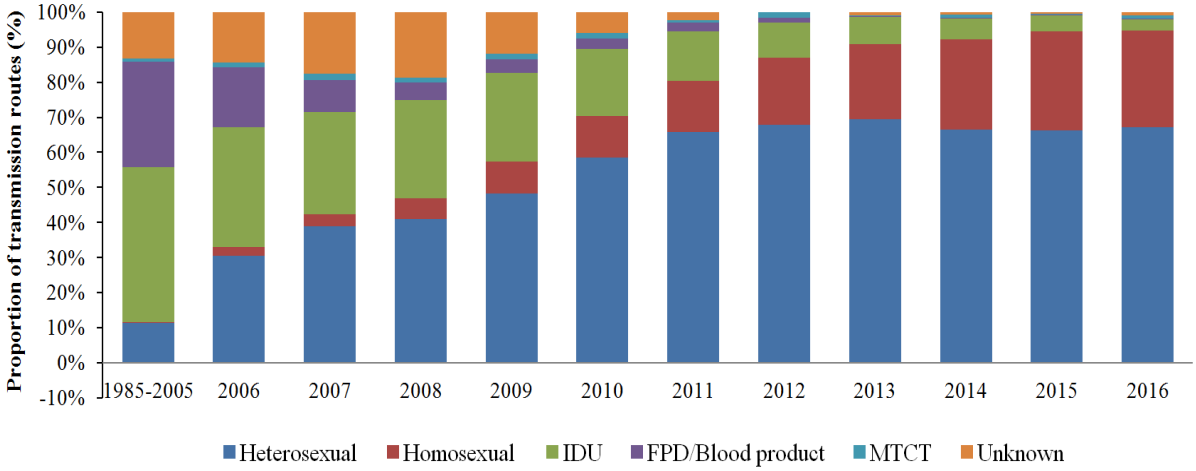

Figure 1.2 The transmission routes of newly reported HIV cases in China, 1985-2016. IDU: injection drug user; FPD: fresh dried plasma; MTCT: mother-to-child transmission;

China's National Free Antiretroviral Therapy Program (NFATP) was initiated among former plasma donors as a pilot project in 2002 and was officially launched in July 2005[39–41]. Free ART medications and HIV-related laboratory tests are provided to all eligible PLWH. In resource-limited settings, WHO recommends a public-health approach to provide ART, which means that only simplified and standardized first- and second-line ART regimen combinations are routinely provided[42]. Currently, six first-line ART medications and one second-line ART medication are provided for free in China, including zidovudine (AZT), lamivudine (3TC), tenofovir (TDF), efavirenz (EFV), nevirapine (NVP), abacavir (ABC), and lopinavir/ritonavir (LPV/r) (LPV/r is used in the second-line ART)[43]. The latest ART implementation policy in China requires initiating first-line ART as soon as possible after HIV diagnosis regardless of CD4 cell count[21,43]. To monitor the ART response and detect treatment failure timely, routine VL tests and CD4 tests should be performed at least once a year. PLWH who fail the first-line ART can be considered to switch to a second-line ART regimen. By the end of 2017, the average ART coverage in China was over 80%[44]. National cohort studies[45,46] reported that the rates of viral suppression (VL<400 copies/mL) ranged from 89% to 91% among PLWH receiving ART.

1.4 VL and VL tests

VL is the preferred approach to monitor ART response and detect treatment failure. The definition of virological failure varies in different guidelines. In some well-resourced settings, a strict approach to define virological failure is VL>50 copies/mL[47]. Instead, the WHO guideline, which is usually applied in LMICs, defines the virological failure as two consecutive VL>1 000 copies/mL within a 3-month interval with adherence support between the two measurements[21].

High HIV viral load is significantly associated with early progression to AIDS, higher mortality, slower viral suppression, and higher HIV transmission rate[48-49].

The assays for quantification of HIV VL require very elaborate lab infrastructure, such as separate rooms for different test processes, temperature control, continuous power, and water and minimal dust[50,51]. Generally, plasma samples, which are obtained after venous blood collection and centrifugation, are used in the VL testing. Viral load assays can be divided into nucleic acids-based test (NAT) and non-acids-based test (non-NAT) technologies (Figure 1.3)[50]. The NAT technologies can directly detect and quantify HIV viral RNA, whereas, the non-NAT technologies detect and quantify HIV viral proteins[50,52]. Generally, there are three steps for VL assays with NAT technologies[50,51]. The first step or pre-amplification step includes sample preparation and nucleic acid extraction. The second step is nucleic acid amplification since the volume of nucleic acids is generally too low to be detected directly. The two main amplification methods for VL detection are target amplification and signal amplification. The target amplification can synthesize more "target nucleic acids" so that even very low-level nucleic acids can be detected. Target amplification mainly includes reverse transcription-polymerase chain reaction (RT-PCR) (e.g. Abbott RealTime and Roche COBAS) and nucleic acid sequence-based amplification (NASBA) (e.g. bioMerieux NucliSENS EasyQ). In the signal amplification, large amounts of signals, which can be detected in the detection process, are attached to the original target nucleic acids. One commonly used technique of signal amplification is branched-chain DNA (bDNA) (e.g. Siemens Versant™). The third step of VL testing is the detection and quantification of amplified products or amplified signals. The non-NAT-based technologies

detect and quantify HIV-specific enzymes and proteins, including reverse transcriptase and p24 antigen[50,52].

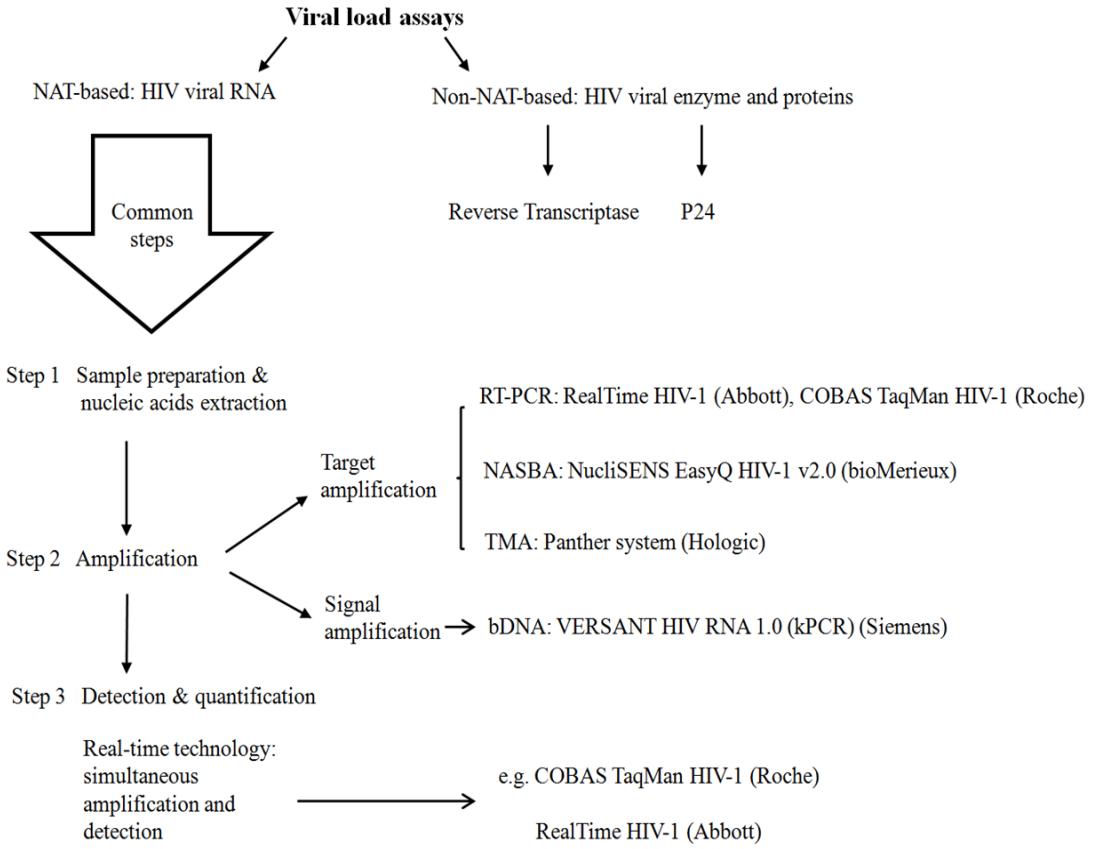

Figure 1.3. Classification and process of viral load assays. NAT: nucleic acids-based test; RT-PCR: reverse transcription-polymerase chain reaction; NASBA: nucleic acid sequence-based amplification; RT-TMA: transcription-mediated amplification; bDNA: branched DNA

1.5 VL tests in LMICs

VL testing needs high-quality infrastructure, sophisticated laboratory equipment, and well-trained technicians[50,53]. In some real-world settings of LMICs, VL testing assays are only centralized in the city- or higher level laboratories, hence, centralized laboratory-based approaches are used for VL tests (Figure 1.4)[54]. PLWH who are retained in ART need to undergo

routine VL tests. Generally, they can visit local ART sites to donate VL testing blood samples. The samples are processed and temporarily stored in the local ART sites. Different local ART sites send blood samples to the same central VL laboratory periodically for VL testing. The technicians in the laboratory are in charge of performing VL tests, and then return the testing reports to the original ART sites. After receiving the reports, the healthcare providers in local ART sites can implement clinical interventions according to the reports and inform PLWH of the VL results. However, in some real-world settings, because of a shortage of healthcare providers and a lack of physician-patient communication, some patients cannot be informed of VL testing results until the next routine ART follow-up visits. Hence, some patients may tend to take the initiative to ask about VL testing results before patient notifications.

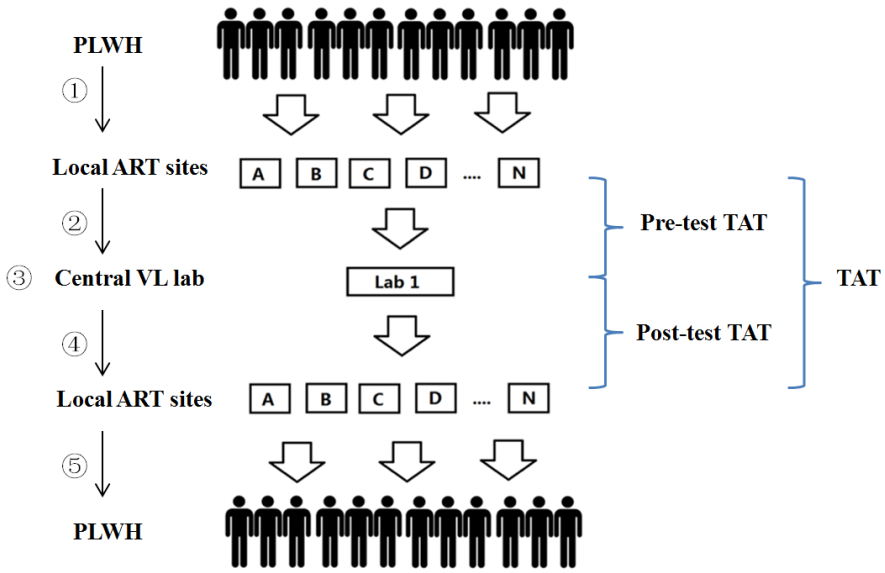

Figure 1.4 The general processes of centralized viral load test and the definition of turnaround time. (1) People living with HIV (PLWH) go to local antiretroviral therapy (ART) sites to donate blood samples; (2) Different ART sites send samples periodically to the central viral load (VL) laboratory; (3) VL tests are performed in the central VL laboratory; (4) Testing results are sent back to the local ART sites; (5) Healthcare providers in the local ART sites inform PLWH of the testing results. TAT: turnaround time.

The time interval between VL blood sample collection and receipt of testing reports by the ART sites is defined as turnaround time (TAT) of the VL test. Specifically, the pre-test TAT refers to the time interval between blood sample collection and VL testing, and the post-time TAT refers to the time interval between VL testing and receipt of VL results by local ART sites (Figure 1.4). The TAT varies considerably among different countries. Studies in well-resourced settings reported the median VL testing TATs ranging from 1 to 8 days[55–57]. A study conducted in seven Sub-Saharan countries reported that the mean TAT in each country ranged from 3 days to 50 days, and the mean TATs in five of the seven countries were close to or longer than 1 month[58,59]. Factors associated with prolonged TAT in LMICs are various. The pre-test TAT can be influenced by weak health and laboratory systems, incomplete sample referral network, and a

shortage of trained staff; and the post-test TAT is mainly influenced by immature results reporting systems[27,58,60–62]. Prolonged TAT results in delayed detection of treatment failure, which further leads to delayed clinical interventions among PLWH with treatment failure. Staying at an unsuppressed VL level for a long time is associated with higher mortality rates, more opportunistic infections, and higher HIV transmission rates[63–67].

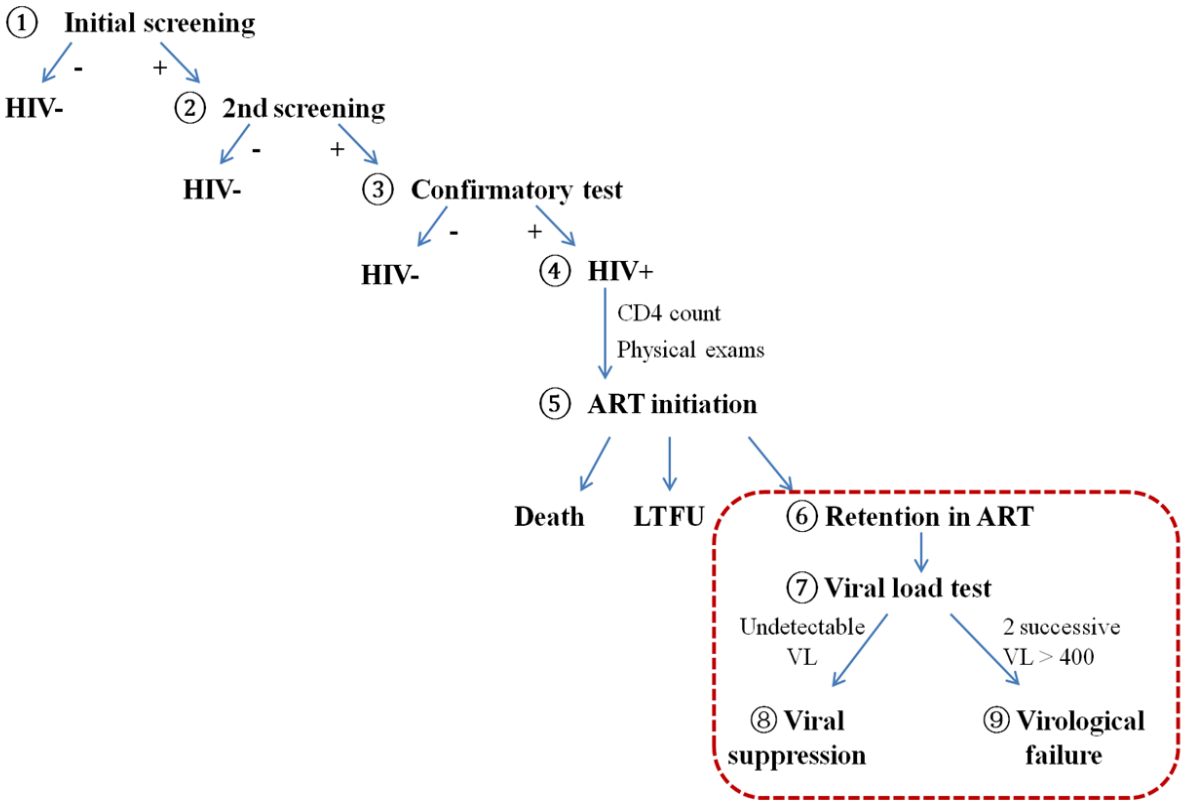

Figure 1.5 HIV care cascade from initial HIV screening to viral load test.
ART=antiretroviral therapy. LTFU=loss to follow-up. VL=viral load (copies/mL).

1.6 VL tests in China

The general HIV care cascade in China is shown in Figure 1.5[43,68–70]. When a patient has positive results in both the first and the second HIV screenings and the HIV confirmatory test, he/she will be diagnosed as HIV infection and should initiate lifelong ART regardless of CD4 cell count[21] (step ①-⑤). After ART initiation, PLWH go on follow-up visits to local ART sites every three or four months for a refill of ART medications and to undergo laboratory tests. Some patients are dead or lost to follow-up (LTFU) during early ART. A nationwide cohort study[46] of the Chinese PLWH reported that on average only 84% of patients were retained in ART for longer than 6 months. Among those who have been retained in ART for longer than 6 months, VL tests will be performed to evaluate the treatment response (step ⑥-⑦). According to WHO guidelines and current Chinese standard practices, the first VL test is usually performed between 6 months and 12 months after ART initiation[21,43]. Based on the Chinese ART guidelines, the viral suppression or virally suppressed is defined as VL<20 copies/mL or undetectable VL and the virological failure is defined as two successive VL>400 copies/mL, which are stricter than the definitions recommended by WHO (step ⑦-⑨)[21,43].

VL testing has been provided in China since 2006 and scaled up since 2008[71]. By 2015, there were about 4 000 healthcare facilities providing ART service around the country, however, only 181 laboratories qualified to perform VL tests[34,72]. In addition, the VL testing laboratories are usually located in the capital region of a city or a province. Hence, the centralized laboratory-based strategy is used for VL tests in China, especially in remote and rural areas. A nationwide serial cross-sectional study among Chinese PLWH[46] reported the coverage of annual VL testing

among PLWH retained in care had increased from 50% in 2009 to 84% in 2015. However, a cohort study[73] in rural China reported only 68% of PLWH had VL tests in their first year of ART. The VL testing TAT in rural China may vary from several weeks to months, however, there has been little published data about this issue. Studies[45,46] reported that the average viral suppression (VL<400 copies/mL) rate among PLWH receiving sustained ART in China had approximately reached 90%.

1.7 Study location: Wenshan Prefecture, Yunnan Province

To investigate the centralized VL testing among PLWH in rural China, Wenshan Prefecture in Yunnan Province was selected as the study location. Yunnan Province is located in Southwest China and borders the countries Vietnam, Laos, and Myanmar. In 2018, the gross domestic product (GDP) of Yunnan was about 250 billion US dollars, ranking the 20th of the 31 provinces in mainland China[74]. Yunnan had a population of 48 million at the end of 2017, among which 53.3% are living in the rural areas[74]. The earliest HIV infected cases in China appeared among the IDUs in the border areas of Yunnan Province in the late 1980s[28]. By 1998, the HIV infection had spread from Yunnan Province to all other provinces of mainland China[31]. Currently, Yunnan is one of the provinces with the most serious HIV/AIDS epidemic and which pioneered ART implementation[34,75]. The cumulative number of PLWH in Yunnan accounted for 21% of the total number in China[76]. The main HIV transmission route has changed from injection drug use to heterosexual contact[76,77], which accounted for over 80% of newly diagnosed cases[75]. By the end of 2016, twenty-two laboratories in Yunnan were capable of VL testing[78].

Wenshan Zhuang and Miao Autonomous Prefecture ("Wenshan" or "Wenshan Prefecture" for short) is located in the eastern mountainous region of Yunnan Province. The total population of Wenshan was about 3.6 million by 2017, among which 61% were living in rural areas[78,79]. The GDP per capita of Wenshan in 2017 was about 3 400 US dollars, which was 35% and 62% lower than the provincial and national averages, respectively[79–81]. Han ethnicity, China's main ethnicity, only accounted for 42.0% of the Wenshan population; Zhuang, Miao, Yi, and other ethnic minorities accounted for 58.0%[79]. Wenshan is one of the regions with the largest number of PLWH within the province[82]. The number of PLWH receiving ART in 2017 was about 8 900. Nine county-level facilities and one prefecture-level facility provide ART and laboratory tests to all PLWH in the prefecture. However, only one central laboratory is eligible to perform VL tests in this prefecture.

1.8 Study aims

The third 90-90-90 target, which required 90% of PLWH receiving ART to achieve viral suppression, was focused on in this dissertation. Three topics during the process of centralized VL testing in rural China were investigated. The first topic was the coverage of timely VL tests among PLWH receiving ART; the second topic was the TAT of the centralized VL testing; the third topic was the association between active concern for VL testing results and the long-term virological outcomes, i.e. SVS.

The specific aims of this study included:

(1) To assess the coverage and timeliness of VL test and identify factors associated with delayed VL testing;

(2) To investigate the current VL testing TAT under the centralized testing strategy in rural China and identify factors associated with prolonged TAT.

(3) To examine whether PLWH's active concern for VL testing results is associated with their SVS status and identify other factors associated with SVS.

(4) To provide suggestions for improving VL testing service and achieving 90-90-90 targets in rural China

1.9 Summary of study methods

Three ART sites and the only central VL testing laboratory in Wenshan Prefecture were selected as the study sites of our study. As shown in Figure 1.6, topic 1 (the coverage and timeliness of VL test) only involved the process of VL blood sample collection; topic 2 (VL testing TAT) involved all processes from blood sample collection to receipt of VL testing reports; topic 3 (association between active concern for VL testing results and SVS) involved the process of patient notification. To address the three topics, three studies were conducted in the study sites.

Study 1:

Parts of PLWH could not undergo VL tests timely after ART initiation. To understand the coverage and timeliness of VL testing and identify factors associated with timely VL tests, a clinic-based retrospective cohort study was conducted among PLWH who initiated ART from 2015 and 2016 in the three ART sites. Each participant was followed up from ART initiation to the date of first valid VL test, death, loss to follow-up (LTFU), transferring to other ART facilities or Dec 31, 2018, whichever came first. Basic characteristics were extracted from medical records. A survey was conducted among a subset of PLWH to supplement more detailed

information. The cumulative rate (%) of VL testing at 12, 18 and 24 months after ART initiation was calculated. Cox proportional hazard models were used to identify factors associated with the time of VL testing.

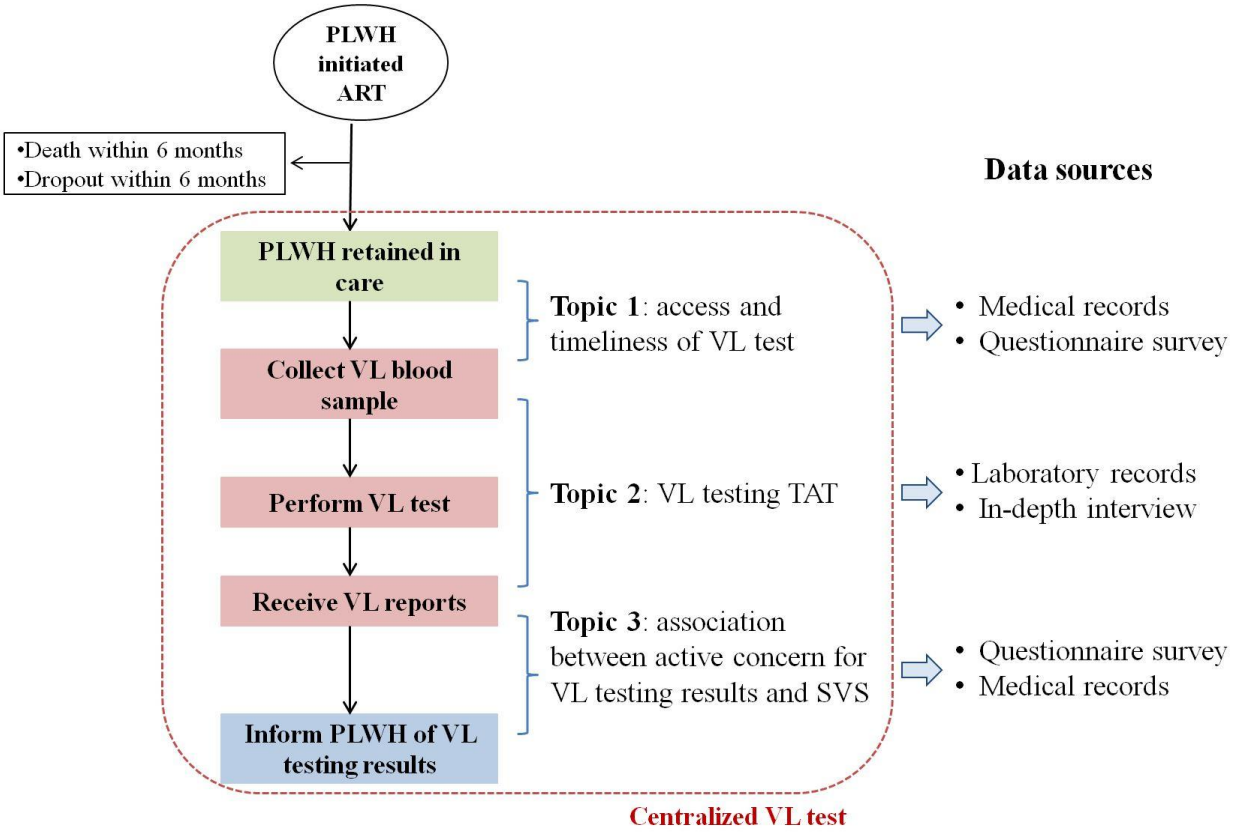

Figure 1.6 Summary of the three sub-study topics and the main data sources
PLWH: people living with HIV; ART: antiretroviral therapy; VL: viral load; TAT: turnaround time; SVS: sustained viral suppression. Dropout was defined as loss to follow-up or discontinuation of ART

Study 2:

The VL testing TAT in rural China was prolonged. To investigate the TAT of centralized VL testing in rural settings, a sequential explanatory mixed-method study was conducted in the three ART sites and the central VL testing laboratory. The study contained a quantitative phase and a subsequent qualitative phase. In the quantitative phase, individual-level TAT was calculated for

all VL blood samples collected in 2018. In the qualitative phase, in-depth interviews were conducted among healthcare providers in the ART sites and technicians in the central VL testing laboratory to identify factors associated with prolonged TAT.

Study 3:

Because of the prolonged TAT and shortages of healthcare providers, some PLWH could not be informed of the VL testing results until long after the VL blood sample collection, so that some of the patients tend to take the initiative to ask about the results before patient notification. To investigate whether active concern for VL testing results is associated with SVS, a clinic-based study was conducted in the three ART sites. PLWH who initiated ART during 2015 and 2016 in these three facilities were recruited to participate in a survey during Jan to Mar in 2019, which collected information about socio-demographic characteristics, VL testing experience, VL-related knowledge, and family/social support using structured questionnaire. In addition, clinical characteristics and VL testing results were extracted from medical records. The rates (%) of viral suppression and SVS at the end of 2018 were calculated. Logistic regressions were conducted to investigate the association between actively asking about VL testing results and SVS, and to identify other factors associated with SVS.

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Chapter 2: Timeliness of viral load tests among people living with HIV on antiretroviral therapy in Wenshan, China

Abstract

Objective: To understand the coverage and timeliness of viral load (VL) testing and identify factors associated with the timing of VL tests among people living with HIV (PLWH) in Wenshan Prefecture, Yunnan Province of China.

Methods: A clinic-based retrospective cohort study was conducted among PLWH who initiated ART between Jan 1, 2015 and Dec 31, 2016 in Wenshan. Each participant was followed up from ART initiation to the date of the first valid VL test, death, loss to follow-up (LTFU), transferring to other ART facilities or Dec 31, 2018, whichever came first. Basic characteristics were extracted from medical records. A survey was conducted among a subset of PLWH to supplement more detailed information. The cumulative rate (%) of VL testing at 12, 18 and 24 months after ART initiation was calculated. Cox proportional hazard models were used to identify factors associated with VL testing timing.

Results: A total of 815 PLWH (source cohort) were recruited, among whom 264 (survey cohort) participated in a survey to collect additional information. In the source cohort, the median age was 42 years (IQR: 32-51), and 60.3% were male. About 94.5% PLWH reported they were infected with HIV through heterosexual contacts. The median CD4 cell count at ART initiation was 316/μL (IQR: 199-447). The cumulative VL testing rates (%) at 12, 18, and 24 months of ART were 58.5%, 78.1%, and 93.0%, respectively. Factors associated with early VL testing

included being older than 45 years (45-60 years: aHR=1.23, CI=1.03-1.48; >60 years: aHR=1.34, CI=1.07-1.70), WHO stage I & II at ART initiating (aHR=1.40, CI=1.18-1.65), initiating ART in 2016 (aHR=1.32, CI=1.14-1.53) and receiving ART at study Site C (aHR=2.20, CI=1.87-2.60). Additionally, multivariate analyses in the survey population (n=264) showed that living farther than 2-hours drive from the ART site (aHR=0.70, CI=0.59-0.83) and had a permanent job (aHR=0.71, CI=0.56-0.90) were associated with undergoing VL testing late.

Conclusions: To improve VL testing timeliness in rural areas, PLWH who are young, live far away from ART facilities, and are at advanced WHO clinical stage should be targeted. HIV health service decentralization in remote rural areas is highly recommended. Efforts of patients, health service providers, governments and non-governmental organizations (NGOs) should be coordinated.

Keywords: HIV/AIDS, antiretroviral therapy, viral load test, timeliness

Introduction

Viral load (VL) is the most important indicator of disease progression in HIV infection[1,2]. To monitor treatment response, VL should be routinely tested on people living with HIV (PLWH) after initiating antiretroviral therapy (ART)[3]. VL test can provide early detection of virologic failure and prompt timely adherence counseling and regimen switching for patients with unsuppressed VL[4–7]. The Joint United Nations Programme on HIV/AIDS (UNAIDS) established 90-90-90 targets for ending the AIDS epidemic, which required 90% of PLWH on ART to achieve viral suppression[8]. One of the prerequisites for those targets was universal access to VL

testing. However, the gap between VL testing demand and VL testing coverage in recourse-limited settings has still been huge. In sub-Saharan Africa, more than 6 million PLWH on ART do not have access to VL testing[7].

VL testing has been scaled up in China since 2008[9]. By 2015, there were 181 laboratories qualified to perform VL tests, covering all provinces across the country[10,11]. China's National Free ART Program (NFATP) provides free VL tests once a year to all PLWH after 6-month continuous ART[12,13]. Patients go on follow-up visits to ART clinics every three or four months for a refill of ART medications and to undergo laboratory testing. A nationwide serial cross-sectional study in Chinese PLWH[14] reported the coverage of annual VL testing among PLWH retained in care had increased from 50% in 2009 to 84% in 2015. According to WHO guidelines and current Chinese standard practices, the first VL test is usually performed between 6 months and 12 months after ART initiation[3,15]. Missing VL monitoring within the first year of ART is associated with worse retention in care, faster disease progression and increased mortality[16–18]. However, a cohort study[18] in rural China reported only 68% of PLWH had VL tests in their first-year on ART.

Reasons for lack of or delayed VL testing can be multifaceted. Structural factors may include insufficient laboratory network system, centralized VL testing strategy, shortage of professionals and limited staff training[4,19,20]. Patient individual-level characteristics have influences on the utilization of healthcare service, which further affect the coverage and timeliness of VL testing. HIV transmission route, CD4 cell count, WHO stage, ART regimen and awareness of VL benefits are all reported as individual-level factors[4,21–23].

Yunnan has been one of the provinces with the most serious HIV/AIDS epidemic and which pioneered ART implementation[10,24]. The cumulative number of PLWH in Yunnan accounted for 21% of the total number in China[25]. The main HIV transmission route has changed from injection drug use (IDU) to heterosexual contact[25,26], which accounted for over 80% of newly diagnosed cases[24]. By the end of 2016, twenty-two laboratories in Yunnan were capable of VL testing, however, only one was located in and provided service for Wenshan Prefecture[27]. Wenshan is located in a mountainous region, where traffic and economy have not been fully developed[28–31]. About 61% of the population is living in rural areas[28,29]. Han ethnicity, China's main ethnicity, only accounted for 42.0% of the Wenshan population; Zhuang, Miao, Yi, and other ethnic minorities accounted for 58.0%[29]. Wenshan is one of the regions with the largest number of PLWH within the province. Nine county-level and one prefecture-level facilities provide ART and laboratory tests to all PLWH in the prefecture. To our knowledge, there have been few studies focusing on the access to VL testing in China's rural areas. To assess the coverage and timeliness of VL test and identified specific factors associated with lack of or delayed VL testing, we did a clinic-based retrospective cohort study in Wenshan. Data used in our study were extracted from medical records and a survey conducted among PLWH.

Methods

Study sites

Three of the ten ART clinics in Wenshan Prefecture were selected as our study sites (Site A, B, and C). Site A was attached to a county-level hospital and had the largest number of PLWH receiving ART in Wenshan. Site B was attached to a prefecture-level hospital, where the only

VL testing laboratory was located. Site C was a county-level independent specialized HIV/AIDS care center with the third largest number of PLWH.

Study design

A clinic-based retrospective study was conducted among a cohort of PLWH who initiated ART between Jan 1, 2015 and Dec 31, 2016 in ART sites A, B and C. Each participant was followed up from ART initiation to the date of the first valid VL test, death, loss to follow-up (LTFU), transferring to other ART facilities or Dec 31, 2018, whichever came first. To supplement more necessary information, a survey was conducted among a subset of participants from Jan to Mar 2019. Cox proportional hazard models were used to identify factors associated with the VL testing timing.

Enrollment

There were two study cohorts. Cohort 1, or the source cohort, included PLWH who (a) initiated ART from Jan 1, 2015 to Dec 31, 2016, (b) initiated ART in Site A, B and C, (c) were aged≥18 years when initiated ART, (d) retained in care for more than 6 months (Figure 2.1). Cohort 2, or the survey cohort, was a subset of the source cohort. A survey was conducted from Jan to Mar 2019 in our three study sites. Criteria for the survey cohort included (a) belonging to the source cohort, (b) visiting the study ART sites during the three-month survey period. When patients visited the study sites during the survey period, the local healthcare providers first preliminarily checked their medical records for eligibility of the source cohort. For eligible patients, the staff then simply introduced the study and invited them to participate in the survey. Since PLWH was a sensitive population, the healthcare providers were in charge of making the initial screen and

contact with patients on behalf of the investigator. If the potential participants agreed to participate, they would be referred to the investigator. The investigator then double-checked the eligibility and conducted a questionnaire survey to the eligible ones. Oral informed consent was obtained before the survey (Figure 2.1).

Data collection

There were two data sources: medical records in ART sites and the survey. From medical records, three types of data were extracted, including important dates (e.g. dates of ART initiation and VL tests), basic socio-demographic information (e.g. gender, age, marital status, ethnicity, and education level), and clinical characteristics (e.g. HIV infection route, baseline CD4 cell count, and WHO clinical stage). All VL testing dates from ART initiation to Dec 31, 2018 were extracted. Data were extracted at the end of 2019 March by healthcare providers who worked in the ART sites and had access to medical records.

The survey was conducted from Jan to Mar 2019 in ART Sites A, B and C using a structured questionnaire. After being recruited in the survey, participants filled out the questionnaire under the investigator's guidance on the same day they visited the ART sites. If the participant was not able to read, the investigator would do a face-to-face survey and fill out the questionnaire according to the participant's answer. Characteristics during the first year of ART were collected for this study, mainly including socio-demographic information (e.g. occupation, location of home and distance from home to ART site). The patient ID was collected to link survey data to medical records, and it was deleted once data were linked. It took 5-10 minutes to complete a

questionnaire. After the survey, each participant received a gift equivalent to 20 Yuan (about 3 USD).

Measurements of VL testing timeliness

According to current standard practice in China, VL testing is not provided before or at the very beginning of ART. Viral load tests within 3 months after ART initiation were excluded since the duration of ART might not be enough to evaluate treatment response. VL tests after 3 months of ART were regarded as valid VL tests. Two measurements were used to assess the timeliness of VL testing. The first measurement was the cumulative rate (%) of valid VL testing at 12 months after ART initiation. The second measurement was VL testing event, which was defined as the first valid VL test after ART initiation. Patients were censored at death, LTFU, transferring to other ART facilities or on Dec 31, 2018. The time of VL testing in our study referred to the date of blood sample collection.

Data analysis

Descriptive analyses were conducted to describe the characteristics of both the source cohort and the survey cohort. Categorical variables were compared by Chi-square tests, and continuous variables were compared by Wilcoxon test. The cumulative rates (%) of having VL test at 12, 18 and 24 months after ART initiation were calculated in both cohorts. VL testing rate (%) at 12 months would be presented by three ART sites. Time to VL testing event was calculated as the difference between the date of ART initiation and the date of first valid VL testing or censored date (person-year [PY]). Cox proportional hazard models were used to identify factors associated

with VL testing timing. Hazard ratio (HR), 95% confidence interval (CI) and p-value were presented. Data were analyzed using SAS 9.4 software (SAS Institute, Cary, NC, US)

Ethics

The study was reviewed and approved by the Institutional Review Board (IRB) of University of California, Los Angeles (UCLA) in the U.S. and by National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Center and Prevention (China CDC).

Results

Figure 2.1 shows the source cohort development and the sampling strategy for the survey cohort. A total of 911 PLWH initiated ART from Jan 1, 2015 to Dec 31, 2016 in the three selected ART sites, and 815 adult PLWH (89.5%) were retained in care for at least 6 months, with 425 in Site A, 124 in Site B and 266 in Site C. Patients who visited the three ART sites during 2019 Jan to Mar and belonged to the source cohort were eligible to be invited to participate in the survey. The numbers of patients who were screened and invited were not recorded by the healthcare providers. There were 293 PLWH accepted the invitation and were referred to the investigator. After double-checking for eligibility, 264 (90.1%) of those patients were included in the survey cohort, with 85 from Site A, 84 from Site B and 95 from Site C. The proportions of PLWH who participated in the survey were 20% at Site A, 67.7% at Site B and 35.7% at Site C, which were significantly different across three study sites ($p<0.001$). Sampling weights were added in the Cox model to correct the unbalance.

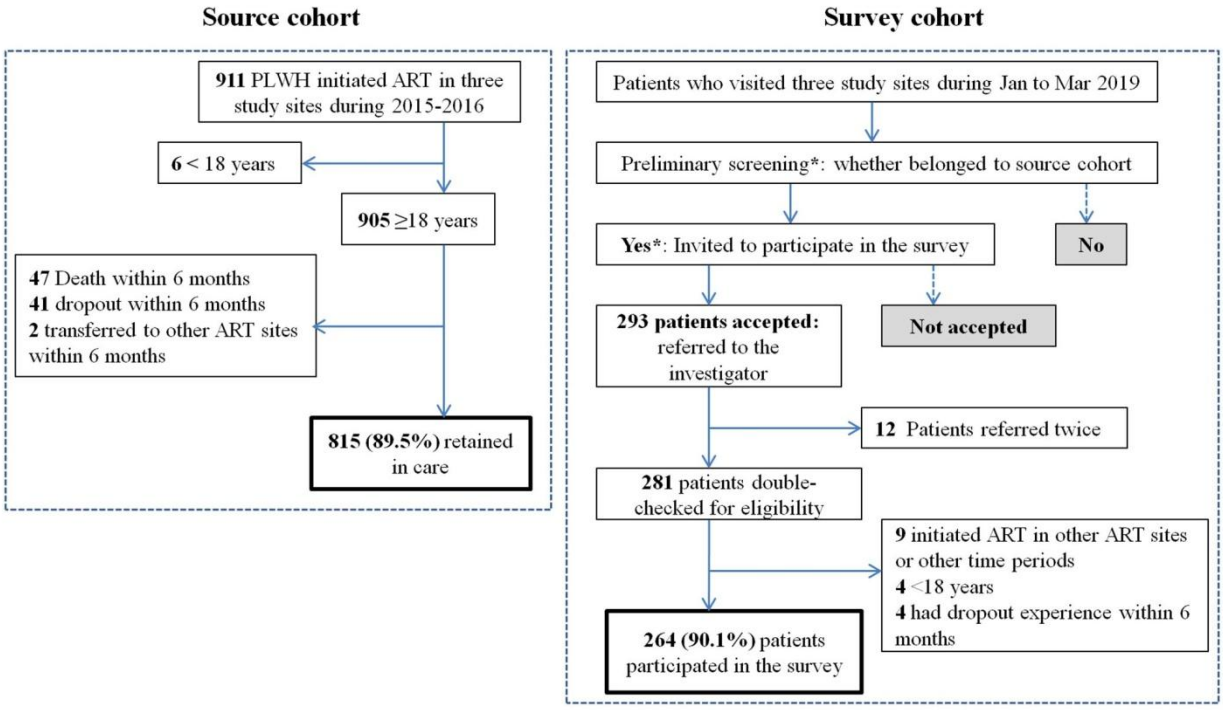

Figure 2.1 Flowchart: the development of source cohort and survey cohort
ART=antiretroviral therapy. PLWH: people living with HIV. Dropout was defined as loss to follow-up or discontinuation ART. The survey cohort (n=264) was a subset of the source cohort (N=815).
*****: The numbers of patients who were preliminary screened by the healthcare providers and invited to participate in the survey was not recorded.

Characteristics of the source cohort and the survey cohort were shown in Table 2.1. In the source cohort, the median age was 42 years (IQR: 32-51), and 60.3% were male. About 94.5% PLWH reported they were infected HIV through heterosexual contacts. About 48% of participants were illiterate or at elementary school level education. The median CD4 cell count at ART initiation was 316/μL (IQR: 199-447). The basic demographic and clinical characteristics of PLWH participated in or not participated in the survey were comparable, except that more PLWH in the survey cohort were at WHO stage I & II than the population who didn't participate in the survey (80% vs. 72%, p=0.019).

Table 2.1 Basic demographic and clinical characteristics of source cohort (N=815), survey cohort (n= 264) and PLWH who didn't participate in the survey (n=551)

Characteristics	Total cohort (n%)	PLWH participated survey (n%)	PLWH not participated survey (n%)	P-value*
Overall	815 (100.0)	264 (100.0)	551 (100.0)	
ART site				
Site A	425 (52.2)	85 (32.2)	340 (61.7)	**<0.001**
Site B	124 (15.2)	84 (31.8)	40 (7.3)	
Site C	266 (33.6)	95 (36.0)	171 (31.0)	
Gender				
Male	491 (60.3)	153 (58.0)	338 (61.3)	0.355
Female	324 (39.7)	111 (42.0)	213 (38.7)	
Age at ART initiation, years				
Median (IQR)	42 (32, 51)	42 (32, 51)	42 (32, 52)	0.574
18-30	167 (20.5)	52 (19.7)	115 (20.9)	0.501
30-45	321 (39.4)	110 (41.7)	211 (38.3)	
45-60	215 (26.4)	72 (27.3)	143 (26.0)	
>60	112 (13.7)	30 (11.4)	82 (14.9)	
Marital status				
Single	230 (28.2)	81 (30.7)	149 (27.0)	0.280
Not single	585 (71.8)	183 (69.3)	402 (73.0)	
Ethnicity				
Han	478 (58.7)	151 (57.2)	327 (59.4)	0.560
Minority	337 (41.3)	113 (42.8)	224 (40.6)	
Education level				
Illiteracy & elementary school	389 (48.0)	124 (47.2)	265 (48.4)	0.171
Middle school	295 (36.4)	89 (33.8)	206 (37.6)	
High school & above	127 (15.6)	50 (19.0)	77 (14.0)	
Occupation**				
Farmer	--	54 (20.5)	--	--
Permanent job	--	40 (15.2)	--	
Temporary job	--	131 (49.6)	--	
No job, housework or others	--	39 (14.7)	--	
Living in the same county with their ART site**				
Yes	--	195 (75.3)	--	--
No	--	64 (24.7)	--	
Distance between home and ART site**				
<2-hour drive	--	156 (59.1)	--	--
>2-hour drive	--	108 (40.9)	--	
Time of ART initiation				
2015	362 (44.4)	128 (48.5)	234 (42.5)	0.106

2016	453 (55.6)	136 (51.5)	317 (57.5)	
Route of HIV transmission				
Heterosexual behaviors	770 (94.5)	252 (95.5)	518 (94.0)	0.398
Other	45 (5.5)	12 (4.5)	33 (6.0)	
CD4 cell count/µL when ART initiated				
Median (IQR)	316 (199, 447)	321 (210, 452)	314 (193, 443)	0.620
<200	238 (29.2)	72 (27.3)	166 (30.1)	0.532
200-350	242 (29.7)	82 (31.1)	160 (29.0)	
350-500	207 (25.4)	63 (23.9)	144 (26.1)	
>500	128 (15.7)	47 (17.8)	83 (14.7)	
WHO clinical stage when ART initiated				
Stage I & II	606 (74.4)	210 (79.6)	396 (71.9)	**0.019**
Stage III & IV	209 (25.6)	54 (20.4)	155 (28.1)	

*: Chi-square test for categorical variables; Wilcoxon rank-sum test for continuous variables

**: Only PLWH who participated the survey provided these information

PLWH: people living with HIV, ART: antiretroviral therapy

Cumulative VL testing rate (%)

Among 815 PLWH in the source cohort, 1 (0.1%) person died, 5 (0.6%) transferred to other ART facilities, 20 (2.5%) were lost to follow-up, and 2 (0.2%) didn't have VL test throughout the observed period. Finally, 787 (96.6%) took at least one valid VL test during our observed period, and 58.5%, 78.1%, 93.0% had VL test by 12, 18, and 24 months of ART, respectively. The median time from ART initiation to the first valid VL test was 10.7 months (IQR: 7.8-16.5). The cumulative VL testing rates in the source population are shown by ART sites in Figure 2.2a. The cumulative rate (%) of VL test at 12 months was highest for Site C (73.3%), followed by site B (61.3%), and lowest for Site A (48.5%), p<0.001. At 24 months of ART, the overall VL testing coverage was 93.0%, with no significant difference among the three ART sites (p=0.480).

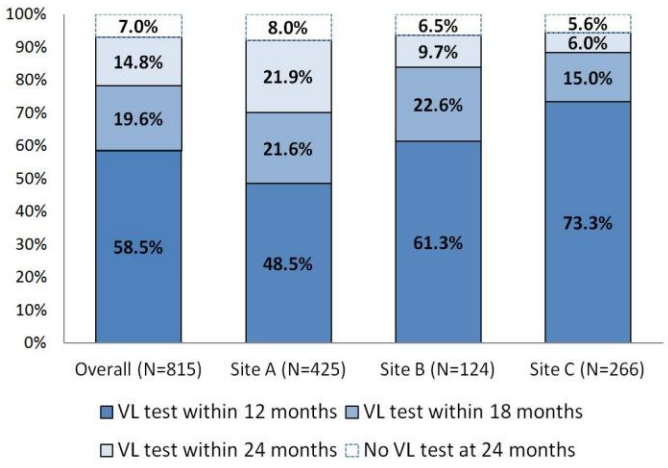

Figure 2.2a Cumulative rates of VL testing at 12, 18 and 24 months after ART initiation: source cohort (N=815)

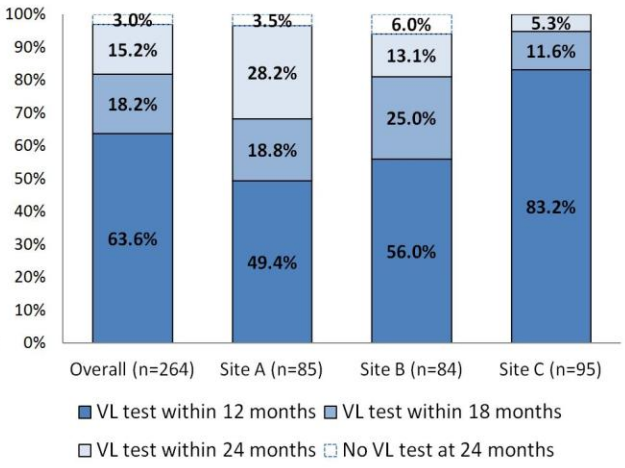

Figure 2.2b Cumulative rates of VL testing at 12, 18 and 24 months after ART initiation: survey cohort (n=264)

Among 264 people in the survey cohort, only 1 (0.4%) was LTFU and 263 (99.6%) had VL tests during the observed period, among whom 63.6%, 81.1%, 97% took VL test by 12, 18, and 24 months of ART, respectively. The median timing of the first VL test was 10.1 months (IQR: 7.3, 15.6). Similar to the source cohort, the cumulative VL testing rates (%) by 12 months in ART Site C was significantly higher than the rates in Site A and B. (Figure 2.2b).

Stratified analysis by three ART sites

Table 2.2a showed participants' characteristics and VL testing rates (%) at 12 months by study sites in source cohort (N=815). Participants in Site C tended to be younger (p=0.009), be ethnic minorities (p<0.001), have a lower education level (p<0.001) and higher CD4 cell count at ART initiation (p<0.001). The cumulative VL testing rates (%) at 12 months in Site A, B and C were different by age group (*18-30* group: p<0.001; *30-45* group: p=0.001; *45-60* group: p=0.002; *>60* group: p=0.557), marital status (*Single* group: p=0.212; *married* group: p<0.001), education level (*Illiteracy & elementary school* group: p=0.008; *Middle school* group: p<0.001; *High school & above* group: p=0.076), baseline CD4 cell count (*<200/μL* group: p=0.374; *200-350/μL* group: p<0.001; *350-500/μL* group: p=0.001; *>500/μL* group: p=0.015) and baseline WHO clinical stages (*Stage I & II* group: p<0.001; *Stage III & IV* group: p=0.248).

Table 2.2a. Participant characteristics by ART site and rates of taking VL test within 12 months after ART initiation: source cohort (N=815)

	Participant characteristics by ART sites, (n%)				VL testing rates at 12 months after ART initiation, %				
Characteristics	**Site A**	**Site B**	**Site C**	**P-value**	**Overall**	**Site A**	**Site B**	**Site C**	**P-value**
Overall	425 (100.0)	124 (100.0)	266 (100.0)		58.5	48.5	61.3	73.3	**<0.001**
Gender									
Male	255 (60.0)	69 (55.7)	167 (62.8)	0.402	59.9	52.6	59.4	71.3	**<0.001**
Female	170 (40.0)	55 (44.4)	99 (37.2)		56.5	42.4	63.6	76.8	**<0.001**
Age at ART initiation, years									
Median (IQR)	43 (33, 54)	42 (32, 50)	39 (30, 49)	**0.009**					
18-30	78 (18.4)	22 (17.7)	67 (25.2)	**0.030**	61.1	42.3	63.6	82.1	**<0.001**
30-45	155 (36.5)	55 (44.4)	111 (41.7)		54.8	44.5	60.0	66.7	**0.001**
45-60	121 (28.5)	32 (25.8)	62 (23.3)		60.9	51.2	65.6	77.4	**0.002**
>60	71 (16.7)	15 (12.1)	26 (9.8)		60.7	59.2	53.3	69.2	0.557
Marital status									
Single	125 (29.4)	42 (33.9)	63 (23.7)	0.084	57.4	54.4	52.4	66.7	0.212
Not single	300 (70.6)	82 (66.1)	203 (76.3)		59.0	46.0	65.9	75.4	**<0.001**
Ethnicity									
Han	271 (63.8)	93 (75.0)	114 (42.9)	**<0.001**	55.2**	47.2	58.1	71.9	**<0.001**
Minority	154 (36.2)	31 (25.0)	152 (57.1)		63.2**	50.7	71.0	74.3	**<0.001**
Education level									
Illiteracy & elementary school	211 (49.7)	33 (27.1)	145 (54.9)	**<0.001**	59.1	52.6	57.6	69.0	**0.008**
Middle school	158 (37.2)	47 (38.5)	90 (34.1)		58.0	43.7	61.7	81.1	**<0.001**
High school & above	56 (13.2)	42 (34.4)	29 (11.0)		57.5	46.4	64.3	69.0	0.076
Time of ART initiation									

2015	183 (43.1)	55 (44.4)	124 (46.6)	0.657	52.8***	36.6	50.9	77.4	**<0.001**
2016	242 (56.9)	69 (56.6)	142 (53.4)		63.1***	57.4	69.6	69.7	**0.027**
CD4 cell count/μL when ART initiated									
Median (IQR)	304 (191, 431)	296 (164, 391)	368 (231, 514)	**<0.001**					
<200	111 (26.1)	39 (31.5)	88 (33.1)	**0.004**	61.3	58.6	56.4	67.1	0.374
200-350	141 (33.2)	38 (30.7)	63 (23.7)		55.4	42.6	55.3	84.1	**<0.001**
350-500	115 (27.1)	34 (27.4)	58 (21.8)		58.0	47.0	67.7	74.1	**0.001**
>500	58 (13.7)	13 (10.4)	57(21.4)		60.2	46.6	76.9	70.2	**0.015**
WHO clinical stage when ART initiated									
Stage I & II	299 (70.4)	103 (83.1)	204 (76.7)	**0.010**	60.9***	50.2	63.1	78.4	**<0.001**
Stage III & IV	126 (29.6)	21 (16.9)	62 (23.3)		48.8***	44.4	52.4	56.5	0.248

VL: viral load; PLWH: people living with HIV, ART: antiretroviral therapy

*：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.1;

**：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.05;

***：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.01;

Among the survey cohort (n=264, Table 2.2b), in addition to characteristics mentioned in the source cohort, participants in Site C were also more likely to be married (p=0.019), have less permanent jobs (p<0.001) and have shorter travel distance between home and ART site (p=0.003). Additional characteristics which could differentiate VL testing rates (%) in three ART sites included occupation (*Farmer* group: p=0.142; *Permanent job* group: p=0.446; *Temporary job* group: p<0.001; *Other* group: p=0.035) and location of home (*In the same county of ART site* group: p<0.001; *Not in the same county of ART site* group: p=0.559).

Table 2.2b Participant characteristics by ART site and VL testing rates (%) at 12 months after ART initiation: survey cohort (N=264)

	Participant characteristics by ART sites, (n%)				VL testing rates at 12 months after ART initiation, %				
Characteristics	**Site A**	**Site B**	**Site C**	**P-value**	**Overall**	**Site A**	**Site B**	**Site C**	**P-value**
Overall	85 (100.0)	84 (100.0)	95 (100.0)		63.6	49.4	56.0	83.2	**<0.001**
Gender									
Male	51 (60.0)	47 (56.0)	55 (57.9)	0.868	63.4	52.9	59.6	76.4	**0.035**
Female	34 (40.0)	37 (44.0)	40 (42.1)		64.0	44.1	51.4	92.5	**<0.001**
Age at ART initiation, years									
Median (IQR)	41 (32, 50)	41 (31, 49)	43 (33, 51)	0.662					
18-30	16 (18.8)	18 (21.4)	18 (18.9)	0.664	65.4	43.8	61.1	88.9	**0.020**
30-45	36 (42.4)	37 (44.1)	37 (39.0)		64.6	52.8	51.4	89.2	**<0.001**
45-60	21 (24.7)	19 (22.6)	32 (33.7)		65.3	47.6	63.2	78.1	0.072
>60	12 (14.2)	10 (11.9)	8 (8.4)		55.5	50.0	50.0	62.5	0.832
Marital status									
Single	27 (31.8)	34 (40.5)	20 (21.1)	**0.019**	66.7	63.0	44.1	70.0	0.131
Not single	58 (68.2)	50 (59.5)	75 (78.9)		56.8	43.1	64.0	86.7	**<0.001**
Ethnicity									
Han	51 (60.0)	63 (75.0)	37 (39.0)	**<0.001**	57.0***	45.1	52.4	81.0	**0.002**
Minority	34 (40.0)	21 (25.0)	58 (61.0)		72.6***	55.9	66.7	84.5	**0.010**
Education level									
Illiteracy & elementary school	43 (50.6)	24 (28.6)	57 (60.6)	**<0.001**	67.7	53.5	58.3	82.5	**0.005**
Middle school	31 (36.5)	29 (34.5)	29 (30.9)		57.3	45.2	44.8	82.8	**0.003**
High school & above	11 (12.9)	31 (36.9)	8 (8.5)		64.0	45.5	64.5	87.5	0.168
Occupation									
Farmer	28 (32.9)	13 (15.5)	13 (13.7)	**<0.001**	64.8	53.6	69.2	84.6	0.142
Permanent job	10 (11.8)	22 (26.2)	8 (8.4)		55.0	50.0	50.0	75.0	0.446
Temporary job	37 (43.5)	43 (51.2)	51 (53.7)		66.4	48.7	58.1	86.3	**<0.001**

No job or others	10 (11.8)	6 (7.1)	23 (24.2)		61.5	40.0	33.3	78.3	**0.035**
Living in the same county with their ART site									
Yes	63 (75.9)	59 (71.1)	73 (78.5)	0.517	64.1	47.6	54.2	86.3	**<0.001**
No	20 (24.1)	24 (28.9)	20 (21.5)		65.6	60.0	62.5	75.0	0.559
Distance between home and ART site									
<2-hour drive	56 (65.9)	37 (44.1)	63 (66.3)	**0.003**	68.0*****	51.8	59.5	87.3	**<0.001**
>2-hour drive	29 (34.1)	47 (55.9)	32 (33.7)		57.0*****	44.8	53.2	75.0	**0.044**
Time of ART initiation									
2015	45 (52.9)	39 (46.4)	44 (46.3)	0.608	55.5*******	37.8	46.2	81.8	**<0.001**
2016	40 (47.1)	45 (53.6)	51 (53.7)		71.3*******	62.5	64.4	84.3	**0.034**
CD4 cell count/μL when ART initiated									
Median (IQR)	346 (225, 455)	294 (156, 390)	329 (221, 485)	0.060					
<200	14 (16.5)	25 (29.8)	3 (34.7)	**0.049**	77.2	64.3	64.0	81.8	0.247
200-350	30 (35.3)	27 (32.1)	25 (26.3)		58.5	46.7	40.7	92.0	**<0.001**
350-500	23 (27.1)	23 (27.4)	17 (17.9)		60.3	52.2	60.8	76.5	0.187
>500	18 (21.2)	9 (10.7)	20 (21.1)		63.8	55.6	66.7	80.0	0.073
WHO clinical stage when ART initiated									
Stage I & II	62 (72.9)	72 (85.7)	76 (80.0)	0.119	66.2*****	51.6	57.0	86.8	**<0.001**
Stage III & IV	23 (27.1)	12 (14.3)	19 (20.0)		53.7*****	43.5	50.0	68.4	0.261

VL: viral load; PLWH: people living with HIV, ART: antiretroviral therapy

*：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.1;

**：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.05;

***：the VL testing rate at 12 months after ART initiation were significantly different within categories of this characteristic, p<0.01;

Determinants of VL testing timeliness

As shown in Table 2.3a, 815 PLWH in the source cohort contributed 851.1 PY of observed time, during which 787 PLWH took at least one VL test. The overall incidence rate of VL testing was 0.92 per PY. PLWH who were older than 45 years (*45-60* group: adjusted HR [aHR]=1.23, 95% CI=1.03-1.48; *>60 group*: aHR=1.34, 95% CI=1.07-1.70), were an ethnic minority (aHR=1.24, 95% CI=1.07-1.45), initiated ART in 2016 (aHR=1.32, 95% CI=1.14-1.53), were at WHO stage I & II (aHR=1.40, 95% CI=1.18-1.65) and received ART in Site C (aHR=2.21, 95% CI=1.89-2.26) were more likely to take VL tests earlier. CD4 cell count was removed from the multivariate model since a correlation between CD4 cell count and WHO stage was identified ($r=-0.404$, $p<0.001$) and CD4 cell count was not significant in univariate analysis.

Table 2.3a Factors associated with VL testing timing by Cox proportional modeling: source cohort, N=815

Variables	Participants who had VL test	Observed time, PY	Rate of VL test, per PY	Crude HR (95% CI)	p-value	Adjusted HR (95%CI)	p-value
Overall	787	851.1	0.92				
Gender							
Male	477	510.9	0.93	1.00		1.00	
Female	310	340.2	0.91	0.98 (0.85, 1.13)	0.743	1.02 (0.88, 1.18)	0.788
Age at ART initiation, years							
18-30	161	172.9	0.93	1.07 (0.88, 1.30)	0.487	1.05 (0.86, 1.28)	0.646
30-45	307	345.3	0.89	1.00		1.00	
45-60	209	220.1	0.95	1.12 (0.94, 1.33)	0.212	1.23 (1.03, 1.48)	**0.025**
>60	110	112.8	0.98	1.19 (0.96, 1.48)	0.120	1.34 (1.07, 1.70)	**0.012**
Marital status							
Single	225	243.4	0.92	1.00 (0.86, 1.17)	0.982	1.03 (0.88, 1.22)	0.698
Not single	562	607.7	0.92	1.00		1.00	
Ethnicity							
Han	458	522.2	0.88	1.00		1.00	
Minority	329	328.8	1.00	1.27 (1.10, 1.46)	**0.001**	1.24 (1.07, 1.45)	**0.005**
Education level							
Illiteracy & elementary school	373	398.9	0.94	1.00		1.00	
Middle school	287	310.1	0.93	0.94 (0.81, 1.10)	0.428	1.11 (0.94, 1.32)	0.218
High school & above	123	138.6	0.89	0.88 (0.72, 1.08)	0.222	1.02 (0.81, 1.27)	0.896
ART site							
Site A	416	511.5	0.81	1.00		1.00	
Site B	121	131.7	0.92	1.24 (1.01, 1.53)	**0.035**	1.24 (1.00, 1.53)	0.050
Site C	250	207.8	1.20	2.21 (1.89, 2.60)	**<0.001**	2.20 (1.87, 2.60)	**<0.001**
Time of ART initiation							
2015	353	415.8	0.85	1.00		1.00	
2016	434	435.3	1.00	1.42 (1.23, 1.63)	**<0.001**	1.32 (1.14, 1.53)	**<0.001**

CD4 cell count/µL when ART initiated							
<200	225	241.0	0.93	1.00			
200-350	234	260.7	0.90	0.91 (0.76, 1.09)	0.310		
350-500	204	218.4	0.93	0.98 (0.81, 1.18)	0.827		
>500	124	131.1	0.95	1.00 (0.80, 1.24)	0.963		
WHO clinical stage when ART initiated							
Stage I & II	590	611.4	0.96	1.31 (1.12, 1.54)	**0.001**	1.40 (1.18, 1.65)	**<0.001**
Stage III & IV	197	239.7	0.82	1.00		1.00	

VL: viral load; PLWH: people living with HIV, ART: antiretroviral therapy; PY: person-year

Among participants in the survey cohort (n=264), the total observed time was 259.1 PY, and 263 had VL testing (Table 2.3b). Similar to the results in the source cohort, PLWH who initiated ART in 2016 (aHR=1.45, 95% CI=1.12-1.89), in ART site C (aHR=3.22, 95% CI=2.28-4.55) and were at WHO stage I & II (aHR=1.44, 95% CI=1.03-2.01) were more likely to undergo VL test earlier. Additionally, participants who lived farther than 2-hour drive from ART site (aHR=0.72, 95% CI=0.54-0.95) and had a permanent job (aHR=0.67, 95% CI=0.45-1.00) were more likely to delay VL testing. After adding sampling weights in the multivariate model, age (*18-30 years* vs. *30-45 years*: aHR=0.78, 95% CI=0.63-0.96), ethnicity group (*ethnic minority* vs. *Han*: aHR=1.39, 95% CI=1.19-1.62) and education level (*high school and above* vs. *illiteracy & elementary school*: aHR=1.29, 95% CI=1.00-1.66) also showed weak effects on VL testing timing. Besides the correlation between CD4 cell count and WHO stage ($r=-0.406$, $r<0.001$), a correlation was also identified within another pair of variables: *living in the same county with their ART site* and *travel distance* ($r=0.608$; $p<0.001$). Only travel distance was included in the multivariate models.

Table 2.3b. Factors associated with VL testing timing by Cox proportional modeling: survey cohort, N=264

Variables	Participants who had VL test	Observed time, PY	Rate of VL test, per PY	Crude HR (95% CI)	p-value	Adjusted HR (95%CI)	p-value	Weighted* adjusted HR (95%CI)	p-value
Overall	263	259.1	1.02						
Gender									
Male	153	150.1	1.02	1.00		1.00		1.00	
Female	110	108.9	1.01	0.97 (0.76, 1.25)	0.825	1.10 (0.84, 1.45)	0.488	1.14 (0.98, 1.34)	0.100
Age at ART initiation, years									
18-30	52	51.8	1.00	0.99 (0.71, 1.38)	0.948	0.95 (0.66, 1.36)	0.952	0.78 (0.63, 0.96)	**0.019**
30-45	110	107.4	1.02	1.00		1.00		1.00	
45-60	71	68.7	1.03	1.05 (0.78, 1.42)	0.741	0.85 (0.60, 1.21)	0.379	0.85 (0.69, 1.04)	0.117
>60	30	31.2	0.96	0.97 (0.64, 1.15)	0.864	0.99 (0.60, 1.64)	0.963	0.92 (0.69, 1.24)	0.589
Marital status									
Single	81	85.1	0.95	0.87 (0.67, 1.13)	0.281	0.96 (0.71, 1.30)	0.795	1.18 (0.99, 1.41)	0.063
Not single	182	174.0	1.05	1.00		1.00		1.00	
Ethnicity									
Han	150	161.3	0.93	1.00		1.00		1.00	
Minority	113	97.8	1.16	1.51 (1.18, 1.93)	**0.001**	1.29 (0.98, 1.68)	0.071	1.39 (1.19, 1.62)	**<0.001**
Education level									
Illiteracy & elementary school	124	115.9	1.07	1.00		1.00		1.00	
Middle school	88	92.4	0.95	0.78 (0.56, 1.02)	**0.072**	0.99 (0.72, 1.37)	0.944	0.93 (0.77, 1.12)	0.437
High school & above	50	50.5	0.99	0.88 (0.63, 1.22)	0.439	1.41 (0.95, 2.13)	0.087	1.29 (1.00, 1.66)	**0.043**
ART site									
Site A	84	100.1	0.84	1.00		1.00		1.00	
Site B	84	94.7	0.89	1.07 (0.79, 1.46)	0.649	1.09 (0.84, 1.45)	0.625	1.02 (0.82, 1.28)	0.833
Site C	95	64.2	1.48	3.07 (2.27, 4.15)	**<0.001**	3.22 (2.28, 4.55)	**<0.001**	3.24 (2.70, 3.90)	**<0.001**
Occupation									
Farmer	54	55.3	0.98	0.85 (0.62, 1.17)	0.316	1.02 (0.72, 1.45)	0.923	1.02 (0.84, 1.24)	0.851

Permanent job	40	44.7	0.89	0.71 (0.50, 1.02)	**0.064**	0.67 (0.45, 1.00)	**0.049**	0.71 (0.56, 0.90)	**0.005**
Temporary job	130	122.9	1.06	1.00		1.00		1.00	
Other	39	36.2	1.08	1.01 (0.71, 1.45)	0.956	0.82 (0.52, 1.29)	0.397	0.86 (0.66, 1.11)	0.253
Living in the same county with their ART site									
Yes	195	187.8	1.04	1.00					
No	63	63.9	0.99	0.92 (0.69, 1.22)	0.564				
Distance between home and ART site									
<2-hour drive	156	143.8	1.08	1.00		1.00		1.00	
>2-hour drive	107	115.3	0.93	0.73 (0.57, 0.94)	**0.016**	0.72 (0.54, 0.95)	**0.019**	0.70 (0.59, 0.83)	**<0.001**
Time of ART initiation									
2015	127	140.0	0.91	1.00		1.00		1.00	
2016	136	119.0	1.14	1.50 (1.17, 1.92)	**0.001**	1.45 (1.12, 1.89)	**0.005**	1.54 (1.32, 1.79)	**<0.001**
CD4 cell count/μL when ART initiated									
<200	72	63.2	1.14	1.00					
200-350	82	82.3	1.00	0.81 (0.59, 1.12)	0.206				
350-500	62	67.6	0.92	0.70 (0.50, 0.98)	**0.039**				
>500	47	46.0	1.02	0.86 (0.59, 1.25)	0.862				
WHO clinical stage when ART initiated									
Stage I & II	209	201.1	1.04	1.27 (0.94, 1.72)	0.12	1.44 (1.03, 2.01)	**0.03**	1.56 (1.30, 1.89)	**<0.001**
Stage III & IV	54	58.0	0.93	1.00		1.00		1.00	

VL: viral load; PLWH: people living with HIV, ART: antiretroviral therapy; PY: person-year

* Sampling weights were added in multivariate Cox model

Discussion

The coverage and timeliness of viral load testing was an important but easily-neglected link in the HIV/AIDS care continuum, especially in resource-limited settings. In our study, 90% of PLWH were retained in care longer than 6 months, among whom 58.5% underwent their first VL test within 12 months after ART initiation, which was in agreement with other studies conducted in rural southeast China[18,32]. Patients initiating ART in 2016 tended to have timelier VL testing than those initiating in 2015 ($p<0.001$). However, universal access to timely VL testing remained a challenge in the real-world settings.

VL tests were performed significantly earlier in ART Site C than Site A & B. Figure 2.2a and 2.2b showed age, marital status, education level, baseline CD4 cell count and WHO stage might modify the association between ART sites and VL testing timing. However, after controlling available variables, Site C was still an independent better predictor for timely VL testing. Hence, the early VL testing in Site C could not be explained by those patient individual-level characteristics. Site C was an independent specialized HIV/AIDS care center with a higher doctor-patient ratio than Site A & B (Site A vs. B vs. C=1/600 vs. 1/600 vs.1/300). Additionally, a policy of *government purchasing public service* was well implemented in Site C. The local government purchased HIV care services from non-governmental organizations (NGOs) as an auxiliary method to improve ART[33]. In Site C, NGO staff organized patient education meetings twice a month and provided peer support to patients, which could be helpful to improve patients' adherence and ART outcomes[34,35].

In our study, PLWH at WHO stage I & II at ART initiation were more likely to undergo VL test timely. Patients could only undergo VL tests when they visited the ART sites, hence the frequency of follow-up visits and healthcare service utilization might directly influence the VL monitoring. Studies[36,37] in resource-limited settings reported advanced WHO stage (Stage III & Stage IV) at ART initiation were associated with fewer follow-up visits. However, a study in Canada indicated HIV disease increased the possibility to visit health care settings[21]. The discrepancy could be due to the different levels of health care system development in resource-limited settings and well-resourced settings. In rural China, further improvement of HIV care service accessibility is needed.

Cox models in the survey cohort showed that distance between home and ART sites significantly adversely affected VL testing timeliness. Although ART coverage in China had been greatly scaled up, ART in rural areas was still mainly centralized in county- and prefecture/city-level facilities. Quite a few PLWH in Wenshan living in mountainous areas without developed traffic, and it took them hours to arrive at the ART site secured. Studies[38–40] reported longer travel distance and time made HIV care less accessible, which resulted in less health service utilization, suboptimal ART outcomes and increased LTFU. The results of our study provided more evidence of the necessity to decentralization ART service to township- or community-level facilities in remote rural regions. Higher level HIV care facilities should cooperate with township hospitals or provide outreach services so that VL testing blood samples could be collected more conveniently[38].

An interesting finding was that ethnic minority PLWH were more likely to undergo VL testing earlier than Han ethnicity. Studies[41–43] reported that the HIV epidemic was more serious among ethnic minority populations in this area. However, the proportion of ethnic minorities in our patient cohort was only 41%, which was much lower than the proportion of ethnic minorities accounted for in the general population (58%). This result might indicate a disproportionally higher pre-ART attrition among ethnic minorities, which was supported by a study conducted in Yunnan[44]. Hence, it was possible that the ethnic minority PLWH in the ART program might have better HIV knowledge and compliance, which could lead to a better VL testing rate found among them.

Our study had limitations. First, only three ART sites were selected in Wenshan, which might limit the generalizability of study results to other rural regions. Second, participants of the survey were only recruited from PLWH who visited the ART sites during the study period and we didn't have accurate records about how many patients were screened and invited to the survey. Although most of the basic characteristics in the source cohort and survey cohort were comparable, it was possible that the results from the survey cohort could not completely represent the characteristics of in total source cohort. Third, only patient individual-level characteristics were analyzed in our study, structural level factors should be the focus of future studies, which should cover more regions and ART facilities.

Our study results demonstrated that 58.5% of PLWH achieved VL testing at 12 months after ART initiation in the Wenshan area. To improve VL testing timeliness, PLWH who were young, at a low level of education, lived far away from ART facilities, and at advanced WHO stage

should be targeted. HIV healthcare decentralization to remote rural areas is highly recommended. UNAIDS 90-90-90 targets could only be achieved by combining efforts of patients, healthcare service providers, governments, and NGOs.

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Chapter 3: Factors affecting the turnaround time of viral load testing in rural China: a mixed-method study in Wenshan

Abstract

Objective: To investigate current viral load (VL) testing turnaround time (TAT) in rural China, and to identify factors associated with prolonged TAT in each step of the VL test.

Methods: A sequential explanatory mixed-method study was conducted in three ART sites and a central VL testing laboratory in Wenshan Prefecture, Yunnan Province of China. The study contained a quantitative phase and a subsequent qualitative phase. In the quantitative phase, individual level TAT was calculated for all VL blood samples collected in 2018. In the qualitative phase, in-depth interviews were conducted among healthcare providers in the ART sites and technicians in the central VL testing laboratory to identify factors associated with prolonged TAT.

Results: In our study sites, a total of 2 892 VL blood samples were collected and tested during 2018. The median TAT was 54 days (IQR: 36, 92), with a median pre-test TAT of 47 days (IQR: 31, 81) and a median post-test TAT of 5 days (IQR: 1, 10). Factors associated with prolonged TAT mainly included the annual urban-to-rural labor migration, the shortage of healthcare professionals and lab technicians, limited VL testing instruments, and the immature reagent procurement system and testing results reporting system.

Conclusion: The current VL testing TATs in the Wenshan area were longer than those in developed countries and in some other low- and mid-income countries (LMICs). To address the challenges in each process of VL testing and shorten VL testing TAT in rural China, the local ART facilities, VL testing laboratories, and healthcare policymakers at all levels of government should collaborate and coordinate their procedures.

Keywords: HIV/AIDS, viral load test, turnaround time, sequential explanatory mixed-method

Introduction

Viral load (VL) directly reflects the disease progression of HIV infection, and the VL test is the gold standard in antiretroviral therapy (ART) monitoring among people living with HIV (PLWH). To achieve the 90-90-90 targets established by the Joint United Nations Programme on HIV/AIDS (UNAIDS), all PLWH continuously receiving ART need to undergo routine VL tests and the testing results should be reported timely[1]. VL testing assays require high-level infrastructure, expensive testing equipment and kits, and well-trained lab technicians[2,3]. Hence, in low- and mid-income countries (LMIC), VL testing is only centralized in city-/prefecture- or higher level laboratories in most cases.

In real-world settings of LMICs, centralized laboratory-based approaches are used for VL tests. The VL testing blood samples collected in different local ART facilities are sent to a central laboratory to perform VL tests. The turnaround time (TAT) of the VL test refers to the time interval between blood sample collection and receipt of testing results by the ART facility. The TAT varies considerably among different countries. Studies in well-resourced settings reported

the median VL testing TATs ranging from 1 to 8 days[4–6]. A study conducted in seven Sub-Saharan countries reported that the mean TAT in each country ranged from 3 days to 50 days, and the mean TATs in five of the seven countries were close to or longer than 1 month[2,7]. Factors associated with prolonged TAT in LMICs are various. The pre-test TAT (time interval between blood sample collection and performing VL tests) can be influenced by weak health and laboratory systems, incomplete sample referral network, and a shortage of trained staff; and the post-test TAT (time interval between performing VL test and testing results receipt by the ART facilities) is mainly influenced by immature results reporting systems[2,3,8–10].

China's National Free ART Program (NFATP) provides once-a-year free VL tests to all PLWH who are retained in ART for longer than 6 months. VL testing had been scaled up in China since 2008, and by 2015 there were 181 qualified laboratories providing VL testing service, covering all regions of the country[11–13]. Since the VL testing laboratories are usually located in the capital region of a city or a province, the centralized laboratory-based strategy is used for VL tests in remote and rural areas. The VL testing TAT in rural China may vary from several weeks to months, however, there has been little published data about this issue. To investigate the current VL testing TAT under the centralized testing strategy in rural China, a mixed-method study was conducted in Wenshan Prefecture, Yunnan Province.

Yunnan Province has been one of the provinces with the most serious HIV/AIDS epidemic and pioneered ART implementation in China[11,14]. By the end of 2016, a total of 22 laboratories in Yunnan were capable of VL testing[15]. Wenshan Prefecture is located in the east of Yunnan Province. The traffic and economy in Wenshan have not been fully developed[16–19], and more

than 60% of the population is living in rural areas[18,19]. There are ten ART sites distributed in the eight counties of Wenshan, however, only one central VL testing laboratory located in the prefecture capital provides VL testing service for all PLWH in Wenshan. The aims of this study included: (1) to understand the current centralized VL testing strategy, (2) to calculate VL testing TAT and present its variation tendency during a whole calendar year; (3) to identify factors associated with prolonged TAT in each process of VL test.

Methods

Study sites

Three local ART facilities (ART Site A, B, and C) and the only central VL testing laboratory in Wenshan Prefecture were selected as study sites. Site A is attached to a county-level general hospital and has the largest number of PLWH receiving ART in Wenshan. Site B is attached to the only prefecture-level general hospital in Wenshan, where the central VL testing laboratory is located. Site C is a county-level specialized HIV/AIDS care center with the third-largest number of PLWH in Wenshan. The number of PLWH receiving ART in Sites A, B and C accounts for about 41% of the total PLWH retained in care in Wenshan.

Study design

A sequential explanatory mixed-method design was used to investigate VL testing TAT and identify factors associated with prolonged TAT. The study contained two phases: a quantitative phase and a subsequent qualitative phase. In the first phase or the quantitative phase, patient-individual level TAT was calculated for all VL blood samples collected in the three selected ART sites during 2018. In the second phase or qualitative phase, in-depth interviews were

conducted among healthcare providers in the selected ART sites and lab technicians in the central VL testing laboratory.

Data collection for the quantitative phase

In the quantitative phase, the dates of VL blood sample collection (T1), VL test performing (T2) and testing results received by the ART sites (T3) of each VL testing sample collected during 2018 were extracted from both medical records and laboratory records. No individual-level identifiable information was collected.

Recruitment and data collection for the qualitative phase

In the qualitative phase, in-depth interviews were conducted among healthcare providers in ART sites and technicians in the VL testing laboratory. Criteria for eligible participants were (a) working in selected ART sites or laboratory for longer than 6 months, (b) having experience of VL testing related work. To fully understand the challenges in each step of the centralized VL testing, participants were recruited from different professions, such as physicians, lab technicians, administrators, nurses, and non-governmental organization (NGO) staff working in ART sites. The investigator first contacted a key person in each of the ART sites and the central VL laboratory, such as the chief physician or the laboratory director. The key person could participate in the study as an interviewee, and she/he could also recommend other eligible participants in the ART site or the laboratory. The recruitment was terminated when no new information was collected. Participation in our study was completely voluntary.

The face-to-face in-depth interviews were conducted by the investigator in an office or conference room at the ART sites or the laboratory. Each of the interviews was one-on-one. Before an interview, the investigator provided full disclosure of study procedures and contents, and obtained signed informed consent from each interviewee. The interviews lasted for 30 – 40 minutes and were recorded by a recording pen. The interview started with socio-demographic information collection, such as age, gender, profession and working experience. Semi-constructed interview outlines with open-ended probe questions were used in the interview. The interview outline towards healthcare providers included 5 main topics about the VL testing procedures at local ART sites: (1) environment and infrastructure, (2) ART follow-up and VL testing policy, (3) VL testing procedures in the ART sites, (4) ART and VL testing related work capacity and workload, (5) reception and utilization of VL testing results, (6) perceived challenges in each process of VL test and suggestions to shorten TAT. The interview outlines administrated to laboratory technicians included 4 topics about the VL testing procedures at the central laboratory: (1) environment, facilities, and reagent supplement, (2) VL testing policy, (3) VL testing procedures in the laboratory, (4) VL testing capacity and workload, (5) perceived challenges in each process of VL test and suggestions to shorten TAT. Participants who completed the interview received 100 Yuan (about $15) for their time and efforts.

Data analysis

In the quantitative phase, VL testing related dates (T1, T2 and T3) were first entered into Excel sheets, in which step the completeness and accuracy of data were checked. Then data sheets were transferred into a SAS data set and analyzed by SAS 9.4 software (SAS Institute, Cary, NC, US). The individual-level TAT, pre-test TAT, and post-test TAT were calculated for each blood

sample. The median of TAT, pre-test TAT and post-test TAT of each quarter during 2018 were presented in a table and compared by rank-sum tests. The monthly median TAT, pre-test TAT, and post-test TAT during 2018 were visualized by figures.

In the qualitative phase, the audio records of in-depth interviews were transcribed verbatim by the investigator, and the transcripts were double checked to ensure the completeness and accuracy. Based on the interview outlines, a set of priori codes were first developed. The codes were then modified from the interview transcripts throughout the coding procedure. Categories were created based on the modified codes, and then theories were summarized from the categories. The original data collection and analysis were completed in Chinese, and the results were translated into English. ATLAS.ti5 software (Berlin, Germany) was used in data analysis.

Ethics

The study was reviewed and approved by Institutional Review Board (IRB) of University of California, Los Angeles (UCLA) in the U.S. and National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Center and Prevention (China CDC).

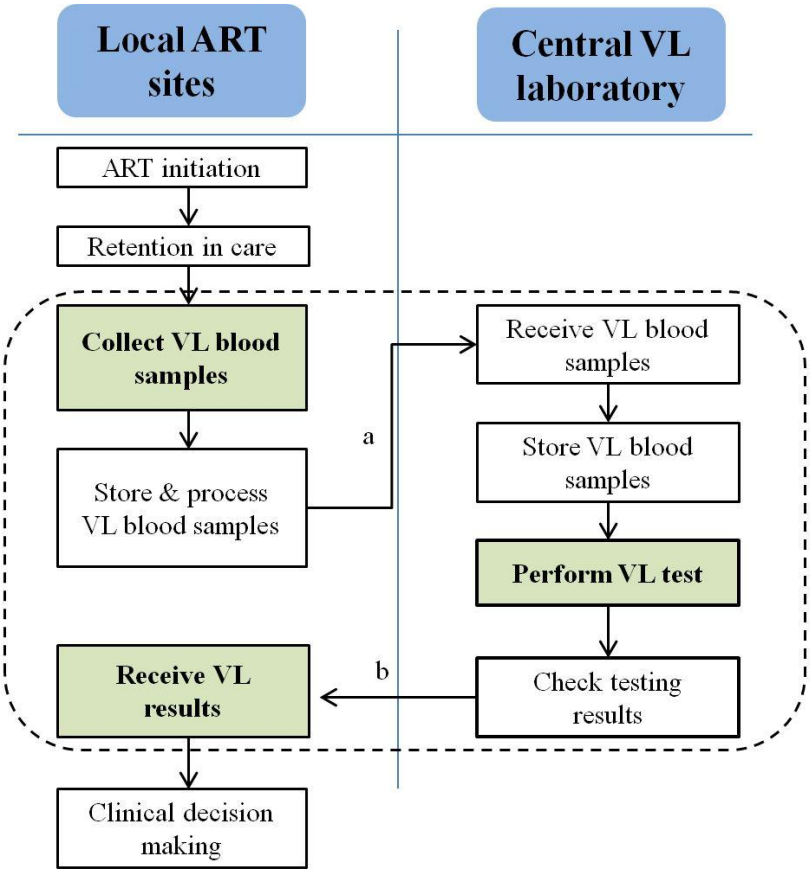

Figure 3.1 Flowchart of centralized viral load testing.
ART: antiretroviral therapy; VL: viral load. a: send plasma samples to the central lab; b: send electronic VL testing reports. The dotted box indicates all steps involving in VL testing turnaround time (TAT).

Results

Centralized VL testing strategy

Figure 3.1 describes the general procedures of centralized VL testing in Wenshan. After confirmation of HIV infection, all PLWH should initiate ART in a designated local ART site regardless of CD4 cell count[20]. PLWH go on follow-up visits to ART sites every three or four months for a refill of ART medications and undergoing laboratory tests. Routine VL tests are

provided once a year for patients who have been retained in care for more than 6 months. To undergo VL tests, patients go to local ART sites to donate blood samples, which will be processed and stored temporarily within the ART sites. In general, the samples are sent to the central VL testing laboratory once a month and the technicians in the central lab perform VL tests according to the order of receiving blood samples. When VL tests are completed and testing results are checked, electronic reports are sent back to the original ART sites in a short period of time. Since the VL testing rate and viral suppression rate are evaluated at the end of each calendar year, all the VL results should be returned to ART sites within the same calendar year as the blood sample collection. After receipt of VL reports, healthcare professionals in ART sites make clinical decisions according to the VL testing results, especially for those patients who have unsuppressed VL.

VL blood sample collection, TAT and pre-test TAT

Totally, 2 892 VL blood samples were collected and tested in the study sites during 2018, and all testing results were sent back to the original ART sites before Dec 31st, 2018. As shown in Table 3.1, the overall median TAT of all VL tests during 2018 was 54 days (IQR: 36, 92), with a median pre-test TAT of 47 days (31, 81). There were 1 501 VL testing blood samples collected during the first quarter of 2018, accounting for 51.9% of the total annual number of samples. During the second quarter of 2018, the number of samples collected dramatically declined to 356, which accounted for only 12.3% of the total annual amount. The numbers of blood samples collected during the third and fourth quarter were 677 (23.4%) and 358 (12.4%), respectively. The median pre-test TAT of the second quarter was 174 days (IQR: 173, 202), which was significantly longer than that of any other quarter ($p<0.001$). On the contrary, the median pre-test

TAT of the fourth quarter was 19 days (IQR: 13, 25), which was the shortest across all quarters (p<0.001). Figure 3.2a shows the monthly number of samples collected and the monthly median of the pre-test TAT.

Table 3.1 Characteristics of viral load testing in three ART sites in Wenshan during 2018 by each quarter, N=2 892

	Sample collected , %	Median pre-test TAT	Sample tested, %	Median post-test TAT	Median TAT
Overall	**2 892 (100)**	**47 (31, 81)**	**2 892 (100)**	**5 (1, 10)**	**54 (36, 92)**
Quarter 1	1 501 (51.9)	43 (26, 57)[a]	784 (27.1)	10 (5, 11)[b]	50 (34, 64)
Quarter 2	356 (12.3)	174 (173, 202)[a]	631 (21.8)	7 (7, 10)[b]	175 (174, 203)
Quarter 3	677 (23.4)	74 (50, 117)[a]	290 (10.0)	6 (4, 23)[b]	78 (55, 118)
Quarter 4	358 (12.4)	19 (13, 25)[a]	1187 (41.0)	1 (1, 3)[b]	22 (15, 27)

a: Corresponding median pre-test TAT of samples collected in the first, second, third and fourth quarter in 2018. b: Corresponding median post-test TAT of samples tested in the first, second, third and fourth quarter in 2018.

VL blood sample tested and post-test TAT

As shown in Table 3.1, the overall median of post-test TAT for all 2 892 samples was 5 days (IQR: 1, 10). During the first quarter, although 1 501 samples were collected, only 784 samples were tested, accounting for 27.1% of the total number of samples. There were 631 (21.8%) samples tested during the second quarter of 2018. During the third quarter, only 290 (10.0%) samples were tested, however, 1 187 (41.0%) samples were tested during the fourth quarter. The median post-test TAT of the fourth quarter was only 1 day (IQR: 1, 3), which was significantly shorter than any of the other quarters (p< 0.001). Figure 3.2b shows the number of samples tested in each month and the corresponding monthly median post-test TAT. Notably, no VL tests were performed in June and August in 2018, and the numbers of samples tested in November sharply increased to 894, which accounted for 30.9% of the total amount in 2018.

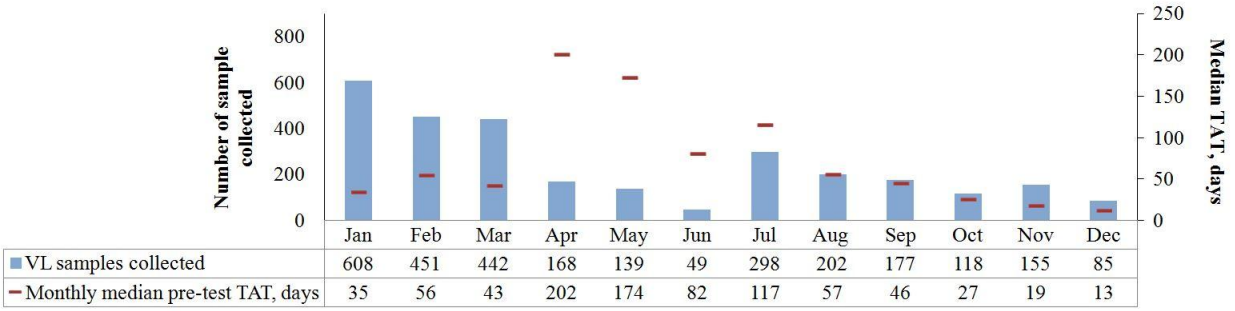

Figure 3.2a Number of viral load samples collected in each month (N=2892) and the corresponding monthly median pre-test TAT. VL: viral load; TAT: turnaround time.

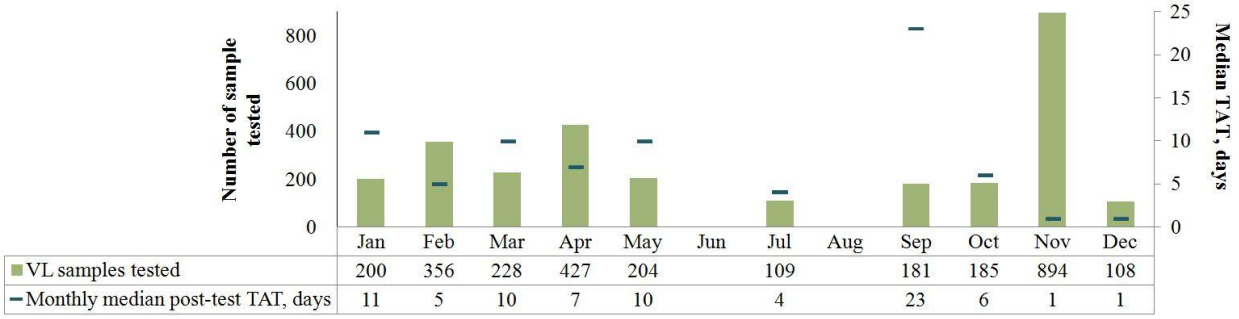

Figure 3.2b Number of viral load samples tested in each month (N=2892) and the corresponding monthly median post-test TAT. VL: viral load; TAT: turnaround time.

Interviewee characteristics

A total of 11 healthcare providers or laboratory technicians participated in the interview, including 4 physicians (36.4%), a nurse (9.1%), an administrator (9.1%), 3 NOG staffs working in ART sites (27.3%), and 2 lab technicians (18.2%). About 36% of participants had engaged in VL testing related work for more than 5 years. (Table 3.2)

Table 3.2 Background characteristics of interviewees, N=11

Characteristics	Number	Percentage of total
Age, years		
<35	4	36.4%
>35	7	63.6%
Gender		
Female	7	63.6%
Male	4	36.4%
Profession		
Physician	4	36.4%
Nurse	1	9.1%
Administrator	1	9.1%
NGO staff	3	27.3%
Lab technician	2	18.2%
Duration of VL test related work, years		
<5	7	63.6%
>5	4	36.4%

Factors associated with TAT in each key process of VL testing

Collect VL blood samples

More than 50% (n=1 501) of the annual total VL samples were collected in the first quarter of 2018, which was beyond the testing capacity of the central VL laboratory. Hence, the pre-test TAT started to increase from March. There could be two main reasons for the unbalanced sample collection.

First, the PLWH who were rural-to-urban migrant workers could only visit their ART sites during the Spring Festival vacation (usually in late January or early February). As a labor export province, a large number of young and mid-aged adults in Yunnan Province went to developed provinces to work as migrant workers and return home only during the Spring Festival every

year. Hence, the HIV infected migrant workers usually undergo their VL tests during the Spring Festival, the only time they were at home. As for the healthcare providers in the ART sites, it was also a priority to collect VL blood samples of those migrant patients during that period.

'About 30% to 40% of patients in our ART site are migrant workers working in other provinces. They don't have to visit the ART site too frequently. We can mail ART medications to them. They can also undergo general laboratory tests locally and fax or mail testing results to us. However, they can only do VL and CD4 tests in our ART site, so they must visit us at least once a year. Usually, they come here during the Spring Festival to donate blood samples (for VL and CD4 testing). If they don't come, we'll call them' (ID 4, physician)

Second, the healthcare professionals in the ART sites tended to do concentrated sample collection to reduce the working burden. The physician/patient ratios in the selected ART sites ranged from 1/600 to 1/300, which were much lower than the national standard, 1/150. As the current VL testing policy for patients who had been on ART for longer than 1 year and already achieved viral suppression, the timing of the annual VL test was not specified. To ensure the universal coverage of the VL test and facilitate patient management, the healthcare providers tended to collect their VL testing samples during the first follow-up visit of this calendar year.

'The majority of patients in our ART site have good adherence and have already achieved undetectable VL. For patients who were virally suppressed in 2017, I tried to collect their VL blood samples during the first quarter of 2018. Totally, about 60%-70% of VL blood

samples were collected (during the first quarter of 2018), so that we had less pressure in the rest of the time of 2018. (ID 8, physician)

'We always received a large number of VL plasma samples in the first three months of a year, which exceeded the testing capacity of our lab. Some of the samples were stored in our lab for longer than 1 or 2 months before VL tests. The (pre-test) TATs for samples collected during that period were relatively long.' (ID 5, lab technician)

Perform VL test

After the scaling-up of VL testing, the VL blood sample can be collected at any time during the work hours in most of the ART sites. However, the frequencies of VL blood sample delivery and VL test performing are low because of low VL testing capacity.

First, a shortage of VL laboratory technicians limited the VL testing capacity. After the VL blood sample collection, the samples were temporarily stored in the local ART sites and sent to the central laboratory once a month (usually in the first half of each month), which seriously extended the pre-test TAT. However, there were only two trained technicians in the central laboratory and they were also in charge of all laboratory examinations in a prefecture-level general hospital. They only had one week during which to perform VL tests each month.

'The frequency of VL sample delivery was determined by the central laboratory. They (technicians in the central VL laboratory) only do VL tests in the third or fourth week in

each month, so that we just send samples there before the VL testing' (ID 1, ART site administrator)

'My colleague and I are in charge of all laboratory tests in our hospital. Although we need to perform VL tests for all blood samples from the ten ART sites across Wenshan Prefecture, it only accounts for 1/4 to 1/3 of our total workload. We arrange the ART sites to send VL samples during the first half of a month, so that we can perform VL tests in the second half of the month. We don't have much time to receive samples too frequently.' (ID 5, lab technician)

'The VL testing samples can be stored at -80 ℃ for several weeks, hence the other urgent tests, such as CD4 test, have priority over the VL tests.' (ID 11, lab technician)

Second, the insufficient number of VL test instruments limited the testing capacity. There was only one COBAS® TaqMan® 48 Analyzer in the central laboratory providing VL tests for all PLWH in Wenshan.

'We only have one VL testing instrument which can process 48 samples at a time. During the VL-testing week, about 500 to 600 samples can be tested. If there are still samples left, we have to store them until the VL-testing week in the next month.' (ID 11, lab technician)

Third, the immature reagent procurement system can seriously prolong the pre-test TAT. The special case in 2018 was that the central laboratory ran out of VL testing kits from June to

August. Only 109 samples from the three selected ART sites were tested during this period, and the pre-test TAT dramatically increased to over 200 days for samples collected in the second quarter of 2018 (Figure 3.2a). As the scaling-up of HIV testing and ART coverage, the number of PLWH who needed to do VL tests in 2018 grew beyond the expectation. However, the procurement plan for VL testing kits was made in 2017 and not updated to reflect changes in 2018. As a result, no VL testing kits were available until September and October in 2018.

'During the period of VL testing kits shortage, a total of 2 500 VL plasma samples from all ten ART sites in Wenshan were stored in our lab. We began to receive new VL testing kits in succession from September 2018' (ID 5, lab technician)

However, the TAT for samples collected during the last quarter of 2018 was short. To evaluate the viral suppression rate of the year, all VL tests should be completed and all testing reports should be returned to ART sites by the end of 2018. Hence, the number of samples tested increased dramatically during November and the TAT for samples collected at the end of the year significantly shortened (Figure 3.2a & 3.2b).

'To return all VL testing results to the ART sites by end of last year (2018), we had to work overtime during October and November.' (ID 5, lab technician)

'They (the lab technicians) might have much pressure on VL test in November and December of 2018, but in fact, they did a very good job. We received VL results within about 2 weeks after we sent samples to the laboratory.' (ID 7, nurse)

Receive VL results

After VL test performing and testing results checking, which usually took one to three days, the electronic testing reports were sent back to the original ART sites through a specialized ART information system. However, the system didn't have a reminder function, so that the healthcare providers in ART sites might delay in checking and receiving electronic testing reports, which could be the main reason for the long post-test TAT in September (Figure 3.2b).

> *'In general, we checked the (VL testing) information system frequently at the end of each month, however, because of the testing kits shortage, we had not received VL reports for several months. Hence, we checked the system less frequently and might miss some reports, especially when the VL testing just re-started (in September 2018).' (ID 3, physician)*

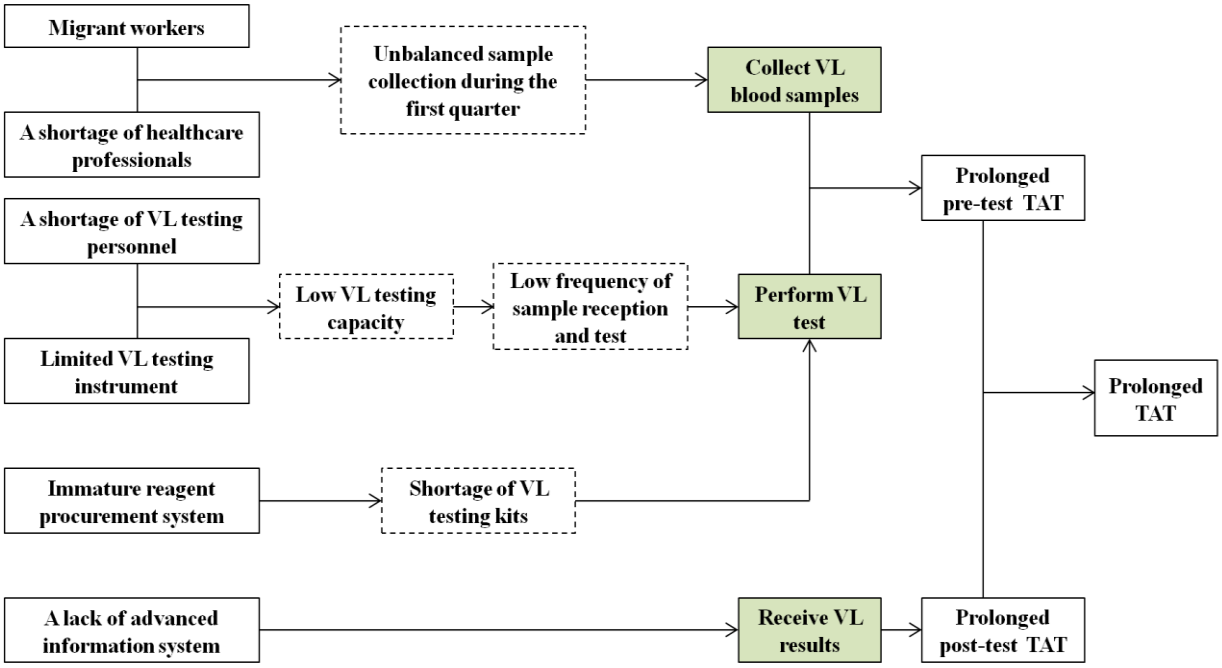

Figure 3.3 Factors associated with prolonged TAT in the processes of centralized VL testing
VL: viral load; TAT: turnaround time. The processes of centralized VL testing included (1) collect VL blood sample, (2) perform VL test, and (3) receive VL results.

Discussion

Although the coverage of the VL test in China had been scaled up since 2008, the prolonged TAT limited the timely use of VL testing results, especially in the rural areas where a centralized VL testing strategy was conducted. As one of the first studies focusing on VL testing TAT and factors associated with prolonged TAT in China, this study identified important structural factors associated with prolonged TAT in each process of VL test and provided preliminary knowledge for further investigation on improving VL testing services among PLWH.

Rural-to-urban migrant workers are one of the vulnerable populations to HIV. The estimated HIV prevalence of migrant workers in China was 0.09% in 2011 and 0.11% in 2015, which were approximately 60% higher than the HIV prevalence of the general population at the same time period[21,22]. The high mobility and more HIV risk behaviors made this population a potential bridge to transmit HIV to the general population[21,23–25]. Since they are highly mobile among provinces, HIV infected migrant workers are more likely to initiate ART in their hometown, where they have permanent residency and regularly go back. Among this population, the migration status can result in late HIV detection, late ART initiation and unsatisfactory ART follow-up[26–28]. Hence, they have been given attention since they were diagnosed as HIV positive and initiated ART. They are advised by local healthcare providers not to go out for work until they achieve viral suppression and are stable on ART. For those who have already gone out for work, the important laboratory tests, like VL tests and CD4 count tests are generally scheduled during Spring Festival, when most of the migrant workers return home.

The heavy working burden of healthcare providers in rural ART sites comes from two factors. First, the physician/patient ratios in the selected ART sites are low, ranging from 1/300 to 1/600, which are much lower than those in some other LMICs[29,30]. Second, the PLWH in rural areas have low HIV-knowledge levels and are highly dependent on healthcare providers, which further adds to the workload of physicians. To improve work efficiency and reduce the working burden, healthcare providers tend to intensively collect VL testing blood samples during the first quarter. In addition, the VL testing TAT has been prolonged in rural areas. To

allow enough time for receiving testing results by the end of the calendar year, physicians have to collect and deliver VL testing samples as early as possible.

The fundamental cause for the low frequency of sample delivery and test was insufficient VL testing capacity in rural areas. First, similar to the healthcare providers in rural ART sites, the VL testing technicians in the laboratory are also overburdened, which occurs widely in LMICs[3,7]. There were only two trained technicians in charge of performing VL tests for all PLWH in Wenshan. Although totally up to 6 900 VL plasma samples were tested in 2018 alone, it only accounted for 1/4 to 1/3 of the technicians' overall workload. Another reason for the weak VL testing capacity is insufficient VL platforms. There was only one VL testing instrument in Wenshan Prefecture providing service for approximate 7000 PLWH. Similarly, a survey in LMICs in Asia and Africa reported on average there was one VL testing instrument per 4 800 PLWH on ART[3,31]. To decentralize VL testing in rural areas, the point-of-care (POC) technologies operated in ART sites are promising supplements for conventional lab-based testing platforms. However, POC may further increase the burden of healthcare providers in local ART sites, and the performance of POC tests are still to be validated[3,9].

A mature reagent procurement system should have the ability to forecast the VL testing kits needed in the next year and cope with any unexpected situation. The shortage of VL testing kits is not uncommon, but the problem is especially noticeable during 2018. The VL testing demand in 2018 was higher than expected. HIV testing had been continuously scaled up and HIV-related mortality remained stable, hence the number of PLWH continuously increased[32–

[34]. In addition, the implementation policy requiring immediate ART was made in 2016[20,35], which directly increased the demand of VL testing. The procurement plan of VL testing kits for 2018 was made by a provincial HIV/AIDS care institution during 2017 and no timely adjustments were made subsequently, which directly led to the shortage of VL testing kits from June to August in 2018.

There are some limitations to our study. First, Wenshan Prefecture was the only study location selected in our study and no comparison groups were included. The VL testing strategy and TAT could vary by different regions, but we were not able to compare the situations in Wenshan with other rural areas in China. Second, the single study location might limit the generalizability of our study results. The factors identified in Wenshan Prefecture might not represent the situations in other rural regions. Third, we only focused on VL testing TAT during 2018, so that the TAT variation trend in recent years could not be investigated. To further investigate VL testing TAT and factors associated with prolonged TAT, larger-scaled studies covering more regions, different VL testing strategies, and a longer study period are needed.

In conclusion, the current VL testing TAT in Wenshan areas is still suboptimal, and challenges exist in each step of the centralized VL testing procedures. There is a need to improve current VL testing service and to shorten TAT by increasing the numbers of healthcare professionals and lab technicians undertaking ART and VL related works. It is also suggested to strengthen the relevant professional training, improve the laboratory network construction, ensure sufficient testing reagent supplies, and improve the ART information

system. The application of new POC VL testing technologies in local ART sites and a flexible ART referral system among migrant PLWH should also be explored in rural China. To achieve the 90-90-90 goals, it is very important to raise the VL testing efficiency and effectively use VL testing results. The local ART facilities, VL testing laboratories, and healthcare policymakers at all levels of government should collaborate and coordinate their procedures.

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Chapter 4: Is active concern for viral load testing results associated with better antiretroviral therapy response among people living with HIV? A clinic-based study in Yunnan Province, China

Abstract

Background: In low- and mid-income countries (LMIC), where the viral load (VL) testing system is not well developed, the HIV-infected patients are not informed of the VL testing results until long after VL blood sample collection. Hence, some patients take the initiative to ask about VL testing results before patient notification.

Objective: To investigate the association between actively asking about VL testing results and antiretroviral therapy (ART) outcomes among people living with HIV (PLWH) in rural China.

Methods: A clinic-based study was conducted in three ART facilities in Wenshan Prefecture, Yunnan Province of China. PLWH who initiated ART between Jan 1, 2015 and Dec 31, 2016 in these three facilities were recruited to participate in a survey from Jan to Mar in 2019, which collected information about socio-demographic characteristics, VL testing experience, VL-related knowledge, and family/social support using structured questionnaire. In addition, clinical characteristics and VL testing results were extracted from medical records. The rates (%) of viral suppression and sustained viral suppression (SVS) at the end of 2018 were calculated. Descriptive analyses and logistic regressions were conducted to investigate the association between actively asking about VL testing results and SVS, and to identify other factors associated with SVS.

Results: A total of 264 PLWH participated in the survey. The median age was 41.5 years (IQR 32.1-50.6), and 58.0% of them (n=153) were male. Most of the participants were infected with HIV by heterosexual contact (95.5%, n=252), and the baseline CD4 count at ART initiation was 321/μL (IQR: 210-452). The median ART duration of all participants was 3.0 years (IQR: 2.4-3.5). Among the participants, 58.3% (n=154) reported they had ever taken the initiative to ask about VL testing results before patient notification. By Dec 31, 2018, 95.1% of participants (n=251) had VL lower than 200 copies/mL, and 61.0% (n=161) had achieved SVS for 18 months. In the multivariable analysis, PLWH who had ever proactively asked about VL testing results were 3 times more likely to achieve SVS compared with those who had never asked (aOR=3.08, 95% CI=1.52-6.26). Other factors associated with SVS included baseline CD4 count, VL-related knowledge, support from non-governmental organizations (NGO), age, and time interval from HIV diagnosis to ART initiation.

Conclusions: Good virological outcomes among PLWH retaining in clinical care were observed in rural China. Under the circumstance of resource-limited settings, PLWH should be encouraged to actively communicate with healthcare providers. Larger-scaled follow-up studies covering more regions and participants are needed in the future.

Keywords: HIV/AIDS, antiretroviral therapy, viral load test, sustained viral suppression, patient notification

Introduction

Viral load (VL) is the most important indicator of HIV disease progression and antiretroviral therapy (ART) response[1–4]. The Joint United Nations Programme on HIV/AIDS (UNAIDS) established 90-90-90 targets, which required 90% of people living with HIV (PLWH) receiving ART to achieve viral suppression[5]. Good viral suppression rates have been observed among PLWH who are continually taking ART in low- and mid-income countries (LMIC)[6]. A study conducted in rural east Africa[7] reported that 90% of PLWH treated had viral suppression (VL<500 copies/mL). Nationwide cohort studies in China[8,9] also reported that the rates of viral suppression (VL<400 copies/mL) ranged from 89% to 91% among PLWH receiving ART. Since ART is a life-long process, it is important to continuously keep VL at an undetectable level. Sustained viral suppression (SVS), which focuses on the long-term ART response has also been investigated. Studies[10–12] conducted in the United States reported that the proportion of SVS among PLWH receiving HIV clinical care had increased from 45% in 2001 (SVS: sustained VL<400 copies/mL) to 68% in 2013 (SVS: sustained VL<200 copies/mL), and the increase was particularly evident in the young PLWH group (18-24 years). In an ethnic minority region of rural China, the SVS among PLWH who were receiving ART and had VL measurements in 2013 was about 41% (sustained VL< 50 copies/mL)[13].

China's National Free Antiretroviral Therapy Program (NFATP) provides free ART and relevant laboratory tests to all PLWH. After at least 6-month successive ART, a free VL test is provided for each patient once a year[14]. Generally, PLWH visit local ART sites every three to four months for routine follow-up visits, during which they can refill ART medications and undertake necessary laboratory tests. Like other LMICs, where the VL testing service has not been fully

developed, a laboratory-based centralized VL testing strategy is used in China[15]. Patients go to local ART sites to donate blood samples for VL testing, which are sent to and tested in a central laboratory periodically. After performing VL tests, the testing reports are sent back to the local ART sites, so that healthcare providers can implement clinical interventions according to the reports and inform patients of the testing results. It usually takes several weeks to months between blood sample collection and testing reports sent back to the original ART sites, so that the patients can only be informed even later[16,17]. Additionally, there has been no uniform system or policy across the country about how and when to inform patients of VL testing results. Hence, some patients tend to take the initiative to ask about the VL testing results before the patient notification. Studies[18–22] have reported that the capacity of obtaining healthcare information and good communication with healthcare providers are important for PLWH to have better ART adherence and achieve optimal virological outcomes. Therefore, it is reasonable to hypothesize that patients who proactively communicate with healthcare providers and ask about VL testing results can be more likely to have better ART outcomes. To investigate the association between actively asking about VL testing results and SVS among PLWH receiving ART in rural China, a cross-sectional study was conducted in Wenshan Prefecture, Yunnan Province.

Yunnan Province has been one of the provinces with the most serious HIV/AIDS epidemic and which pioneered ART implementation in China[23,24]. Wenshan Prefecture is located in the eastern mountainous region of Yunnan. The economy and education in Wenshan have not been fully developed, and more than 60% of the population are living in rural areas[25,26]. The ten ART sites are distributed in the eight counties of Wehshan and provide ART-related service to all PLWH across the prefecture. Generally, the PLWH are informed of VL testing results during their next

routine follow-up visits. However, when the patients take the initiative to ask about VL testing results in person or by phone, the healthcare providers will provide and explain the results to them immediately, if the testing results have already been received by the ART sites. For a small number of patients who have obviously elevated VL testing results, the healthcare providers tend to contact them and provide adherence support as soon as possible. The specific aims of this study include: (1) to calculate the rates (%) of viral suppression and SVS at the end of 2018 among PLWH in Wenshan; (2) to investigate the association between actively asking about VL results and SVS; (3) to identify other factors associated SVS.

Methods

Study sites

Three ART sites (Site A, B, and C) in Wenshan Prefecture were selected as study sites. Site A is attached to a county-level general hospital and has the largest number of PLWH receiving ART in Wenshan. Site B is attached to the only tertiary-level (the highest level in China) hospital in Wenshan and accepts PLWH coming from the whole prefecture. Site C is a county-level specialized HIV/AIDS care center with the third largest number of PLWH in Wenshan. The total number of PLWH retaining in care at the three ART sites accounts for more than 40% of the whole PLWH population in the Wenshan Prefecture.

Study design

A clinic-based study was conducted among PLWH who initiated ART between Jan 1, 2015 to Dec 31, 2016 in the three study sites. Information about socio-demographic characteristics, VL testing experience, VL-related knowledge, and family/social support, was collected from a

survey using a structured questionnaire. Basic clinical characteristics and VL testing results were extracted from medical records. Logistic regression models were conducted to assess the association between actively asking about VL testing results and SVS.

Enrollment

The survey was conducted from Jan to Mar 2019. During the 3-month study period, patients who went to the ART sties A, B and C for routine follow-up visits or education meetings were screened for eligibility. The eligibility criteria included (a) initiated ART from Jan 1, 2015 to Dec 31, 2016, (b) initiated ART in Site A, B, and C, (c) were aged≥18 years when initiated ART, (d) had remained in care for more than 6 months. When patients visited the study sites, healthcare providers in the ART sites would first check their medical records for eligibility. Since PLWH was a sensitive population, healthcare providers in the ART sites were in charge of making the initial screen and contact to patients on behalf of the investigator. For eligible patients, the healthcare providers then simply introduced the study and invited them to participate in the survey. If the potential participants agreed to participate, they would be referred to the investigator. The investigator then double-checked the eligibility and conducted a questionnaire survey to the eligible ones on the same day of recruitment (Figure 4.1).

Data collection

There were two main data sources: structured questionnaire surveys and medical records. Oral informed consent was obtained before the survey. After being recruited, participants filled out the questionnaire under the investigator's guidance. If the participant was not able to read, the investigator did a face-to-face survey and filled out the questionnaire according to the

participant's answers. The patient ID was collected to link survey data to medical records, and it was deleted once data were linked. It took 10-15 minutes to complete a questionnaire. After the survey, each participant received a gift equivalent to 20 Yuan (about 3 USD).

The questionnaire consisted of four parts. The first part was socio-demographic information, including gender, age, and education level. The second part was about the experience of VL testing. The participants were asked whether they only underwent VL tests in the same sites in which they received ART (yes/no/I don't know), whether they only underwent VL tests during routine follow-up visits (yes/no/I don't know), how did they evaluate the current patient notification strategy of VL testing results (good/fair/too slow/I don't know), had they ever taken the initiative to ask about VL testing results before patient notification (yes/no/ I don't know what is viral load test), and what was their overall satisfaction with the ART service (good/fair/poor). The variable of whether having ever taken the initiative to ask about VL testing results was the main exposure of our study. Both the participants who answered "no" and "I don't know" were regarded as "never asked". The third part was the awareness of VL-related knowledge. There were 5 questions about VL or VL test, including the frequency of routine VL test, the optimal result of VL test, the most important reason for ART failure, whether a patient with successive optimal VL results needs to continue ART, and whether a patient with successive optimal VL results can infect others. Each question was worth one point. The total score of each participant and the median score for the survey population were calculated and a new binary variable indicating higher (> median score) or lower score (≤ median score) was generated accordingly. The last question for this part was the source of the knowledge (learning from healthcare providers/self-studying/learning from other patients/and other ways). The fourth

part of the questionnaire was about the family support and social support for the patients. The questions included the patient's marital status, the HIV status of the patient's spouse or partner, the current living status of the patient (living alone/living only with the spouse or partner/living with other family members). If the patients were not living alone, they would be further asked about whether they had disclosed their HIV status to people who were living with them and whether they had got support from people who were living with them. The last question was whether they had ever got support from non-governmental organizations (NGOs) (got much support/got some support/never got any support).

After completing the questionnaire survey, the participants' clinical characteristics and VL testing results were extracted from medical records in the ART sites. The clinical characteristics included the HIV transmission route, date of HIV diagnosis, date of ART initiation, baseline CD4 cell count, and baseline ART regimen. The date and result of each VL test between ART initiation and Dec 31, 2018 were extracted and the SVS rates (%) by Dec 31, 2018 were calculated. All data were extracted by healthcare providers working in the three study sites.

The measurement of sustained viral suppression (SVS)

In our study, viral suppression or virally suppressed status was defined as undetectable HIV VL or VL<20 copies/mL. SVS was defined as maintaining viral suppression for 18 months by Dec 31, 2018, i.e. maintaining viral suppression from Jul 1, 2017 to Dec 31, 2018. Specifically, the following two situations could be regarded as SVS: (1) patients who had two or more VL tests during that 18-month period and all testing results were virally suppressed; (2) patients who had only one VL test during that 18-month period and the testing result was virally suppressed,

meanwhile, the last VL test prior to that 18-month period was also virally suppressed. All other situations were considered as a failure to SVS.

Statistical analysis

Descriptive analyses were conducted to describe the characteristics of our survey population, including demographic characteristics, basic clinical characteristics, family/social support, and VL-related knowledge. The results of descriptive analyses were presented by the different statuses of the exposure, i.e. whether having ever proactively asked about VL testing results before patient notification. Categorical variables were compared by the Chi-square test, and continuous variables were compared by the Wilcoxon test. Stratified analyses of each covariate were conducted for the exposure. The associations between the exposure and SVS were estimated by univariate and multivariate logistic regression models. Covariates included in the multivariate models were selected based on the results of univariate analyses ($p<0.1$) and prior knowledge. Crude odds ratios (cOR) and adjusted odds ratios (aOR) were presented with 95% confidence intervals (95% CI) and p-values. Furthermore, sensitivity analyses were conducted. First, the definition of SVS was changed from maintaining viral suppression for 18 months to maintaining viral suppression for 12 months; second, a new multivariate analysis was conducted among a sub-group, in which participants who had no awareness of VL test were excluded. Data were analyzed using SAS 9.4 software (SAS Institute, Cary, NC, US)

Ethics

The study was reviewed and approved by Institutional Review Board (IRB) of University of California, Los Angeles (UCLA) in the U.S. and National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Center and Prevention (China CDC).

Results

Recruitment

Figure 4.1 shows the procedures of participant recruitment for the survey. Patients who visited the three study sites during the 2019 January to March were first screened for eligibility by healthcare providers working in the ART sites. The eligible individuals were invited to participate in the survey and those who accepted the invitation were referred to the investigator. A total of 293 patients passed the preliminary screening, agreed to participate in the survey and were referred to the investigator. Twelve of the 293 patients were referred twice so that 281 patients were double-checked for eligibility by the investigator. Seventeen patients were ruled out during the double-check procedure and finally, 264 (90.1%) of patients participated in the survey.

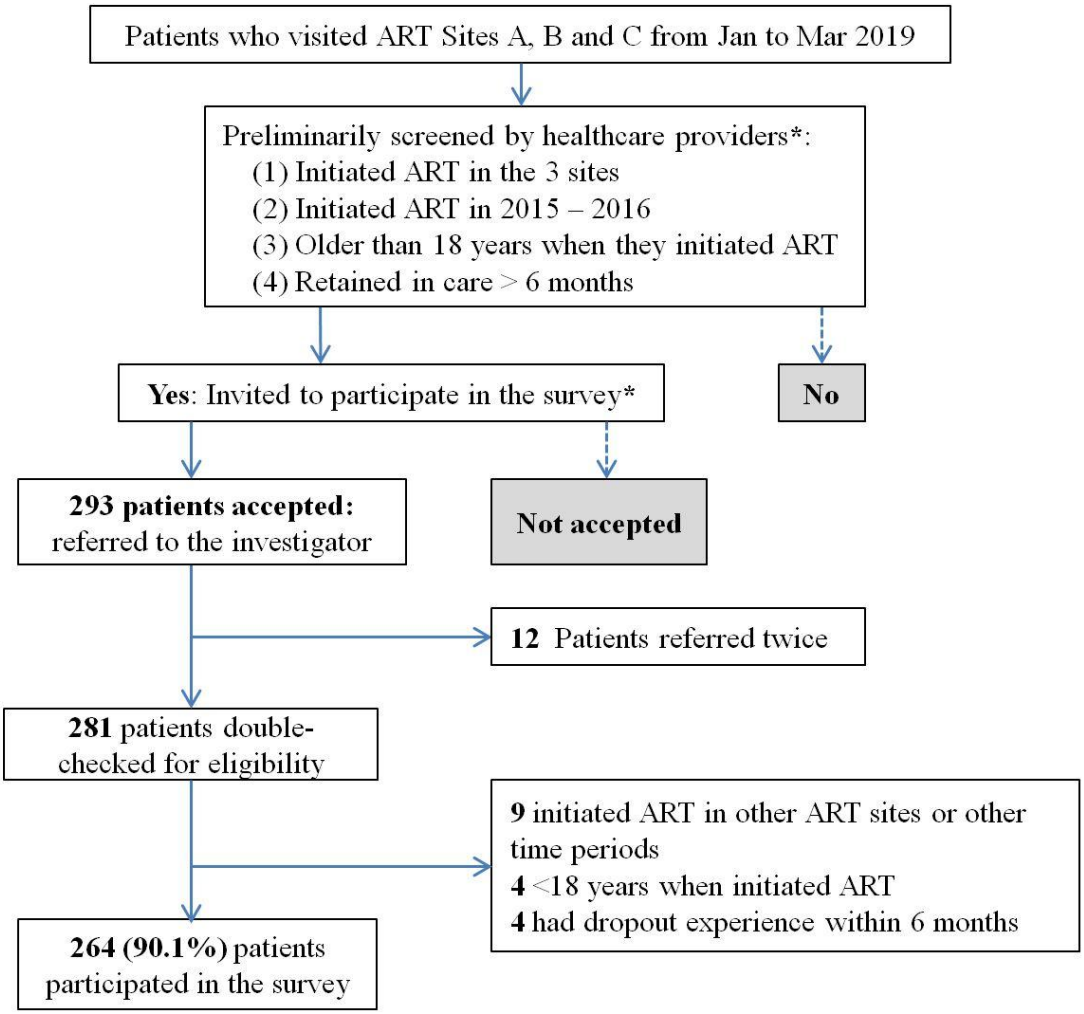

Figure 4.1 Flowchart: participants recruitment procedures of the survey
ART=antiretroviral therapy. Dropout was defined as loss to follow-up or discontinuation ART.
***:** The numbers of patients who were preliminarily screened by the healthcare providers and invited to participate in the survey was not recorded.

Participants' characteristics

As shown in Table 4.1, the median age of all 264 participants was 41.5 years (IQR 32.1-50.6), and 58.0% of them (n=153) were male. The proportion of participants who were illiterate or had only elementary school education was up to 47.2% (n=124). Most participants were infected

with HIV by heterosexual contacts (95.5%, n=252), and 61.4% (n=162) of them had initiated ART within 1 month after HIV diagnosis. The median baseline CD4 count at ART initiation was 321/μL (IQR: 210-452) and the two main baseline ART regimens were Tenofovir(TDF)/Lamivudine(3TC)/Efavirenz(EFV) and zidovudine(AZT)/3TC/EFV, which accounted for 39.4% (n=104) and 34.1% (n=90), respectively. By Dec 31, 2018, the median ART duration of all participants was 3.0 years (IQR: 2.4-3.5).

All of the 264 participants had at least one VL test after ART initiation. Among them, 86.0% (n=227) reported they had undergone all VL tests in the same ART sites and 48.1% (n=127) of them had undergone VL tests only during routine follow-up visits. Only 20.5% (n=54) of participants thought the current notification strategy of VL testing results was good. A total of 58.3% (n=154) of participants had ever taken the initiative to ask about the VL testing results, and 41.7 % (n=110) had never asked. Among these 110 participants, 40% (n=44) had no awareness of VL test. Overall, 89.4% (n=236) of participants reported they were very satisfied with the current ART service.

The socio-demographic and clinical characteristics of the survey population were also compared by the status of whether they had asked about the VL testing results before patient notification (Table 4.1). Patients who were more likely to ask about VL testing results tended to have higher education levels ($p<0.001$), received ART in Site B ($p<0.001$), and use TDF/3TC/EFV as the baseline ART regimen ($p<0.001$).

Table 4.1 The socio-demographic and clinical characteristics of the participants by the status of whether they had ever asked about VL testing results before patient notification, N=264.

Variables	All participants (n%)	Ever asked about VL testing results before patient notification		
		Yes (n%)	No (n%)	P-value
Overall	264 (100)	154 (100)	110 (100)	
Gender				
Male	153 (58.0)	89 (57.8)	64 (58.2)	0.950
Female	111 (42.0)	65 (42.2)	46 (41.8)	
Age at ART initiation, years				
Median (IQR)	41.5 (32.1, 50.6)	40.9 (31.9, 49.6)	42.7 (32.3, 51.4)	0.507
18-30	52 (19.7)	29 (18.8)	23 (20.9)	0.587
30-45	110 (41.7)	69 (44.8)	41 (37.3)	
45-60	72 (27.3)	41 (26.6)	31 (28.2)	
>60	30 (11.4)	15 (9.7)	15 (13.6)	
Education level				
Illiteracy & elementary school	124 (47.2)	57 (37.3)	67 (60.9)	**<0.001**
Middle school	89 (33.8)	58 (37.9)	31 (28.2)	
High school & above	50 (19.0)	38 (24.8)	12 (10.9)	
ART site				
Site A	85 (32.2)	22 (14.3)	63 (57.3)	**<0.001**
Site B	84 (31.8)	72 (46.7)	12 (10.9)	
Site C	95 (36.0)	60 (39.0)	35 (31.8)	
Time of ART initiation				
2015	128 (48.5)	72 (46.8)	56 (50.9)	0.505
2016	136 (51.5)	82 (53.2)	54 (49.1)	
Route of HIV transmission				
Heterosexual behaviors	252 (95.5)	147 (95.5)	105 (95.5)	1.000
Other	12 (4.5)	7 (4.5)	5 (4.5)	
Interval between HIV diagnosis and ART initiation				
Median (IQR), days	13 (5, 161)	11 (5, 102)	17 (5, 239)	0.363
<7 days	108 (40.9)	65 (42.2)	43 (39.1)	0.726
7 days - 1 month	54 (20.5)	33 (21.4)	21 (19.1)	
1 month - 1 year	56 (21.2)	29 (18.8)	27 (24.5)	
>1 year	46 (17.4)	27 (17.5)	19 (17.3)	
CD4 cell count/µL at ART initiation				
Median (IQR)	321 (210, 452)	327 (212, 451)	313 (202, 452)	0.823
<200	72 (27.3)	42 (27.3)	30 (27.3)	0.602
200-350	82 (31.1)	46 (29.9)	36 (32.7)	
350-500	63 (23.9)	41 (26.6)	22 (20.0)	
>500	47 (17.8)	25 (16.2)	22 (20.0)	
Baseline ART regimen				
TDF+3TC+EFV	104 (39.4)	75 (48.7)	29 (26.4)	**<0.001**
AZT+3TC+EFV	90 (34.1)	44 (28.6)	46 (41.8)	

LPV/r+other drugs	29 (11.0)	19 (12.3)	10 (9.1)
Other combinations	41 (15.5)	16 (10.4)	25 (22.7)

VL: viral load; ART: antiretroviral therapy; TDF: tenofovir; 3TC: lamivudine; EFV: efavirenz; AZT: zidovudine; LPV/r: lopinavir/ritonavir

The results of the five questions about VL-related knowledge are shown in Table 4.2. The accuracy rate for each question was 45.1%, 35.6%, 66.3%, 79.2% and 30.7%, respectively. Patients who had ever proactively asked about VL testing results tended to have a higher accuracy rate for each of the five questions ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$ and $p=0.018$, respectively). The total score for the five questions ranged from 0 to 5, and the median of the total score was 3 points (IQR: 1.5 - 4). The median score of patients who had ever asked about VL testing results was significantly higher than those who had never asked ($p<0.001$). As the total score went up, the patients were also more likely to asked about VL testing results (0 point: 10.3%, 1 point: 29.6%, 2 points: 59.7%, 3 points: 69.8%, 4 points: 79%, and 5 points: 84.6%, $P_{trend}<0.001$). A total of 67.8% (n=179) of participants reported they learned this knowledge only from healthcare providers in the ART sites, and 32.2% (n=85) had other ways to learn.

Table 4.2 Awareness of VL testing-related knowledge by the status of whether they had ever asked about VL testing results before patient notification, N=264

Questions	All participants (n%)	Ever asked about VL testing results before patient notification		
		Yes (n%)	No (n%)	P-value
Overall	264 (100)	154 (100)	110 (100)	
1) What's the frequency of free VL test?				
Correct	119 (45.1)	93 (60.4)	26 (23.6)	**<0.001**
Wrong	145 (54.9)	61 (39.6)	84 (76.4)	
2) What's the optimal result of VL test?				
Correct	94 (35.6)	79 (51.3)	15 (13.6)	**<0.001**
Wrong	170 (64.4)	75 (48.7)	95 (86.4)	
3) What's the most important reason for ART failure?				
Correct	175 (66.3)	126 (81.8)	49 (44.6)	**<0.001**
Wrong	89 (33.7)	28 (18.2)	61 (55.4)	
4) If a patient has got the optimal viral load for several successive years, does he/she need to continue ART?				
Correct	209 (79.2)	139 (90.3)	70 (63.4)	**<0.001**
Wrong	55 (20.8)	15 (9.7)	40 (36.4)	
5) If a patient has got the optimal viral load for several successive years, can he/she still infect others?				
Correct	81 (30.7)	56 (36.4)	25 (22.7)	**0.018**
Wrong	183 (69.3)	98 (63.4)	85 (77.3)	
Total score				
Median (IQR)	3.0 (1.5, 4.0)	3.0 (2.0, 4.0)	2.0 (0, 3.0)	**<0.001**
Lower score (<3)	123 (46.6)	46 (29.9)	77 (70.0)	**<0.001**
Higher score (>=3)	141 (53.4)	108 (70.1)	33 (30.0)	
Source of the knowledge				
Only learning from healthcare providers in the ART site	179 (67.8)	90 (58.4)	89 (80.9)	**<0.001**
Other Sources (including learning from healthcare providers, self-study, learning from other PLWH, learning from NGOs, etc.)	85 (32.2)	64 (41.6)	21 (19.1)	

VL: viral load; ART: antiretroviral therapy; PLWH: people living with HIV; NGO: non-governmental organizations

Table 4.3 showed the family and social support for the participants. Among all the participants, 68.3% (n=179) were married, and 31.3% (n=82) had a HIV positive spouse. Most of the participants (76.9%, n=203) were living with their spouse or other family members, and only 23.1% (n=61) were living alone. Among the 203 participants who are not living alone, 84.7% (n=172) had disclosed their HIV status to family members. And among those 172 participants, 89.5% (n=154) reported they were supported by their family members. In addition to the family support, 70.5% (n=186) of the total 264 participants reported they had got help from NGOs to some extent. Participants who had ever asked about VL testing results tended to be supported more by NGOs ($p<0.001$).

Table 4. 3 The family and social support for the participants by the status of whether they had ever asked about VL testing results before patient notification, N=264.

Variables	All participants (n%)	Ever asked about VL testing results before patient notification		
		Yes (n%)	No (n%)	P-value
Overall	264 (100)	154 (100)	110 (100)	
Current marital status				
Single	83 (31.7)	49 (31.8)	33 (30.6)	0.718
Married, with an HIV negative spouse	97 (37.0)	54 (35.1)	43 (39.8)	
Married, with an HIV positive spouse	82 (31.3)	51 (33.1)	32 (29.6)	
Living status				
Live alone	61 (23.1)	31 (20.1)	30 (27.3)	0.278
Live with a spouse/partner	81 (30.7)	52 (33.8)	29 (26.4)	
Live with family members (including the spouse/partner)	122 (46.2)	71 (46.1)	51 (46.4)	
Among the participants NOT live alone (n=203)				
Do people living with you know your HIV status?				
Yes, they all know	*107 (52.7)*	*64 (52.0)*	*43 (53.8)*	*0.408*
Yes, some of them know	*65 (32.0)*	*37 (30.1)*	*28 (35.0)*	
No, they don't know	*31 (15.3)*	*22 (17.9)*	*9 (11.2)*	
If people living with you know your HIV status (n=172), *do they provide you any support about ART?*				
Yes, much help	*74 (43.0)*	*46 (45.5)*	*28 (39.4)*	*0.727*
Yes, some help	*80 (46.5)*	*45 (44.6)*	*35 (49.3)*	
Not at all	*18 (10.5)*	*10 (9.9)*	*8 (11.3)*	
Support from NGOs				
Got much support	110 (41.7)	81 (52.6)	29 (26.4)	**<0.001**
Got some support	76 (28.8)	40 (26.0)	36 (32.7)	
Never got any support	78 (29.5)	33 (21.4)	45 (40.9)	

VL: viral load; ART: antiretroviral therapy; NGO: non-governmental organizations

VL status by Dec 31, 2018

By Dec 31, 2018, 95.5% (n=252) of the 264 participants' VL testing results fell below 1000 copies/mL, 95.1% (n=251) fell below 200 copies/mL, and 85.6% (n=226) fell below 20 copies/mL or were undetectable, i.e. at viral suppression status. A total of 61.0% (n=161) participants had achieved SVS status for 18 months.

Stratified analysis

As shown in Table 4.4, the SVS rate (%) among patients who had ever asked about VL testing results before the patient notification was significantly higher than those who never asked (66.9% vs. 52.7%, p=0.02). Participants with a higher education level (p<0.1) and who initiated ART in 2015 (p<0.1) were more likely to have SVS status at the end of 2018. Meanwhile, participants who were older than 60 years (p<0.1), receiving ART in Site C (p<0.1), and had baseline CD4 < 200 count/μL (p<0.1) were less likely to have SVS status. The SVS rates (%) for participants who had ever asked about VL testing results and who had never asked about VL testing results were different by gender (*Male*: p=0.002), age group (*>60*: p=0.003), education level (*Middle school*: p=0.009), ART sites (*Site A*: p=0.004), time of ART initiation (*2016*: p=0.029), baseline ART regimen (*TDF+3TC+EFV*: p=0.049), VL knowledge score (*Lower*: p=0.022), living status (*Live alone*: p=0.014) and NGO supports (*Some support*: p=0.012).

Table 4.4 Stratified analysis of SVS for 18 months by whether they had ever asked about VL testing results before patient notification, N=264

Variables	Participants achieved SVS for 18 months, (%)			
	Overall	Ever asked about VL testing results before patient notification		
		Yes	No	P-value
Overall	161 (61.0)	103 (66.9)	58 (52.7)	**0.020**
Gender				
Male	92 (60.1)	63 (70.8)	29 (45.3)	**0.002**
Female	69 (62.2)	40 (61.5)	29 (63.0)	0.872
Age at ART initiation, years				
18-30	36 (69.2)*	22 (75.9)	14 (60.9)	0.245
30-45	68 (61.8)*	45 (65.2)	23 (56.1)	0.341
45-60	45 (62.5)*	26 (63.4)	19 (61.3)	0.854
>60	12 (40.0)*	10 (66.7)	2 (13.3)	**0.003**
Education level				
Illiteracy & elementary school	67 (54.0)*	32 (56.1)	35 (52.2)	0.664
Middle school	59 (66.3)*	44 (75.9)	15 (48.4)	**0.009**
High school & above	35 (70.0)*	27 (71.1)	8 (66.7)	0.773
ART site				
Site A	56 (65.9)*	20 (90.9)	36 (57.1)	**0.004**
Site B	56 (66.7)*	50 (69.4)	6 (50.0)	0.186
Site C	49 (51.6)*	33 (55.0)	16 (45.7)	0.382
Time of ART initiation				
2015	85 (66.4)*	51 (70.8)	34 (60.7)	0.229
2016	76 (55.9)*	52 (63.4)	24 (44.4)	**0.029**
Interval between HIV diagnosis and ART initiation				
<7 days	74 (68.5)	48 (73.9)	24 (55.8)	0.052
7 days - 1 month	30 (55.6)	19 (57.6)	10 (47.6)	0.474
1 month - 1 year	37 (66.1)	21 (72.4)	16 (59.3)	0.299
>1 year	23 (50.0)	15 (55.6)	8 (42.1)	0.369
CD4 cell count/μL at ART initiation				
<200	36 (50.0)*	25 (59.5)	11 (36.7)	0.059
200-350	57 (69.5)*	36 (78.3)	21 (58.3)	0.052
350-500	36 (57.1)*	24 (58.5)	12 (54.5)	0.760
>500	32 (68.1)*	18 (72.0)	14 (63.6)	0.539
Baseline ART regimen				
TDF+3TC+EFV	69 (66.4)	54 (72.0)	15 (51.7)	**0.049**
AZT+3TC+EFV	49 (54.4)	25 (56.8)	24 (52.2)	0.658
LPV/r+other drugs	20 (69.0)	14 (73.7)	6 (60.0)	0.449
Other combinations	23 (56.1)	10 (62.5)	13 (52.0)	0.509
Total score of VL related knowledge				
Lower	77 (62.6)	33 (71.7)	44 (57.1)	0.105

Higher	84 (59.6)	70 (64.8)	14 (42.4)	**0.022**
Current marital status				
Single	48 (57.8)	32 (62.8)	16 (50.0)	0.252
Married, with an HIV negative spouse	57 (58.8)	35 (64.8)	22 (51.2)	0.175
Married, with an HIV positive spouse	55 (67.1)	36 (73.5)	19 (57.6)	0.133
Living status				
Live alone	36 (59.0)	23 (74.2)	13 (43.3)	**0.014**
Live with a spouse/partner	52 (64.2)	35 (67.3)	17 (58.6)	0.434
Live with family members (including the spouse/partner)	73 (59.8)	45 (63.4)	28 (54.9)	0.346
Support from NGOs				
Got much support	68 (61.8)	52 (64.2)	16 (55.2)	0.391
Got some support	49 (64.5)	31 (77.5)	18 (50.0)	**0.012**
Never got any support	44 (56.4)	20 (60.6)	24 (53.3)	0.522

SVS: sustained viral suppression; ART: antiretroviral therapy; TDF: tenofovir; 3TC: lamivudine; EFV: efavirenz; AZT: zidovudine; LPV/r: lopinavir/ritonavir; NGO: non-governmental organization;

*: the rates of 18-month SVS were significantly different within categories of this characteristic, $p<0.1$;

Factors associated with SVS

As shown in Table 4.5, after controlling for all potential confounders and effect measure modifiers, the odds of SVS for participants who had ever asked about VL testing results was higher than 3 times (aOR=3.08, 95%CI=1.52-6.26) of those who had never asked. Participants who had higher baseline CD4 count (*200-350/μL vs. <200/μL*: aOR=3.32, 95% CI=1.47-7.53; *>500/μL vs. <200/μL*: aOR=2.90, 95% CI=1.13-7.91), had got support from NGOs (*Much support vs. No support:* aOR= 2.54, 95% CI=1.07-6.02), and had lower score for VL-related knowledge (*Lower score vs. Higher score:* aOR=4.37, 95% CI=1.85-10.32) were more likely to achieve SVS. In addition, participants who were older than 60 years *(>60 vs. 18-30*: aOR=0.19, 95% CI=0.06-0.65), initiated ART later than 1 year after HIV diagnosis *(>1 year vs. <7 days*: aOR=0.24, 95% CI=0.10-0.60), and were receiving ART in Site C (*Site C vs. Site A*: aOR=0.28, 95% CI=0.10-0.76) were less likely to have SVS. When the definition of SVS was changed from maintaining at viral suppression for 18 months to 12 months, the experience of asking about VL testing results was still strongly associated with SVS (aOR=2.76, 95% CI=1.37-5.55) (Supplement Table 4.1). After excluding the participants who had no awareness of VL test (n=44), a positive association between asking about VL results and SVS was still observed (OR=5.7, 95%CI=2.4-13.5).

Table 4.5 Factors associated with sustained viral suppression for 18 months by logistic regression modeling, N=264

Variable	Sustained viral suppression (n%)	Univariate model		Multivariate model	
		cOR (95% CI)	P-value	aOR (95% CI)	P-value
Overall	161 (100)				
Ever asked about VL testing results before patient notification					
Yes	103 (64.0)	1.81 (1.10, 3.00)	**0.021**	3.08 (1.52, 6.26)	**0.002**
No	58 (36.0)	--		--	
Gender					
Male	92 (57.1)	0.92 (0.56, 1.52)	0.738	1.12 (0.57, 2.18)	0.739
Female	69 (42.9)	--		--	
Age at ART initiation, years					
18-30	36 (22.4)	--		--	
30-45	68 (42.2)	0.72 (0.36, 1.45)	0.359	0.81 (0.35, 1.91)	0.633
45-60	45 (28.0)	0.74 (0.35, 1.58)	0.438	1.21 (0.46, 3.19)	0.707
>60	12 (7.5)	0.30 (0.12, 0.76)	**0.011**	0.19 (0.06, 0.65)	**0.008**
Education level					
Illiteracy & elementary school	67 (41.6)	--		--	
Middle school	59 (36.7)	1.67 (0.95, 2.94)	0.074	1.23 (0.62, 2.44)	0.561
High school & above	35 (21.7)	1.99 (0.99, 4.00)	0.055	1.53 (0.62, 3.79)	0.354
ART site					
Site A	56 (34.8)	--		--	
Site B	56 (34.8)	1.04 (0.55, 1.96)	0.914	0.97 (0.32, 2.96)	0.962
Site C	49 (30.4)	0.55 (0.30, 1.01)	0.053	0.28 (0.10, 0.76)	**0.012**
Time of ART initiation					
2015	85 (52.8)	1.56 (0.95, 2.57)	0.081	1.78 (0.98, 3.25)	0.059
2016	76 (47.2)	--		--	
Interval between HIV diagnosis and ART initiation					
<7 days	72 (44.7)	--		--	
7 days - 1 month	29 (18.0)	0.58 (0.30, 1.13)	0.110	0.50 (0.23, 1.11)	0.087
1 month - 1 year	37 (23.0)	0.97 (0.49, 1.93)	0.939	0.77 (0.34, 1.76)	0.534
>1 year	23 (14.3)	0.50 (0.25, 1.01)	0.053	0.24 (0.10, 0.60)	**0.002**
CD4 cell count/μL at ART initiation					
<200	36 (22.4)	--		--	
200-350	57 (35.4)	2.28 (1.18, 4.40)	**0.014**	3.32 (1.47, 7.53)	**0.004**
350-500	36 (22.4)	1.33 (0.68, 2.63)	0.407	0.92 (0.40, 2.11)	0.874
>500	32 (19.9)	2.13 (0.99, 4.60)	0.053	2.90 (1.13, 7.91)	**0.028**
Baseline ART regimen					
TDF+3TC+EFV	69 (42.9)	--		--	
AZT+3TC+EFV	49 (30.4)	0.61 (0.34, 1.08)	0.091	0.78 (0.34, 1.81)	0.567

LPV/r+other drugs	20 (12.4)	1.13 (0.47, 2.73)	0.791	2.59 (0.74, 8,98)	0.135
Other combinations	23 (14.3)	0.65 (0.31, 1.36)	0.350	0.57 (0.22, 1.49)	0.247
Total score of ART/VL related knowledge					
Lower	77 (47.8)	1.14 (0.69, 1.87)	0.615	4.37 (1.85, 10.32)	**<0.001**
Higher	84 (52.2)	--		--	
Current marital status					
Single	48 (30.0)	--		--	
Married, with an HIV negative spouse	57 (35.6)	1.04 (0.57, 1.88)	0.900	1.06 (0.45, 2.49)	0.895
Married, with an HIV positive spouse	55 (34.4)	1.49 (0.79, 2.80)	0.221	1.87 (0.73, 4.82)	0.196
Living status					
Live alone	36 (22.4)	1.20 (0.67, 2.15)	0.532	0.94 (0.40, 2.24)	0.891
Live with a spouse/partner	52 (32.3)	0.97 (0.52, 1.81)	0.915	0.77 (0.38, 1.58)	0.475
Live with family members (including the spouse/partner)	73 (45.3)	--		--	
Support from NGOs					
Got much support	68 (42.2)	1.25 (0.69, 2.26)	0.457	2.54 (1.07, 6.02)	**0.034**
Got some support	49 (30.4)	1.40 (0.73, 2.68)	0.307	1.76 (0.75, 4.10)	0.193
Never got any support	44 (27.3)	--		--	

ART: antiretroviral therapy; TDF: tenofovir; 3TC: lamivudine; EFV: efavirenz; AZT: zidovudine; LPV/r: lopinavir/ritonavir; NGO: non-governmental organization;

Discussion

This study is one of the first studies to investigate factors associated with SVS among PLWH in rural China. In our study, more than 95% of participants who initiated ART from 2015 to 2016 had a VL < 200 copies/mL by the end of 2018, and 61.0% of the participants had been at SVS status for at least 18 months. Patients who had taken the initiative to ask about VL testing results were significantly more likely to achieve SVS. In addition, some important factors associated with SVS were also identified, which could be helpful to add new evidence for current ART policy and to make new policies on improving ART service in rural China.

Most participants in the three study sites were not been informed of their VL testing results until the next routine follow-up visits, which were generally 3 to 4 months after VL blood collection. More than half (58.3%, n=154) of participants had ever taken the initiative to ask about VL testing results before the patient notification. After controlling all potential confounders and potential effect modifiers, proactively asking about VL testing results became a much stronger predictor of SVS (Table 4.5). PLWH who had asked about VL testing results might have positive attitudes towards ART and feel less HIV-related stigma. Patients with positive attitudes towards ART could be more concerned about their ART response and more likely to communicate with healthcare providers to ask about testing results. Studies conducted in African countries and a systematic review considering Asian populations[27–29] reported that PLWH with positive attitudes towards ART had better treatment adherence, which could directly improve their virological outcomes. In addition, previous studies[30–33] reported that perceived stigma among PLWH was associated with the delayed presentation in care and less usage of healthcare service, leading to diminished ART adherence and adverse health outcomes. However, it also should be pointed out

that PLWH with unsuppressed VL might be informed earlier than others, which would reduce the likelihood that they would feel a need to inquire about the results.

Participants who were at a higher level of VL-related knowledge were more likely to proactively ask about VL testing results, and an obvious dose-response relationship was observed in our study population ($P_{trend}<0.001$). A qualitative study[34] in rural African settings showed that a lack of HIV/ART-related knowledge could challenge the patient-provider relationship, which reduced patients' willingness to communicate with healthcare providers. However, it is still controversial whether HIV/ART-related knowledge is positively associated with good treatment outcomes. Some previous studies[27,35–37] reported a high level of HIV/ART-related knowledge was associated with better ART adherence, which directly contributed to SVS. Whereas, our study reported the opposite result, which indicated participants with better VL-related knowledge were less likely to achieve SVS. Among the 154 participants who had ever actively asked about VL testing results, the VL-related knowledge was not associated with SVS in both univariate and multivariate analyses. This result was supported by a qualitative study conducted in the United States[38], which indicated that most PLWH just simply followed the physicians' instruction, and good knowledge of HIV and ART was not associated with good treatment adherence. Noticeably, among the 110 participants who had never asked about VL results, people with higher VL-related knowledge score were significantly less likely to have SVS (aOR=0.11, 95% CI=0.02-0.57). A possible explanation of this finding was that PLWH who had better HIV/ART-related knowledge were more likely at higher socioeconomic status (SES) and thus to have higher levels of perceived stigma towards HIV infection, especially in the rural settings[39,40], so that the ART adherence and outcomes might be adversely affected[31,41,42]. Additionally, it was

also possible that patients who had suboptimal virological outcomes were more likely to receive patient education, which helped them get a higher score for VL-related knowledge in the survey which did not translate to a higher probability of achieving SVS.

The current standard ART practice in China does not require the VL test at baseline, thus the baseline CD4 cell count is regarded as the important predictor of ART response[14,43,44]. Our study suggested that participants with a baseline CD4 cell count > 200/μL at ART initiation were more likely to achieve SVS, but no dose-response was observed. This finding was supported by some previous studies[45,46], which reported baseline CD4 cell count lower than 200/μL was significantly associated with virologic failure. In each of the three study sites, there were one to three NGO staff assisting the physicians in daily work and providing peer support to patients. A total of 110 participants reported they had "got much support" from the NGO staff, and they were more likely to achieve SVS compared to those who had "never got any support" from local NGOs. Studies[47,48] conducted in LMICs demonstrated the local NGO staff could provide service that was very helpful to improve PLWH's adherence and ART outcomes. In addition, there was a trend that the participants who initiated ART in 2015 were more likely to achieve SVS by the end of 2018 compared to those who initiated ART in 2016, although the association was not statistically significant in our main analyses (*SVS for 18 months*: aOR=1.78, 95% CI=0.98, 3.25; *SVS for 12 months*: aOR=1.91, 95% CI=1.03, 3.46). Generally, PLWH who remained in clinical care for a longer time would have better virological outcomes.

A new ART policy requiring immediate ART regardless of CD4 count was implemented in the second half of 2016 in China and gradually scaled up to the whole country[14,49]. Since only

PLWH who initiated ART in 2015 and 2016 were included in our study, the majority of participants still used the old ART initiating criteria, which required initiating ART after CD4 cell count dropped to < 500/μL. In our study, a total of 62.7% of the participants initiated ART within 1 month after HIV diagnosis. Participants who initiated ART later than 1 year after HIV diagnosis were significantly less likely to achieve SVS. A nationwide cohort study conducted in China[9] also reported that delayed ART initiation could significantly increase the risk of treatment failure. Another finding in our study was that participants initiated ART after the age of 60 years were less likely to achieve SVS compared to those who initiated ART at the age of 18 to 30. Previous cohort studies[9,50] conducted in Asian populations indicated that older age could be an independent predictor for suboptimal ART outcomes. In addition, participants recruited from ART Site C were less likely to achieve SVS. About one-third of participants from Site C were recruited from patient education meetings, which tended to recruit PLWH who had suboptimal adherence and VL results. Hence, it was possible that the overall SVS rate (%) among participants in Site C was influenced.

There are some limitations to our study. First, the recall bias could exist since the information about previous VL testing experience was collected based on participants' recollection and might have caused non-differential misclassification of the study exposure. To minimize the influences of the recall bias, we didn't recruit PLWH who initiated ART before 2015. Second, because the data on the temporal relationships between the exposure and other covariates (e.g. VL-related knowledge) and the outcome were collected cross-sectionally, causal relationships between these factors and SVS could not be determined. Third, there might be a selection bias in our study. The total numbers of patients who were screened for eligibility and invited to participate in the

survey were unknown, so that an accurate participation rate could not be calculated. However, the participants' recruitment was completed prior to VL-related data extraction, so the recruitment was not influenced by the SVS status. In addition, as shown in Supplement Table 4.2, most of the basic demographic and clinical characteristics were comparable between the participants and those who were eligible but didn't participate in the survey. Therefore, the participants in the survey could have certain representativeness of the whole eligible population. Fourth, only the PLWH who were still alive and retained in care during the survey-period could be recruited in the study, so that a survival bias might exist and the rates (%) of viral suppression and SVS were overestimated. Finally, since we only selected Wenshan as the study location, the generalizability of the findings in our study might be limited.

Good virological outcomes of ART among PLWH retaining in care were observed in our study. In resource-limited settings where ART service has not been fully developed, it is crucial to encourage patients to actively communicate with healthcare providers and raise patients' concerns of ART and VL testing. HIV/ART-related knowledge is not sufficient for optimal treatment outcomes. Patients who are older than 60, have baseline CD4 counts < 200/μL and initiate ART later than 1 year after HIV diagnosis need to be targeted. The local NGOs should be recruited to assist in the support of ART services as a strategy to increase SVS. To further investigate factors contributing to better virological outcomes among rural PLWH, larger-scaled follow-up studies covering more regions and participants are needed in the future.

Supplement Table 4.1 Factors associated with sustained viral suppression for 12 months by logistic regression modeling, N=264

Variable	Sustained viral suppression (n%)	Univariate model		Multivariate model	
		cOR (95% CI)	P-value	aOR (95% CI)	P-value
Overall	164 (100)				
Ever asked about VL testing results before patient notification					
Yes	104 (63.4)	1.73 (1.05, 2.87)	**0.033**	2.76 (1.37, 5.55)	**0.004**
No	60 (36.6)	--		--	
Gender					
Male	94 (57.3)	0.93 (0.56, 1.55)	0.789	1.10 (0.57, 2.13)	0.772
Female	70 (42.7)	--		--	
Age at ART initiation, years					
18-30	36 (22.0)	--		--	
30-45	69 (42.1)	0.75 (0.37, 1.51)	0.419	0.88 (0.38, 2.06)	0.770
45-60	46 (28.0)	0.79 (0.37, 1.68)	0.536	1.25 (0.48, 3.26)	0.654
>60	13 (7.9)	0.34 (0.13, 0.86)	**0.023**	0.25 (0.08, 0.84)	**0.025**
Education level					
Illiteracy & elementary school	70 (42.7)	--		--	
Middle school	59 (36.0)	1.80 (0.89, 2.63)	0.100	1.19 (0.56, 2.21)	0.750
High school & above	35 (21.3)	1.52 (0.86, 2.67)	0.148	1.41 (0.58, 3.45)	0.452
ART site					
Site A	56 (34.2)	--		--	
Site B	56 (34.2)	1.04 (0.55, 1.96)	0.914	0.96 (0.32, 2.87)	0.936
Site C	52 (31.7)	0.63 (0.34, 1.15)	0.129	0.36 (0.14, 0.96)	**0.042**
Time of ART initiation					
2015	87 (53.1)	1.63 (0.98, 2.69)	0.058	1.91 (1.05, 3.46)	**0.034**
2016	77 (46.9)	--		--	
Interval between HIV diagnosis and ART initiation					
<7 days	74 (45.1)	--		--	
7 days - 1 month	30 (18.3)	0.57 (0.29, 1.13)	0.106	0.53 (0.24, 1.17)	0.115
1 month - 1 year	37 (22.6)	0.90 (0.45, 1.78)	0.751	0.72 (0.31, 1.63)	0.426
>1 year	23 (14.0)	0.46 (0.23, 0.93)	**0.031**	0.25 (0.10, 0.61)	**0.002**
CD4 cell count/μL at ART initiation					
<200	38 (23.2)	--		--	
200-350	57 (34.8)	2.04 (1.06, 3.95)	**0.034**	2.87 (1.27, 6.45)	**0.011**
350-500	37 (22.6)	1.27 (0.64, 2.52)	0.488	0.90 (0.39, 2.05)	0.801
>500	32 (19.5)	1.91 (0.89, 4.12)	0.099	2.62 (1.00, 6.84)	**0.049**
Baseline ART regimen					
TDF+3TC+EFV	71 (43.3)	--		--	

AZT+3TC+EFV	50 (30.5)	0.58 (0.32, 1.04)	0.069	0.62 (0.27, 1.42)	0.258
LPV/r+other drugs	20 (12.2)	1.03 (0.43, 2.51)	0.943	2.10 (0.62, 7.13)	0.234
Other combinations	23 (14.0)	0.59 (0.28, 1.25)	0.169	0.46 (0.18, 1.19)	0.109
Total score of ART/VL related knowledge					
Lower	79 (48.2)	1.18 (0.72, 1.95)	0.510	4.43 (1.89, 10.37)	**<0.001**
Higher	85 (51.8)	--		--	
Current marital status					
Single	50 (30.7)	--		--	
Married, with an HIV negative spouse	57 (35.0)	0.94 (0.52, 1.71)	0.840	0.99 (0.42, 2.30)	0.976
Married, with an HIV positive spouse	56 (34.3)	1.42 (0.75, 2.70)	0.281	1.85 (0.73, 4.75)	0.198
Living status					
Live alone	38 (23.2)	1.27 (0.71, 2.28)	0.421	1.12 (0.47, 2.64)	0.799
Live with a spouse/partner	53 (32.3)	1.11 (0.59, 2.09)	0.748	0.83 (0.41, 1.69)	0.607
Live with family members (including the spouse/partner)	73 (44.5)	--		--	
Supports from NGOs					
Got much supports	70 (42.7)	1.28 (0.71, 2.32)	0.410	2.50 (1.07, 5.86)	**0.035**
Got some supports	49 (29.9)	1.33 (0.70, 2.55)	0.389	1.72 (0.74, 3.96)	0.205
Never got any support	45 (27.4)	--		--	

ART: antiretroviral therapy; TDF: tenofovir; 3TC: lamivudine; EFV: efavirenz; AZT: zidovudine; LPV/r: lopinavir/ritonavir; NGO: non-governmental organization;

Supplement Table 4.2 The comparison of demographic and clinical characteristics for eligible PLWH who participated in and didn't participate in the survey

Characteristics	Total eligible PLWH (n%)	PLWH participated survey (n%)	PLWH not participated survey (n%)	P-value
Overall	815 (100)	264 (100)	551 (100)	
Gender				
Male	491 (60)	153 (58)	338 (61)	0.355
Female	324 (40)	111 (42)	213 (39)	
Age at ART initiation, years				
Median (IQR)	41.8 (32.2, 51.4)	41.9 (32.3, 51.9)	41.5 (32.1, 50.6)	0.574
Education level				
Illiteracy & elementary school	389 (48)	124 (47)	265 (48)	0.171
Middle school	295 (36)	89 (34)	206 (38)	
High school & above	127 (16)	50 (19)	77 (14)	
Route of HIV transmission				
Heterosexual behaviors	770 (94)	252 (95)	518 (94)	0.398
Other	45 (6)	12 (5)	33 (6)	
Interval between HIV diagnosis and ART initiation				
Median (IQR), days	14 (5, 173)	13 (5, 161)	14 (6, 182)	0.224
CD4 cell count/μL when ART initiated				
Median (IQR)	316 (199, 447)	321 (210, 452)	314 (193, 443)	0.620
Baseline ART regimen				
TDF+3TC+EFV	278 (34.1)	104 (39.4)	174 (31.6)	**0.008**
AZT+3TC+EFV	315 (38.7)	90 (34.1)	225 (40.8)	
LPV/r+other drugs	66 (8.1)	29 (11.0)	37 (6.7)	
Other combinations	156 (19.1)	41 (15.5)	115 (20.9)	

PLWH: people living with HIV, ART: antiretroviral therapy; TDF: tenofovir; 3TC: lamivudine; EFV: efavirenz; AZT: zidovudine; LPV/r: lopinavir/ritonavir

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Chapter 5: Conclusions

Despite great achievements in HIV/AIDS epidemic control that have been made in China, there is still a gap with the 90-90-90 targets. This dissertation focuses on the centralized viral load (VL) test in rural China. Three important but easily-neglected topics throughout the whole process of VL testing are respectively investigated in three sub-studies. First, universal access to timely VL testing is the foundation of antiretroviral therapy (ART) response evaluation. Second, poor efficiency and prolonged turnaround time (TAT) of VL testing greatly reduce the value of VL testing. Third, the process of patient notification of VL testing is also associated with ART outcomes. To our knowledge, this is one of the first studies specifically targeting the VL testing in rural PLWH of China.

Results of the first study show that 58.5% of people living with HIV (PLWH) receiving ART underwent their first VL test within the first year of ART. In the rural areas, where the traffic and economy are not fully developed, it is highly recommended to decentralize HIV healthcare service to remote areas, so that PLWH who are living far away from ART facilities and have suboptimal health status are more likely to utilize the ART services. The working burden of rural healthcare providers is quite heavy since the physician-patient ratio is low and rural PLWH are highly dependent on the healthcare providers. It is important to increase the number of healthcare providers and providing them more training opportunities. In addition, the local non-governmental organizations (NGOs), which provide service and peer support for PLWH in local ART sites, can be enhanced to act as an auxiliary method to improve ART uptake and adherence. In our study, PLWH who are younger than 30 years and at a low education level need to be particularly targeted to improve the VL testing rates.

The median TAT among all VL tests conducted in the study sites during 2018 is 54 days (IQR: 36-92), which is still suboptimal. Challenges to increase the VL testing efficiency exist in each process of the centralized VL testing. Both the number of healthcare providers at local ART sites and the number of technicians at the VL testing laboratory should be increased. It is suggested to strengthen the relevant professional training, improve the laboratory network construction, ensure sufficient testing reagent supplies, and improve the ART information system. The application of new point of care (POC) VL testing technologies in local ART sites and a flexible ART referral system among migrant PLWH should also be explored in rural China.

Good virological outcomes of ART among PLWH retaining in care are observed in the third study. By the end of 2018, more than 95% of participants who initiated ART from 2015 to 2016 had achieved a VL<200 copies/mL, and 61.0% had achieved sustained viral suppression (SVS) for at least 18 months. In resource-limited settings where ART service has not been fully developed, it is more important to raise patients' concerns for ART and encourage patients to actively communicate with healthcare providers than only imparting the HIV/ART-related knowledge. To improve SVS, the local NGOs should be recruited to assist in the support of ART services. In addition, PLWH who are older than 60, have baseline CD4 counts < 200/μL, and initiate ART later than 1 year after HIV diagnosis are key populations for improving long-term virological outcomes.

To achieve the 90-90-90 targets, the local ART facilities, VL testing laboratories, healthcare policymakers at all levels of government and the NGOs should collaborate and coordinate their

procedures. Larger-scaled follow-up studies covering more regions and participants are needed in the future to investigate how to improve ART service among rural PLWH in China.