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Analysis of the immunological response to antiviral therapy in patients with different subtypes of HIV/AIDS: a retrospective cohort study

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Abstract

Objective: This study was conducted to evaluate efficacy of standardized antiretroviral therapy (ART) among different HIV subtypes in people living with HIV/AIDS (PLWHA), as well as to screen the best medications for PLWHA.

Methods: Based on a historical cohort study, PLWHA living in Huzhou, China from 2018 to 2020 were enrolled and data on their demographic characteristics and laboratory assessments were collected. Recovery of immune system was used to assess the curative effect of ART, and increased percentage of $CD4^+$ T lymphocyte (CD4) count over 30% after receiving ART more than 1 year was determined as immunopositive. Multiple logistic regression model was performed to comprehensively quantify the association of PLWHA immune response status with the virus subtype. In addition, the potential interaction and joint effects of the subtype and treatment regimen on immune response status were further investigated.

Results: Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC and other subtypes, the percentages of immunopositive were 74.0%, 65.6% and 69.6%, respectively. Depending on multivariate logistic regression models, adjusted odds ratio (OR) [95% confidence interval (CI)] were 0.8 (0.4,1.4) for CRF07_BC vs. other subtypes vs. CRF01_AE, and 1.2 (0.6, 2.3) for other subtypes vs. CRF07_BC, respectively. No significant association between immunological response and HIV subtype was observed after adjusting for some potential confounders. We also did not detect obvious interaction and joint effects of HIV subtype with ART strategies on the immune response ($P_{multiplicative\ interaction}$ =0.37, $P_{additive\ interaction}$ =0.80).

Conclusion: Standardized ART was beneficial to all PLWHA regardless of their HIV subtypes though PLWHA with CRF01_AE were possible to have better efficacy to some extent.

Strengths and limitations of this study

- Our findings were based on a population-based cohort study rather than a case-control or descriptive study.
- The confounding factors were solved by adjusting for multi - factor, hierarchical analysis and other methods.
- HIV-1 subtypes are regionally relevant and sampling bias is inevitable.
- This study contained a small sample size because HIV-1 genotyping is not routinely tested in China.
- Viral load is missing from a large number of records.

Introduction

AIDS is one of the world's major public health problems[1]. As of 2019, 38 million people worldwide are living with HIV and 690,000 people have died from HIV-related illnesses[2]. HIV harms human health primarily by infecting the body and destroying immune cells, and people living with HIV often live as asymptomatic carriers for decades or more before eventually developing AIDS and secondary comorbidities[3]. In China, the HIV epidemic has generally stabilised from a year-on-year increase at the beginning of the 21st century[4]. However, there is a wide variation in the type of epidemic and geographical spread, which poses a greater challenge to the prevention and control of AIDS in China[5].

Although there is still no effective cure for AIDS, previous studies have shown that ART has been widely used to control HIV/AIDS with good results[6]. Since 2016, all people living with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell level, have been eligible to receive free state ART medication and regular follow-up visits by health service staff[7]. Until 2019, the world has entered an era of universal access to ART, and many developing countries, including China, have largely achieved full coverage of ART treatment, with an increasing number of PLWHA receiving free ART and receiving effective viral control[8]. Despite this, the HIV epidemic in China shows no sign of slowing down[9, 10].

HIV, as a highly diverse virus, has significant genetic variability and a high viral replication rate, which may produce biological variability that affects treatment outcomes[11]. HIV is divided into two main groups, HIV-1 and HIV-2, of which HIV-2 tends to have a lower viral load than HIV-1 infected individuals, which could explain the low transmission rate and the almost complete absence of mother-to-child transmission of HIV-2. HIV-1 is by far the most prevalent type, with almost 95% of global HIV infections are of type 1. It is made up of four different spectrums, including groups M, O, N and P. Group M is the world's leading group of HIV infections[12]. Group M is the world's leading HIV/AIDS epidemic agent and is further divided into ten subtypes (A,B,C,D,F,G,F,J,K and L) and a series of circulating recombinant forms (CRF)[2]. The CRFs have been developed in China. In China, circulating recombinant forms of HIV-1 are the most common, with HIV-1 CRF01_AE and CRF07_BC accounting for 36.2% and 40.8% of the population reported to be infected that year, respectively, according to the results of the 2018 China Molecular Epidemiology Survey[13], and these two branches have become the most predominant HIV CRFs in China[14]. Studies on the effectiveness of antiretroviral therapy in patients with the major subtypes of HIV-1 are scarce and inconclusive. An analysis of the impact of HIV-1 subtype diversity on the long-term clinical outcomes of ART in Guangxi Province suggests that patients with CRF01_AE may benefit more from immediate ART compared to CRF07_BC[15]. In contrast, studies in southern Nigeria and the UK did not observe an association between

HIV-1 subtypes and immunological or virological response after treatment[16, 17]. We speculate that these differences may be region and population related. As the efficacy analysis of HIV-1 subtypes in Huzhou, China is in a gap, this study aimed to analyze the immune recovery status of HIV/AIDS patients with several major subtypes after ART in Huzhou from 2018-2021 to help refine the ART regimen.

Methods

Study design and participants

The data used in this study were extracted from 625 new HIV/AIDS patients in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou region from 2018-2020. The study was approved by the Ethics Committee of the Centre for Disease Control and Prevention (CDC) in Wenzhou. All participants identified in AIDS-PCIS will receive a combination antiretroviral regimen containing at least 3 antiretroviral drugs and they sign an informed consent form at the time of initiation of antiretroviral therapy, allowing the use of clinical records in future epidemiological studies. Inclusion criteria for study participants were as follows: 1) complete laboratory blood tests prior to ART and no missing data on CD4 cell counts at baseline ; 2) at least one clinic visit; 3) residing in the Lakeland area, including temporary residents; 4) starting ART between January 1, 2018 and December 31, 2020; 5) having a complete records of CD4 cell counts from 9-15 months after receiving ART. Only 326 study participants ultimately met the inclusion criteria, and they better represented the prevalence of different subtypes of HIV/AIDS patients in the Huzhou region during 2018-2020. The flow chart of participants is shown in Figure 1. In the database, we set patients with subtype CRF01_AE as subtype I; patients with subtype CRF07_BC as subtype II; and the remaining subtypes as subtype III.

Patient and public involvement

None.

Demographic characteristics and laboratory information

Data on the demographic and clinical characteristics of the study participants were collected at the time of their registration in a face-to-face survey or extracted from their medical records using a structured questionnaire designed specifically for AIDS-PCIS. Information included age, sex, height, weight, marital status, occupation, history of STIs, disease status, source of sample, clinical staging by the World Health Organization (WHO), route of infection, etc. The Body mass index (BMI) is calculated by the formula: BMI = weight (kg)/height $(m)^2$. Information on laboratory tests was obtained from the Huzhou CDC or local hospital. Tests included CD4 , $CD8^+$ T lymphocytes (CD8), viral load (VL), white blood cell (WBC), platelet (PLT), haemoglobin (HB), serum creatinine (SCR), triglyceride (TG), total cholesterol (TC), fasting plasma Glucose (GLU), aminotransferase (ALT), aspartate (AST), total bilirubin (TBIL), etc. All laboratory parameters are assessed at the local hospital or at the central laboratory of the Huzhou CDC and are carried out by trained technicians in strict accordance with clinical guidelines.

ART regimens

In this study, 326 patients with HIV/AIDS were under treatment, of whom 253 (77.6%) were on the lamivudine + efavirenz + tenofovir (3TC + EFV + TDF) regimen, while 53 (16.2%) were on the zidovudine + efavirenz + lamivudine (AZT + EFV + 3TC) regimen (53/326). The remaining regimens were lamivudine + nevirapine + tenofovir (3TC + NVP + TDF) and lamivudine + efavirenz + clindamycin (3TC + EFV + clindamycin), and a few other regimens totalling 20 cases, which we grouped together when collating the data.

Study outcomes

While there are many different outcomes for assessing the effectiveness of antiretroviral therapy, such

as all-cause mortality, disease progression from HIV to AIDS, immunological and virological responses[7]. However, since only one of the 326 HIV/AIDS patients died during treatment and there were no variables associated with HIV-to-AIDS transition due to the large number of missing viral load data, immune recovery status was chosen as the only outcome in this study. We defined immunological response as an increase in CD4 cell counts of more than 30% from baseline at 12 months after initiation of ART. For those patients who did not receive CD4 cell count testing 12 months after starting ART treatment, we selected their test results measured between 9-15 months for analysis, which was the closest estimate to 12 months[18]. Although immune response could also be assessed by normalisation of the CD4 /CD8 ratio, too few patients met a CD4 /CD8 ratio <0.8 at baseline to proceed.

Statistical analysis

Since missing values can lead to biased results to some extent, this study first eliminated variables with a proportion of missing values greater than 30% and then used multiple imputation by chained equation methods to impute (5 times) missing baseline data[19]. To assess the effect of missing value filling, a sensitivity analysis was also conducted to compare the differences before and after filling (see Table S1).

Baseline demographic characteristics and laboratory tests were compared between patients with good and poor outcomes. Continuous type variables, which did not follow a normal distribution, used medians (quartile 1, quartile 3) to describe their baseline characteristics and the Mann-Whitney U test was used to compare differences between the two groups. Categorical variables were then described using frequencies (composition ratios) and compared between groups using either the chi-square test on an R x C scale or the Fisher exact probability method. Univariate and multivariate logistic regression models were used to evaluate differences in immunological response between these major subtypes, correcting for variables that were statistically significant (p value < 0.05) in Table 1 as covariates and not including CD4 as a covariate as the outcome was defined using CD4 cell counts. Subgroup analysis was used to explore the impact of different demographic characteristics and different levels of laboratory tests on the above association effects. Given the large heterogeneity in the other subtypes, we selected only the two main subtypes, CRF01_AE and CRF07_BC. The categorical variables in the subgroup analysis were selected as four indicators: sex, route of infection, education, and marital status. The remaining indicators, such as occupation, WHO clinical stage, and whether anaemia was present, were not assessed as subgroup variables because the number of subgroups was too small. The numerical variable BMI was based on the WHO definition of obesity in Asian populations[20].The cut-off value of 25 kg/m^2 was used. Other numerical indicators were determined based on median. As the original aim of this study was to refine treatment regimens for different subtypes of patients, we went on to explore the interaction and combined effects between subtypes and initial treatment regimens in an attempt to uncover the link between the two.

All the above tests were two-sided and P<0.05 was considered significant. All data management and statistical analyses were performed using Stata/MP 15.1 for windows (Copyright 1985-2017 Stata Crop LLC, College Station, Texas 77845 USA) and SAS 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA.) was completed.

Results

Description of baseline characteristics

The retrospective cohort study of this study ultimately included a total of 326 participants for analysis, with the majority of PLWHA being male (84.4%) and Han Chinese (96.9%). The mean age was 41.9±15.0

years, with 20% of the study participants being under 42 years of age. The median (quartile 1, quartile 3) baseline CD4 cell and CD8 cell counts were 279.5 (175.0-382.0) and 657.75 (481.0-913.0) cells/uL, respectively. In addition, the median (quartile 1, quartile 3) baseline leukocyte counts were 5.6 (4.5, 6.7)10^9/L. A comparison of the baseline characteristics of the two populations (well-treated vs. poorly-treated PLWHA) is shown in Table 1, where the immunologically responsive PLWHA had significantly higher baseline CD4, CD8, and WBC levels than the immunologically unresponsive PLWHA, and a significantly shorter time from HIV diagnosis to treatment. We also observed that most of the study participants were in HIV-infected status at the time of ART and did not transition to AIDS status. Sexual contact that occurred out of wedlock was also more common among patients. Other characteristics, such as marital status and history of sexually transmitted diseases, these were similar or not significantly different between the two groups. In addition, gender and age were not statistically significant between the group of patients with good outcomes and the group of patients with poor outcomes, which avoids the impact of known and unknown confounding bias on the analysis of differences in outcomes to some extent.

Association of immune response status with virus subtypes in PLWHA participants

The association of immune response status with viral subtypes in PLWHA subjects is shown in Table 2. When uncorrected for confounding factors, no difference in immunological response was found between the three subtypes, using the CRF01_AE subtype as a reference (CRF07_BC subtype: P=0.158, OR (95%CI) = 0.7(0.4,1.2); other subtypes P=0.527, OR(95%CI)=0.8(0.4,1.6)). After correcting for variables such as infection status, white blood cell count, and time from diagnosis to treatment, the association between immunological response status and HIV subtype did not change significantly, and the difference in efficacy between the three subtypes remained non-significant (CRF07_BCsubtype: P=0.405, OR (95%CI) = 0.8(0.4,1.4); other subtypes: P=0.955, OR (95%CI) =1.0(0.5,2.0)). After removing patients with the CRF01_AE subtype, the remaining two subtypes were assessed with CRF07_BC as the reference variable and no significant results were found either (univariate analysis, other subtypes: P=0.561, OR (95% CI) = 1.2(0.7,2.2); multivariate analysis, other subtypes: P=0.545, OR (95% CI) =1.2(0.6,2.3)).

Subgroup analysis

Subgroup analyses were performed to identify changes in the association between subtype and ART efficacy across subgroups. Figure 2 shows that no effect was observed on the association between different HIV-1 subtypes and immunological response in all subgroups, including variables such as gender, route of infection, education, marital status, ALT, AST, SCR, WBC, and BMI.

Interaction and joint effect of treatment regimens and subtypes

Although the above analysis did not reveal any differences in the immune recovery status of patients with these subtypes after ART treatment, in order to make the whole analysis more comprehensive, we have further explored the possible treatment regimens for different subtypes so that clinicians can prescribe precise treatment for a specific subtype of patients. Due to sample size limitations, we only analysed the multiplicative interaction, additive interaction and combined interaction between CRF01_AE and CRF07_BC for the two main subtypes (see Table S2). The treatment efficiency of different regimens for different subtypes was first obtained and then the results of the interactions were obtained uncorrected ($P_{\text{multiplicative interaction}} = 0.27$, $P_{\text{additive interaction}} = 0.79$) and corrected for covariates ($P_{\text{multiplicative interaction}} = 0.37$, $P_{\text{additive interaction}} = 0.80$). Neither interaction was seen to be statistically significant, indicating that there was no interaction between the two variables of treatment regimen and subtype. Further assessing the combined effect of the two main subtypes and the three treatment regimens (see

Table S3), the results of the combined effect showed better efficacy for the subtype CRF01_AE subtype and the treatment regimen (3TC+EFV+TDF) compared to the treatment regimen (3TC+AZT+EFV) when uncorrected for any variable (P = 0.052, OR (95% CI) = 3.4(1.0,11.8)), but the combined effect disappeared after correction for covariates such as disease status and white blood cell count. The remaining combinations were not statistically significant with or without correction for covariates. This suggests that there was also no joint relationship between the three treatment regimens and the three subtypes.

Discussion

The global distribution of HIV-1 genotypes is highly heterogeneous and varies geographically[21]. In addition, the distribution of HIV-1 may be related to different routes of infection[22], forms of population mobility, etc. Various aspects of disease progression, all-cause mortality, viral suppression status, and immune recovery status following ART treatment in PLWHA may also be influenced by the diversity of HIV-1 genotypes. Therefore, it is important to understand the epidemiological characteristics of HIV-1 subtypes in a given region[23] to allow for more targeted treatment of the disease and control of the epidemic. This study showed that CRF01_AE (30.7%) was the predominant HIV-1 genotype in Huzhou City, followed by CRF07_BC (17.2%). This is similar to the findings of previous studies examining HIV-1 subtype diversity in Shanghai, Jiangsu and Guangxi provinces[11, 15, 24]. However, there are also studies with different results, such as a survey on HIV-1 in Yunnan Province that found CRF08_BC to be the most common subtype etc[25]. Previous studies have shown that the prevalence of CRF01_AE is significantly higher in southern provinces[26]. This is consistent with the location of Huzhou City. The difference in distribution may be related to the route of transmission. In our study, CRF07_BC was the most common genotype in the homosexual transmission population. In fact, it has been officially reported by the state that heterosexual transmission has become a major risk factor for PLWHA in China.

China has one of the highest number of HIV-1 genotypes, with 10 circulating recombinant forms (CRF) all identified for the first time in China (CRF01_AE, CRF07_BC, CRF08_BC, CRF55_01B, CRF57_BC, CRF59_01B, CRF61_BC, CRF62_BC, CRF64_BC, CRF65_cpx). CRF61_BC, CRF62_BC, CRF64_BC, CRF65_cpx). Previous studies by Taylor BS et al. have shown that HIV-1 subtype diversity is associated with response to antiretroviral therapy[27]. A comprehensive assessment of the impact of HIV-1 subtype diversity on long-term clinical outcomes during antiretroviral therapy (ART) can help to inform planning recommendations. Our study aims to investigate the differences in efficacy between subtypes and to find the most appropriate treatment regimen for each subtype in order to achieve precise treatment. Previous studies have found that baseline CD4 cell counts are significantly higher in CRF07_BC-infected patients than in CRF01_AE-infected patients. Our study also yielded consistent results. The available covariates (white blood cell count, disease status, etc.) were adjusted for during the analysis, but ultimately no differences were observed between several major subtypes in the population as a whole or in subgroups of the population. This is not quite in line with the results of some previous investigations, which found that CRF01_AE-infected patients experienced a faster rate of CD4 cell decline and more rapid HIV/AIDS progression in natural infection[28-30]. We speculate that this may be related to region and sample size. In addition, our study did not find interactions and combinations between different subtypes and different treatment regimens. However, further studies in larger populations are necessary to demonstrate this due to the limitations of sample size.

We must point out that there are certain limitations to this study. Firstly, HIV-1 subtypes are regionally relevant and sampling bias is inevitable. Secondly, our study was based on a retrospective cohort and

relied on historical records, which are prone to selection bias and information bias, and the records were also often lacking in confounding factors affecting the relationship between exposure and outcome, making it difficult to control for confounding. We tried to avoid potential confounding factors by stratified analysis . Third, as HIV-1 genotyping is not routinely tested in China, we included only a small proportion of patients with sequence data. The sample size was therefore limited and the patients in the cohort may not be representative of all patients on ART in Huzhou City during 2018-2020. Fourth, HIV viral load (VL) provides a valid reflection of viral replication in PHWLA, but VL is missing from a large number of records. Likewise, the number of deaths in this sample was too small to explore all-cause mortality in patients of different subtypes. Therefore, we ultimately chose immune recovery status as the study outcome. In fact, although we are now in the era of "full treatment" for HIV, long-term detection CD4 cell counts are necessary to determine disease progression and changes in immune status. Fifth, this study is a longitudinal cohort, but we did not assess time due to the inconsistency of the PLWHA follow-up time points, resulting in large time differences, and opted instead for a logistic regression model. However, comparisons of efficacy between subtypes that limit expected confounders are a strength of this study, and it is the first study in Huzhou to explore the interaction between HIV-1 subtypes and different treatment regimens. However, the applied value of our findings needs to be validated in a larger sample cohort.

Conclusion

In summary, we found no evidence of an association between HIV-1 subtypes and immunological treatment response, suggesting that currently widely used antiretroviral drugs have similar efficacy in the subtypes that predominate in the Huzhou city area. There was also no association between HIV-1 subtypes and several of the main treatment regimens, suggesting that treatment regimens are not very specific to several of the main subtypes. Moreover, the effect of new PLWHA in Huzhou City between 2018 and 2020 after receiving ART (69% of treatment effective) is evident. And in the future of AIDS prevention and control work, regular monitoring CD4 cell counts is of great significance for improving the quality of life of HIV/AIDS patients and reducing the risk of transmission.

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Author Contributions

J.M.H., L.J., L.X.Q. and Y.Z.R.: Conceptualization, funding acquisition, supervision, draft and editing. W.Y.N.: Conceptualization, data management, data analysis, methodology, draft and editing. T.Z.W., L.X.F., W.Z.Q., R.F.L. and Z.X.J.: Conducting a research and investigation process, data management, data collection. M.G.Y.: Conceptualization, data management, data analysis, methodology, writing-review and editing, supervision. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics Statement

The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease Control and Prevention (CDC) , and for which the batch number is HZ2021001.

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FIGURE LEGENDS

Figure1. A flowchart of the nascent selection of people living with HIV/AIDS (PLWHA) with different subtypes in Huzhou City, 2018-2020

Figure2. Forest plot of subgroup analysis

Adjusted for infection status, white blood cell count, time from diagnosis to treatment, and CD8 lymphocytes cell counts (when grouped by sex, route of infection, education, marital status, BMI, ALT, AST, SCR).

Abbreviations: OR=Odds ratio; CI=confidence interval; MSM=men who have sex with men; HR=Heterosexual transmission; BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Numerical variables in the subgroup analysis were grouped according to cut-off values (BMI) determined in previous studies or according to the median values of the indicators taken in the current study (ALT, AST, SCR).

Table1. Comparison of demographic and laboratory characteristics of PLWHA in different immune response states

Variables	Negative immunological response (n=101)	Positive immunological response (n=225)	P
Categorical variables			
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.267
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.189
Farmers	33 (32.7)	84 (37.3)	
Service industry	11(10.9)	30(13.3)	
Worker category	35 (34.7)	49(21.8)	
To be employed	7 (6.9)	20(8.9)	
Other	15 (14.9)	42(18.7)	
Education level			0.227
Primary school or under	35 (34.7)	62 (27.6)	
Middle and high school	53 (52.5)	119 (52.9)	
College or above	13(12.9)	44(19.6)	
History of venereal disease			0.576
None	84 (83.2)	182 (80.9)	
Yes	16(15.8)	42(18.7)	
Not available	1(1.0)	1(0.4)	
Route of infection			0.505
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.359
History of men who have sex with men	37 (36.6)	73(32.4)	
Sexual contact occurring out of wedlock	60 (59.4)	134(59.6)	
Sexual contact with a partner	4(4.0)	18(8.0)	
Marital status			0.368
Unmarried	31(30.7)	70 (31.1)	
Married or with a spouse	46(45.5)	116(51.6)	
Divorced or widowed	24 (23.8)	39(17.3)	
Regimens			0.876
3TC+AZT+EFV	18 (17.8)	35(15.6)	
3TC+EFV+TDF	77 (76.2)	176 (78.2)	
Other	6(5.9)	14(6.2)	
Infection status			0.002
AIDS	21 (20.8)	86(38.2)	

HIV	80 (79.2)	139(61.8)	
Sample source			0.964
Pre-operative testing	24 (23.8)	53(23.6)	
Testing Consultancy	20 (19.8)	50 (22.2)	
Other attendee testing	25 (24.8)	52(23.1)	
Other	32 (31.7)	70 (31.1)	
WHO Clinical Classification			0.228
I or II	99 (98.0)	224 (99.6)	
III or IV	2(2.0)	1(0.4)	
Continuous variables			
Age (years)	43.0 (30.0,56.0)	40.0 (29.0,52.0)	0.368
BMI (kg/m^2)	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.392
First CD4 cell counts(pcs/uL)	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.001
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.027
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.844
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.495
SCR (μmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.539
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.240
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.287
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.015
Time from diagnosis to start of treatment (days)	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.059

Note: Information on continuous variables: none followed a normal distribution, statistical description using median (lower quartile, upper quartile) and Mann-Whitney test to compare differences between groups. Information on categorical variables: statistical description using frequency (composition ratio), chi-square test on R x C scale or Fisher's exact probability method to compare differences between groups.

Abbreviations: PLWHA=people living with HIV/AIDS; MSM=men who have sex with men; HR=Heterosexual transmission;BMI=body mass index; CD4=CD4lymphocyte; CD8=CD8 lymphocyte; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine; 3TC+AZT+EFV=zidovudine+efavirenz+lamivudine; 3TC+EFV+TDF=lamivudine + efavirenz + tenofovir.

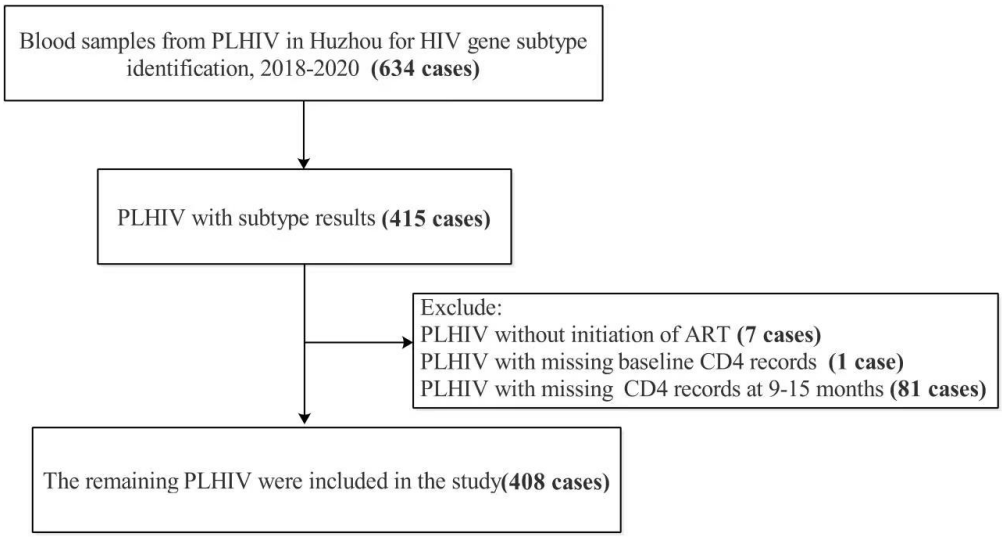
Table2. Association of immune response status with viral subtypes in PLWHA

			Univariate logistic regression model		Multivariable logistic regression model	
Variables	**N**	**# (%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Three subtypes						
CRF01_AE	100	74(74.00)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF07_BC	157	103 (65.6)	0.7(0.4,1.2)	0.158	0.8(0.4,1.4)	0.405
Other	69	48(69.6)	0.8(0.4,1.6)	0.527	1.0(0.5,2.0)	0.955
Two subtypes						
CRF07_BC	157	103 (65.6)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
Other	69	48(69.6)	1.2 (0.7,2.2)	0.561	1.2 (0.6,2.3)	0.545

Note: Three subtypes are compared using the CRF01_AE subtype as the reference variable and two subtypes are compared using the CRF07_BC subtype as the reference variable.

subtypes were included in the multivariable logistic regression model,with adjustment for Infection status, white blood cell count, time from diagnosis to treatment and CD8 lymphocyte cell.

Abbreviations: OR=Odds ratio; CI=confidence interval



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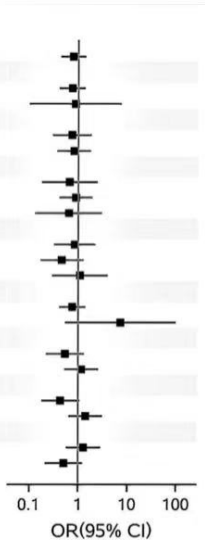
Subgroup	CRF01_AE	CRF07_BC	OR(95% CI)	P-Value
Total	74(28.79)	103(40.08)	0.79(0.44-1.41)	0.4320
Gender				
Male	67(73.63)	87(63.50)	0.75(0.41-1.38)	0.3500
Female	7(77.78)	16(80.00)	0.87(0.10-7.47)	0.9020
Infection pathway				
MSM	34(75.56)	40(60.61)	0.74(0.30-1.83)	0.5090
HR	40(72.73)	63(69.23)	0.82(0.37-1.78)	0.6070
Education				
primary school or under	69(75.00)	89(67.42)	0.66(0.18-2.47)	0.5370
middle and high school	5(62.50)	14(56.00)	0.89(0.41-1.91)	0.7660
college or above	43(72.88)	54(65.85)	0.64(0.13-3.01)	0.5680
Marital status				
Unmarried	19(82.61)	18(69.23)	0.85(0.32-2.24)	0.7440
Married	12(66.67)	31(63.27)	0.46(0.17-1.27)	0.1350
Divorced or widowed	12(66.67)	20(66.67)	1.08(0.29-4.01)	0.9040
BMI				
BMI<25	30(69.77)	31(67.39)	0.75(0.41-1.40)	0.3670
BMI ≥ 25	32(82.05)	52(64.20)	7.27(0.54-98.42)	0.1360
ALT				
ALT<25.1	32(78.05)	53(61.63)	0.54(0.22-1.32)	0.1740
ALT ≥ 25.1	42(71.19)	50(70.42)	1.16(0.52-2.57)	0.7210
AST				
AST<24.3	39(81.25)	47(58.75)	0.43(0.18-1.07)	0.0700
AST ≥ 24.3	35(67.31)	56(72.73)	1.41(0.64-3.12)	0.3930
SCR				
SCR<71.5	34(68.00)	54(69.23)	1.27(0.57-2.85)	0.5610
SCR ≥ 71.5	40(80.00)	49(62.03)	0.51(0.21-1.22)	0.1290



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TableS1. Sensitivity analysis of missing values

Variables with missing values	Before filling	After filling	P-value
BMI (kg/m^2)	22.0 (20.0,24.2)	22.0 (20.2,23.9)	0.931
AST (U/L)	23.0 (19.0,29.0)	24.2 (19.1,30.3)	0.236
SCR (μmol/L)	70.6 (61.0,79.5)	70.9 (60.9,80.6)	0.735
HB (g/L)	149.0 (135.0,158.0)	149.0 (135.8,157.0)	0.861
PLT (10^9/L)	206.0 (170.0,241.0)	203.0 (170.0,237.0)	0.798
WBC (10^9/L)	5.6 (4.5,6.7)	5.7 (4.5,6.7)	0.918
ALT (U/L)	25.0 (17.0,38.0)	25.1 (17.4,39.9)	0.570
CD8 cell counts (pcs/uL)	657.8 (481.0,913.0)	664.5 (479.0,919.0)	0.894

Abbreviations: BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

TableS2. Treatment effectiveness of different regimens for different subtypes and the interaction of subtypes with treatment regimens

Models	Positive immune response CRF01_AE				Positive immune response CRF07_BC				Positive immune response Other subtypes				$P_{multiplicative\ interaction}$	$P_{additive\ interaction}$
	Total [n(%)]	Option 1 [n(%)]	Option 2 [n(%)]	Option 3 [n (%)]	Total [n(%)]	Option 1 [n(%)]	Option 2 [n(%)]	Option 3 [n(%)]	Total [n(%)]	Option 1 [n(%)]	Option 2 [n(%)]	Option 3 [n(%)]		
Single–factor model	74/100 (74)	6/12 (50)	65/84 (77)	3/4 (75)	103/157 (65)	19/27 (70)	79/121 (65)	5/9 (56)	48/69 (70)	10/14 (71)	32/48 (67)	6/7 (86)	0.27	0.79
Multiple–factor model*	74/100 (74)	6/12 (50)	65/84 (77)	3/4 (75)	103/157 (65)	19/27 (70)	79/121 (65)	5/9 (56)	48/69 (70)	10/14 (71)	32/48 (67)	6/7 (86)	0.37	0.80

Note: Adjusted for infection status, white blood cell count, time from diagnosis to treatment and CD8 lymphocytes cell counts

Option 1: 3TC+AZT+EFV; Option 2: 3TC+EFV+TDF; Option 3: Other options

TableS3. Joint effect of subtype and treatment regimen

				Unadjusted		Adjusted	
Subtype	**Treatment**	**n**	**#(%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
CRF01_AE	Option 1	12	6(50.0)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF01_AE	Option 2	84	65 (77.4)	3.4(1.0,11.8)	0.052	2.8 (0.7,10.4)	0.127
CRF01_AE	Option 3	4	3 (75.0)	3.0(0.2,37.7)	0.395	3.3(0.2,44.1)	0.368
CRF07_BC	Option 1	27	19(70.4)	2.4(0.6,9.6)	0.226	2.3(0.5,10.1)	0.261
CRF07_BC	Option 2	121	79 (65.3)	1.9(0.6,6.2)	0.299	1.9 (0.5,6.8)	0.315
CRF07_BC	Option 3	9	5 (55.6)	1.3(0.2,7.1)	0.801	1.1(0.2,6.9)	0.906

Note: Adjusted for infection status, white blood cell count, CD8 cells, time from diagnosis to treatment

Option 1: 3TC+AZT+EFV; Option 2: 3TC+EFV+TDF; Option 3: Other options

Abbreviations: OR=Odds ratio; CI=confidence interval.

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Analysis of the immunological response to antiviral therapy in patients with different subtypes of HIV/AIDS: a retrospective cohort study

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Keywords: HIV/AIDS, ART, CD4 T lymphocyte count, CRF07_BC, CRF01_AE, immunological response

Abstract

Objective: To evaluate the effectiveness of standardized antiretroviral therapy (ART) among different HIV subtypes in people living with HIV/AIDS (PLWHA), and to screen the best ART regimen for this patient population.

Methods: Based on a historical cohort study, PLWHA residing in Huzhou, China, between –2018 and 2020, were enrolled. Data regarding demographic characteristics and laboratory investigation results were collected. Immune system recovery was used to assess the curative effectiveness of ART, and an increased percentage of $CD4^+$ T lymphocyte (CD4) counts> 30% after receiving ART for > 1 year was determined as immunopositive. A multiple logistic regression model was used to comprehensively quantify the association between PLWHA immunological response status and virus subtype. In addition, the joint association between different subtypes and treatment regimens on immunological response status was investigated.

Results: Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate logistic regression models, there was no difference in the immunological response between patients with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy [CRF07_BC: aOR(95%CI) = 0.8 (0.4,1.4); other subtypes: aOR(95%CI) = 1.2 (0.6, 2.3)]. There was no evidence of an obvious joint associationbetween HIV subtypesand ART regimens on immunological response.

Conclusion: Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01_AE.

Strengths and limitations of this study

- Findings of the present study were based on a population-based cohort rather than case-control or descriptive investigations.
- This was the first study in East China to analyze the association between HIV subtypes and immunological responses, considering the joint association of subtypes and ART regimens.
- HIV-1 subtypes were regionally relevant and sampling bias was inevitable.
- This study had a small sample size because HIV-1 genotyping is not routinely performed in China.
- Data regarding viral load was missing from a large number of records.

Introduction

AIDS is a major public health problems[1].[1] As of 2019, 38 million individuals worldwide are living with HIV, and 690,000 have died from HIV-related illnesses[2].[2] HIV harms human health primarily by infecting the body and destroying immune cells, and individuals living with HIV often live as asymptomatic carriers for decades or more before eventually developing AIDS and secondary comorbidities[3].[3] In China, the HIV epidemic has generally stabilized from anannual increase at the beginning of the 21st century[4].[4] However, there is a wide variation in the type of epidemic and geographical spread, which poses a great challenge to the prevention and control of AIDS in China[5].[5]

Although there is still no effective cure for AIDS, previous studies have shown that antiretroviral therapy (ART)is widely used to control HIV/AIDS, with good results[6].[6] Since 2016, all people living with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell levels, have been eligible to receive free-state ART and regular follow-up visits by health service staff[7].[7] In 2019, the world has entered an era of universal access to ART, and many developing countries, including China, have in large part achieved full coverage of ART, with an increasing number of PLWHA receiving free ART and achieving viral control[8].[8] However, the HIV epidemic in China has shown no signs of slowing down[9].[9]

HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate, which may produce biological variability that affects treatment outcomes[10].[10] There are 2 major HIV types— HIV type 1 (HIV-1) and HIV type 2 (HIV-2) , and PLWHA with HIV-2 tend to have a lower viral load than those with HIV-1. HIV-1 is by far the most prevalent type, with almost 95% of global HIV infections are of type 1, and HIV-1 is further divided into four groups, including groups M, O, N and P. The Group M is the world's major epidemic pathogen of HIV[11],[11] and is further divided into 10 subtypes (A,B,C,D,F,G,F,J,K, and L), a series of circulating recombinant forms (CRFs) [12] and unique recombinant forms(URFs). Currently, CRFs formed by recombination among subtypes B,C, and CRF01_AE are the most common in China. HIV-1 CRF01_AE and CRF07_BC account for 36.2% and 40.8% of the population reported to be infected in 2018, respectively, according to the results of the 2018 China Molecular Epidemiology Survey[13],[13] and these 2 branches have become the most predominant CRFs of HIV in China[14].[14] CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses[15],[15] which contributed to rapid CD4 T-lymphocyte count depletion in natural infection [16, 17] and suboptimal CD4 restoration during ART[18].[18] Studies investigating the effectiveness of ART in patients with major HIV-1 subtypes have been inconclusive. An analysis of the impact of HIV-1 subtype diversity on long-term clinical outcomes of ART in Guangxi Province suggested that patients with CRF01_AE may benefit more from immediate ART than those with CRF07_BC[19].[19] However, studies

in southern Nigeria and the UK did not report an association between HIV-1 subtypes and immunological or virological responses after treatment[20, 21]. [20 21] Therefore , we hypothesized that the effectiveness of ART differs among HIV-1 subtypes. Accordingly, we aimed to analyze the associaiation between patients with HIV/AIDS with different subtypes and immunological responses after ART in Huzhou, China, between 2018 and 2021, and to further explore whether different ART regimens have a modifying effect on the association between different HIV subtypes and immunological responses.

Methods

Study design and participants

The present investigation was a retrospective cohort study. Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed. This study was approved by the Ethics Committee of the Centre for Disease Control and Prevention (CDC) in Huzhou. All participants identified in the AIDS-PCIS receive a combination antiretroviral regimen containing at least three antiretroviral drugs and sign an informed consent form at the time of initiation of ART, allowing the use of clinical records in future epidemiological studies. The Inclusion criteria for the study were as follows:1) complete laboratory blood tests before ART; 2)temporary residents; 3) starting ART between January 1, 2018, and December 31, 2020; 4) having a complete record of CD4 cell counts to 9-15 months after receiving ART. Ultimately, data from 326 PLWHA were included in the present study.

Patient and public involvement

None.

Demographic characteristics and laboratory information

Data on the demographic and clinical characteristics of the study participants were collected at the time of their registration in a face-to-face survey interview or extracted from their medical records using a structured questionnaire designed specifically for AIDS-PCIS. Information collected included age, sex, height, weight, marital status, occupation, history of sexually transmitted infections (STIs), disease status, sample source, clinical staging by the World Health Organization (WHO), and route of infection. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Information on laboratory tests was obtained from the Huzhou CDC or a local hospital. Tests included $CD4^+$ T lymphocytes (CD4) , $CD8^+$ T lymphocytes (CD8), viral load (VL), white blood cell (WBC), platelets, hemoglobin , serum creatinine (SCR), triglyceride, total cholesterol, fasting plasma glucose , aminotransferase (ALT), aspartate (AST), and total bilirubin. All laboratory parameters were assessed at the local hospital or central laboratory of the Huzhou CDC by trained technicians in strict accordance with clinical guidelines.

HIV subtypes

HIV subtypes among the 326 PLWHA included in the present study were distributed as follows: CRF01_AE (n=100); CRF07_BC (n=157); and other (n=69).

Study outcomes

Only 1 of the 326 patients with HIV/AIDS died during treatment, a large amount of VL data ~~were~~ was missing, and immune recovery status was chosen as the only outcome in this study. Immunological response was defined as an increase in CD4 cell counts >30% from baseline at 12 months after initiation of ART. For those patients who did not undergo CD4 cell count testing 12 months after starting ART treatment, test results obtained at 9-15 months were selected for analysis, which was the closest estimate to 12 months[22].[22]

Statistical analysis

Because missing values introduced some bias in the results, all variables with missing ratios >30% were eliminated from the final working dataset. Otherwise, the missing values were filled using a 5-fold multiple imputation approach. Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (seeTable S1). We described continuous variables using mean ± standard deviation (SD) or median and interquartile range (IQR), and the Student t-test or Wilcoxon rank sum test was used to compare the differences of patients with and without immunological responses. Categorical variables were shown as proportions, and chi-square or Fisher's exact tests were used for their comparisons. A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response, adjusting for potential confounders, including WBC count, CD8, infection status, and time from diagnosis to treatment(p-value <0.05 in the univariate analysis).Subgroup analysis was used to explore the impact of various demographic characteristics and different laboratory investigation results on this association. Given the large heterogeneity in the other subtypes, we selected only the two main subtypes, CRF01_AE and CRF07_BC. Patients were stratified according to sex, route of infection, education, marital status, BMI, AST, ALT and SCR. According to the WHO definition of obesity in Asian populations[23],[23] the BMI cut-off value was 25 kg/m^2. Other numerical variables were determined based on the median values. In addition, the joint association between HIV– subtypes and ART regimens on immunological response were estimated.

All tests were two-sided, and difference with P<0.05 were considered to be statically significant. All data management and statistical analyses were performed using Stata/MP version 15.1 for windows (Copyright 1985-2017 Stata Crop LLC, College Station, Texas 77845 USA) and SAS version 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA.) was completed.

Results

Description of baseline characteristics

A flow chart of participants is shown in Figure 1. A total of 326 PLWHA– were included in the present study, with a~~n~~ mean follow-up of about 1 year. The mean age was 41.9±15.0 years, with 20% of participants < 42 years of age. The median (quartile 1, quartile 3) baseline CD4 cell and CD8 cell counts were 279.5 (175.0-382.0) and 657.75 (481.0-913.0) cells/uL, respectively. In addition, the median (quartile 1, quartile 3) baseline WBC counts were 5.6 (4.5- 6.7)× 10^9/L. Characteristics of the study participants according to immunological responses are shown in Table 1.Compared with negative immunologically responsive participants, those with positive immunological response were more likely to exhibit higher baseline CD4, CD8, and WBC levels. They also tended to have a shorter time between HIV diagnosis and treatment. In addition, the majority of study participants ~~were~~ was in HIV-infected at the time of ART and did not transition to AIDS status. Furthermore, gender, age, marital status and history of STIs were not significantly different between the 2 groups.

Association between immunological response and different HIV subtypes in PLWHA

The associations between immunological responses and the different subtypes of HIV are summarized in Table 2. The proportion of positive immunological responses in PLWHA withCRF01_AEwas obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs 65.6% vs 69.6%). Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other

subtypes: aOR (95%CI) =1.0(0.5,2.0)], in which adjusted for the infection status, WBC, time from diagnosis to treatment and CD8. After removing patients withCRF01_AE, there was also no significant difference in the immunological response between the 2 remaining subtypes [other subtypes: OR (95% CI) =1.2(0.6,2.3)].

Subgroup analysis

Results of subgroup analysis revealed no difference in immunological response between patients with the CRF07_BC versus CRF01_AE subtype in any subgroup (Figure 2), which was consistent with the main statistical analysis.

Joint association between treatment regimens and HIV subtypes on immunological response

Due to the large heterogeneity of patients with other subtypes, only 2 other subtypes were retained. The joint associations between the two subtypes and the three ART regimens are summarized in Table S2. Among PLWHA with CRF01_AE, the effectiveness of ART for PLWHA receiving 3TC+EFV+TDF increased by 24% for those receiving 3TC+AZT+EFV [P = 0.052, OR (95% CI) = 3.4(1.0,11.8)]. But this effect disappeared after adjusting for infection status, WBC, CD8 and time from diagnosis to treatment. None of the other associations were significant.

Discussion

The global distribution of the HIV-1 genotypes is highly heterogeneous and varies geographically[24].[24] In addition, the distribution of HIV-1 may be related to different routes of infection [25] and forms of population mobility, etc. Various aspects of disease progression, all-cause mortality, viral suppression status, and immune recovery status following ART treatment in PLWHA may also be influenced by the diversity of HIV-1 genotypes. Therefore, it is important to understand the epidemiological characteristics of HIV-1 subtypes in a given region [26] to enable more targeted treatment and control of the epidemics. Results of the present study demonstrated that CRF01_AE (30.7%) was the predominant HIV-1 genotype in Huzhou, followed by CRF07_BC (17.2%). This is similar to findings reported in previous studies examining HIV-1 subtype diversity in Shanghai, Jiangsu, and Guangxi provinces[27].[27] However, there are also studies reporting different results, such as a survey of HIV-1 in Yunnan Province, which found CRF08_BC to be the most common subtype[28].[28] Previous studies have shown that the prevalence of CRF01_AE is significantly higher in the southern provinces of China[29].[29] This is consistent with its location of Huzhou. The difference in distribution may be related to the route of transmission. In our study, CRF07_BC was the most common genotype in the population with homosexual transmission. In fact, it has been officially reported by the state that heterosexual transmission has become a major risk factor for PLWHA in China.

China has one of the highest numbers of HIV-1 genotypes, with 10 circulating recombinant forms (CRFs) identified for the first time in this country (CRF01_AE, CRF07_BC, CRF08_BC, CRF55_01B, CRF57_BC, CRF59_01B, CRF61_BC, CRF62_BC, CRF64_BC and CRF65_cpx). A previous studies by Taylor BS et al. reported that HIV-1 subtype diversity is associated with the response to ART[30].[30] A comprehensive assessment of the effect of HIV-1 subtype diversity on long-term clinical outcomes during ART can help inform planning recommendations. Our study found that PLWHA with CRF07_BC had significantly higher baseline CD4 cell counts than those with CRF01_AE. Previous studies reported that PLWHA with CRF01_AE experience a faster rate of CD4 cell decline and faster progression to HIV/AIDS in natural infection[31-33].[31-33] This phenomenon indicates that patients with CRF01_AE may benefit more

from ART. However, we did not find any differences in the immunological responses of the different subtypes among PLWHA. We hypothesize that this may be related to the short follow-up period. In the future, it will be necessary for us to continue to follow the CD4 records of this cohort to test our hypotheses.

Our study had several limitations, the first of which were its small sample size and short follow-up period. However, identification of HIV-1 genotyping is not a routine practice in testing programs in China, and the subtyping of this group of patients was performed in a pilot study in Huzhou. Second, HIV-1 subtypes are regionally relevant and sampling bias is inevitable. Third, there was a large amount of missing data regarding VL, but we chose immunological response as the study endpoint. Although we are currently in the era of "total treatment" for HIV, long-term serial CD4 cell counts are necessary to determine disease progression and changes in immune status to assess the effectiveness of treatment. Finally, although the study population was a longitudinal cohort, we were unable to determine an accurate survival time because our outcome was an immunological response, so we constructed a multifactorial logistic regression model. Nevertheless, this is the first study from East China to analyze the association between HIV subtypes and immunological responses, considering the joint association between subtypes and ART regimens. Therefore, our findings must to be validated in cohorts with larger samples sizes.

Conclusion

In summary, we found no evidence of an association between HIV-1 subtypes and immunological responses, suggesting that currently widely used antiretroviral drugs have similar effectiveness in the subtypes that predominate in the Huzhou area. Moreover, treatment effectiveness of ART in newly diagnosed PLWHA (69%) in Huzhou between 2018 and 2020 was evident. As such, in the future of AIDS prevention and control work, regular monitoring CD4 cell counts is of great significance for improving the quality of life of PLWHA and reducing the risk for transmission.

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Author Contributions

J.M.H., L.J., L.X.Q. and Y.Z.R.: Conceptualization, funding acquisition, supervision, draft and editing. W.Y.N.: Conceptualization, data management, data analysis, methodology, draft and editing. T.Z.W., L.X.F., W.Z.Q., R.F.L. and Z.X.J.: Conducting a research and investigation process, data management, data collection. M.G.Y.: Conceptualization, data management, data analysis, methodology, writing-review and editing, supervision. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics Statement

The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease Control and Prevention (CDC) , and for which the batch number is HZ2021001.

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FIGURE LEGENDS

Figure1. A flowchart of PLWHA with different subtypes in Huzhou , 2018-2020

Figure2. Forest plot of subgroup analysis

Adjusted for infection status, white blood cell count, time from diagnosis to treatment, and CD8 lymphocytes cell counts (when grouped by sex, route of infection, education, marital status, BMI, ALT, AST, SCR).

Abbreviations: OR=Odds ratio; CI=confidence interval; MSM=men who have sex with men; HR=Heterosexual transmission; BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Numerical variables in the subgroup analysis were grouped according to cut-off values (BMI) determined in previous studies or according to the median values of the indicators taken in the current study (ALT, AST, SCR).

Table1. Comparison of demographic and laboratory characteristics of PLWHA in different immunological response states

Variables	Negative immunological response (n=101)	Positive immunological response (n=225)	P
Categorical variables			
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.267
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.189
Farmers	33 (32.7)	84 (37.3)	
Service industry	11(10.9)	30(13.3)	
Worker category	35 (34.7)	49(21.8)	
To be employed	7 (6.9)	20(8.9)	
Other	15 (14.9)	42(18.7)	
Education level			0.227
Primary school or under	35 (34.7)	62 (27.6)	
Middle and high school	53 (52.5)	119 (52.9)	
College or above	13(12.9)	44(19.6)	
History of venereal disease			0.576
None	84 (83.2)	182 (80.9)	
Yes	16(15.8)	42(18.7)	
Not available	1(1.0)	1(0.4)	
Route of infection			0.505
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.359
History of men who have sex with men	37 (36.6)	73(32.4)	
Sexual contact occurring out of wedlock	60 (59.4)	134(59.6)	
Sexual contact with a partner	4(4.0)	18(8.0)	
Marital status			0.368
Unmarried	31(30.7)	70 (31.1)	
Married or with a spouse	46(45.5)	116(51.6)	
Divorced or widowed	24 (23.8)	39(17.3)	
Regimens			0.876
3TC+AZT+EFV	18 (17.8)	35(15.6)	
3TC+EFV+TDF	77 (76.2)	176 (78.2)	
Other	6(5.9)	14(6.2)	
Infection status			0.002
AIDS	21 (20.8)	86(38.2)	

HIV	80 (79.2)	139(61.8)	
Sample source			0.964
Pre-operative testing	24 (23.8)	53(23.6)	
Testing Consultancy	20 (19.8)	50 (22.2)	
Other attendee testing	25 (24.8)	52(23.1)	
Other	32 (31.7)	70 (31.1)	
WHO Clinical Classification			0.228
I or II	99 (98.0)	224 (99.6)	
III or IV	2(2.0)	1(0.4)	
Continuous variables			
Age (years)	43.0 (30.0,56.0)	40.0 (29.0,52.0)	0.368
BMI (kg/m^2)	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.392
First CD4 cell counts(pcs/uL)	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.001
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.027
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.844
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.495
SCR (μmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.539
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.240
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.287
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.015
Timefrom diagnosis to start of treatment (days)	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.059

Note: Information on continuous variables: none followed a normal distribution, statistical description using median (lower quartile, upper quartile) and Mann-Whitney test to compare differences between groups. Information on categorical variables: statistical description using frequency (composition ratio), chi-square test on R x C scale or Fisher's exact probability method to compare differences between groups.

Abbreviations: PLWHA=people living with HIV/AIDS; MSM=men who have sex with men; HR=Heterosexual transmission;BMI=body mass index; CD4=CD4lymphocyte; CD8=CD8 lymphocyte; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine; 3TC+AZT+EFV=zidovudine+efavirenz+lamivudine; 3TC+EFV+TDF=lamivudine + efavirenz + tenofovir.

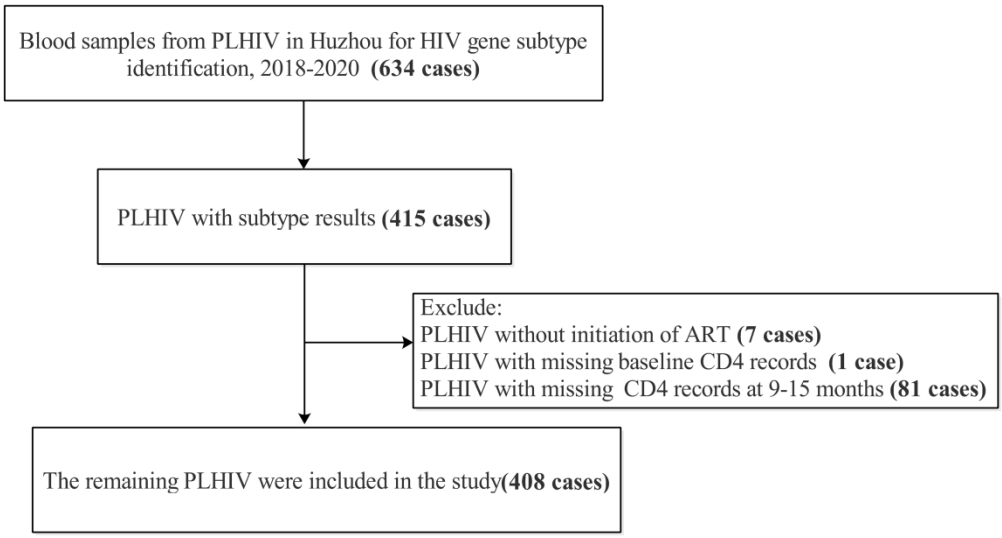
Table2. Association of immunological response status with viral subtypes in PLWHA

			Univariate logistic regression model		Multivariable logistic regression model	
Variables	**N**	**# (%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Three subtypes						
CRF01_AE	100	74(74.00)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF07_BC	157	103 (65.6)	0.7(0.4,1.2)	0.158	0.8(0.4,1.4)	0.405
Other	69	48(69.6)	0.8(0.4,1.6)	0.527	1.0(0.5,2.0)	0.955
Two subtypes						
CRF07_BC	157	103 (65.6)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
Other	69	48(69.6)	1.2 (0.7,2.2)	0.561	1.2 (0.6,2.3)	0.545

Note: Three subtypes are compared using the CRF01_AE as the reference variable and two subtypes are compared using the CRF07_BC as the reference variable.

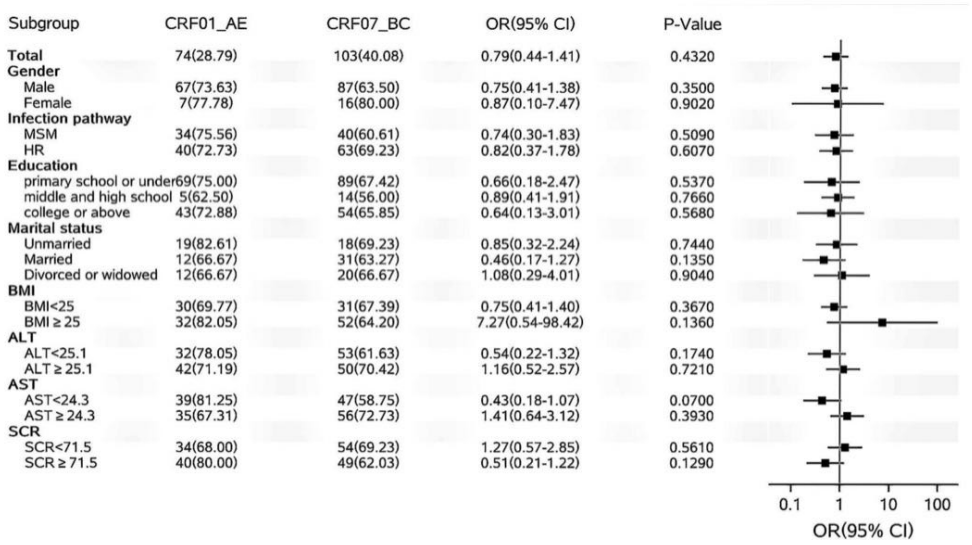
subtypes were included in the multivariable logistic regression model,with adjustment for Infection status, WBC, time from diagnosis to treatment and CD8.

Abbreviations: OR=Odds ratio; CI=confidence interval



A flowchart of PLWHA with different subtypes in Huzhou , 2018-2020

161x86mm (600 x 600 DPI)



Forest plot of subgroup analysis

381x213mm (72 x 72 DPI)

TableS1. Sensitivity analysis of missing values

Variables with missing values	Before filling	After filling	P-value
BMI (kg/m^2)	22.0 (20.0,24.2)	22.0 (20.2,23.9)	0.931
AST (U/L)	23.0 (19.0,29.0)	24.2 (19.1,30.3)	0.236
SCR (μmol/L)	70.6 (61.0,79.5)	70.9 (60.9,80.6)	0.735
HB (g/L)	149.0 (135.0,158.0)	149.0 (135.8,157.0)	0.861
PLT (10^9/L)	206.0 (170.0,241.0)	203.0 (170.0,237.0)	0.798
WBC (10^9/L)	5.6 (4.5,6.7)	5.7 (4.5,6.7)	0.918
ALT (U/L)	25.0 (17.0,38.0)	25.1 (17.4,39.9)	0.570
CD8 cell counts (pcs/uL)	657.8 (481.0,913.0)	664.5 (479.0,919.0)	0.894

Abbreviations: BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Table S2. Joint associations between subtypes and treatment regimens on immunological response

				Unadjusted		Adjusted	
Subtype	**Treatment**	**n**	**#(%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
CRF01_AE	3TC+AZT+EFV	12	6(50.0)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF01_AE	3TC+EFV+TDF	84	65 (77.4)	3.4(1.0,11.8)	0.052	2.8 (0.7,10.4)	0.127
CRF01_AE	Others	4	3 (75.0)	3.0(0.2,37.7)	0.395	3.3(0.2,44.1)	0.368
CRF07_BC	3TC+AZT+EFV	27	19(70.4)	2.4(0.6,9.6)	0.226	2.3(0.5,10.1)	0.261
CRF07_BC	3TC+EFV+TDF	121	79 (65.3)	1.9(0.6,6.2)	0.299	1.9 (0.5,6.8)	0.315
CRF07_BC	Others	9	5 (55.6)	1.3(0.2,7.1)	0.801	1.1(0.2,6.9)	0.906

Note: Adjusted for infection status, WBC, CD8, time from diagnosis to treatment

Abbreviations: OR=Odds ratio; CI=confidence interval.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,line 25	a historical cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1,lines 40-45	Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01_AE
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 2, lines 79-84	CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses[15], which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and suboptimal CD4 restoration during ART[18].
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 3, lines 86-87	Therefore，we hypothesized that the effectiveness of ART differs among HIV-1 subtypes.
Methods				
Study design	4	Present key elements of study design early in the paper	Page 3, line 94	The present investigation was a retrospective cohort study.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 3, lines 94-96	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-

				PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 3, lines 100-103	The Inclusion criteria for the study were as follows:
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Page 3, lines 117-120	HIV subtypes among the 326 PLWHA included in the present study were distributed as follows: CRF01_AE (n=100); CRF07_BC (n=157); and other (n=69).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 3, lines 123-125	Immunological response was defined as an increase in CD4 cell counts > 30% from baseline at 12 months after initiation of ART.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 3, lines 94-96	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Bias	9	Describe any efforts to address potential sources of bias		
Study size	10	Explain how the study size was arrived at	Page 3, lines 94-	Data from 625 patients, who

	96	were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 3, lines 12-13	Information on laboratory tests was obtained from the Huzhou CDC or a local hospital.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 4, lines 36-39	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response
		(*b*) Describe any methods used to examine subgroups and interactions	Page 4, lines 44-46	Subgroup analysis was used to explore the impact of various demographic characteristics and different laboratory investigation results on this association.
		(*c*) Explain how missing data were addressed	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*e*) Describe any sensitivity analyses	Page 4, lines 31-32	Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (see Table S1).
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed		

		(b) Give reasons for non-participation at each stage		
		(c) Consider use of a flow diagram	Page 4, line 154	A flow chart of participants is shown in Figure 1.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 4 line 158-159	Characteristics of the study participants according to immunological responses are shown in Table 1.
		(b) Indicate number of participants with missing data for each variable of interest		
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 4, line 154-155	A total of 326 PLWHA were included in the present study, with an mean follow-up of about 1 year.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 4, line 168-169	The proportion of positive immunological responses in PLWHA with CRF01_AE was obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs 65.6% vs 69.6%).
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 8 4-5, Lines 170-175	Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other subtypes: aOR (95%CI) =1.0(0.5,2.0)], in which adjusted for the infection status, WBC, time from diagnosis to treatment and CD8.
		(*b*) Report category boundaries when continuous variables were categorized		

	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Analysis of the immunological response to antiviral therapy in patients with different subtypes of HIV/AIDS: a retrospective cohort study

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Keywords: HIV/AIDS, ART, CD4 T lymphocyte count, CRF07_BC, CRF01_AE, immunological response

Abstract

Objective: To evaluate the effectiveness of standardized antiretroviral therapy (ART) among different HIV subtypes in people living with HIV/AIDS (PLWHA), and to screen the best ART regimen for this patient population.

Research design and methods: Based on a historical cohort study, PLWHA residing in Huzhou, China, between 2018 and 2020, were enrolled. Data regarding demographic characteristics and laboratory investigation results were collected. Immune system recovery was used to assess the effectiveness of ART, and an increased percentage of $CD4^+$ T lymphocyte (CD4) counts > 30% after receiving ART for > 1 year was determined as immunopositive. A multiple logistic regression model was used to comprehensively quantify the association between PLWHA immunological response status and virus subtype. In addition, the joint association between different subtypes and treatment regimens on immunological response status was investigated.

Results: Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate logistic regression models, there was no difference in the immunological response between patients with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy [CRF07_BC: aOR(95%CI) = 0.8 (0.4,1.4); other subtypes: aOR(95%CI) = 1.2 (0.6, 2.3)]. There was no evidence of an obvious joint association between HIV subtypes and ART regimens on immunological response.

Conclusion: Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01_AE.

Strengths and limitations of this study

- Findings of the present study were based on a population-based cohort rather than case-control or descriptive investigations.
- This was the first study in East China to analyze the association between HIV subtypes and immunological responses, considering the joint association of subtypes and ART regimens.
- HIV-1 subtypes were regionally relevant and sampling bias was inevitable.
- This study had a small sample size because HIV-1 genotyping is not routinely performed in China.
- Data regarding viral load was missing from a large number of records.

Introduction

AIDS is a major public health problems.[1] As of 2019, 38 million individuals worldwide are living with HIV, and 690,000 have died from HIV-related illnesses.[2] HIV harms human health primarily by infecting the body and destroying immune cells, and individuals living with HIV often live as asymptomatic carriers for decades or more before eventually developing AIDS and secondary comorbidities.[3] In China, the HIV epidemic has generally stabilized from an annual increase at the beginning of the 21st century.[4] However, there is a wide variation in the type of epidemic and geographical spread, which poses a great challenge to the prevention and control of AIDS in China.[5]

Although there is still no effective cure for AIDS, previous studies have shown that antiretroviral therapy (ART) is widely used to control HIV/AIDS, with good results.[6] Since 2016, all people living with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell levels, have been eligible to receive free-state ART and regular follow-up visits by health service staff.[7] In 2019, the world has entered an era of universal access to ART, and many developing countries, including China, have, in large part achieved full coverage of ART, with an increasing number of PLWHA receiving free ART and achieving viral control.[8] However, the HIV epidemic in China has shown no signs of slowing down.[9]

HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate, which may produce biological variability that affects treatment outcomes.[10] There are 2 major HIV types— HIV type 1 (HIV-1) and HIV type 2 (HIV-2) , and PLWHA with HIV-2 tend to have a lower viral load than those with HIV-1. HIV-1 is by far the most prevalent type, with almost 95% of global HIV infections are of type 1, and HIV-1 is further divided into four groups, including groups M, O, N and P. The Group M is the world's major epidemic pathogen of HIV,[11] and is further divided into 10 subtypes (A,B,C,D,F,G,F,J,K, and L), a series of circulating recombinant forms (CRFs)[12] and unique recombinant forms(URFs). Currently, CRFs formed by recombination among subtypes B,C, and CRF01_AE are the most common in China.. HIV-1 CRF01_AE and CRF07_BC account for 36.2% and 40.8% of the population reported to be infected in 2018, respectively, according to the results of the 2018 China Molecular Epidemiology Survey,[13] and these 2 branches have become the most predominant CRFs of HIV in China.[14] CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses,[15] which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and suboptimal CD4 restoration during ART.[18] Studies investigating the effectiveness of ART in patients with major HIV-1 subtypes have been inconclusive. An analysis of the impact of HIV-1 subtype diversity on long-term clinical outcomes of ART in Guangxi Province suggested that patients with CRF01_AE may benefit more from immediate ART than those with CRF07_BC.[19] However, studies in

southern Nigeria and the UK did not report an association between HIV-1 subtypes and immunological or virological responses after treatment.[20, 21] Therefore, we hypothesized that the effectiveness of ART differs among HIV-1 subtypes. Accordingly, we aimed to analyze the assocaition between patients with HIV/AIDS with different subtypes and immunological responses after ART in Huzhou, China, between 2018 and 2021, and to further explore whether different ART regimens have a modifying effect on the association between different HIV subtypes and immunological responses.

Methods

Study design and participants

The present investigation was a retrospective cohort study. Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed. This study was approved by the Ethics Committee of the Centre for Disease Control and Prevention (CDC) in Huzhou. All participants identified in the AIDS-PCIS receive a combination antiretroviral regimen containing at least three antiretroviral drugs and sign an informed consent form at the time of initiation of ART, allowing the use of clinical records in future epidemiological studies. The Inclusion criteria for the study were as follows: 1) complete laboratory blood tests before receiving ART; 2)living in Huzhou area including temporary residents; 3) starting ART between January 1, 2018, and December 31, 2020; 4) having a complete record of CD4 cell counts to 9-15 months after receiving ART. Ultimately, data from 326 PLWHA were included in the present study.

Patient and public involvement

None.

Demographic characteristics and laboratory information

Data on the demographic and clinical characteristics of the study participants were collected at the time of their registration in a face-to-face survey interview or extracted from their medical records using a structured questionnaire designed specifically for AIDS-PCIS. Information collected included age, sex, height, weight, marital status, occupation, history of sexually tranmitted infections (STIs), disease status, sample source, clinical staging by the World Health Organization (WHO), and route of infection. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Information on laboratory tests was obtained from the Huzhou CDC or a local hospital. Tests included $CD4^+$ T lymphocytes (CD4) , $CD8^+$ T lymphocytes (CD8), viral load (VL), white blood cell (WBC), platelets, hemoglobin , serum creatinine (SCR), triglyceride, total cholesterol, fasting plasma glucose , aminotransferase (ALT), aspartate (AST), and total bilirubin. All laboratory parameters were assessed at the local hospital or central laboratory of the Huzhou CDC by trained technicians in strict accordance with clinical guidelines.

Study outcomes

Immunological response was defined as an increase in CD4 cell counts > 30% from baseline at 12 months after initiation of ART. For those patients who did not undergo CD4 cell count testing 12 months after starting ART treatment, test results obtained at 9-15 months were selected for analysis, which was the closest estimate to 12 months.[22].

Statistical analysis

Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset. Otherwise, the missing values were filled using a 5-fold multiple imputation approach. Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (see Table S1).

We described continuous variables using mean ± standard deviation (SD) or median and interquartile range (IQR), and the Student t-test or Wilcoxon rank sum test was used to compare the differences of patients with and without immunological responses. Categorical variables were shown as proportions, and chi-square or Fisher's exact tests were used for their comparisons. All factors with p-values, which were calculated based on univariate logistic regression model, below 0.05 were included from multivariate logistic regression model. And the multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response, adjusting for potential confounders, including WBC count, CD8, infection status, and time from diagnosis to treatment. Subgroup analysis was used to explore the impact of various demographic characteristics and different laboratory investigation results on this association. Given the large heterogeneity in the other subtypes, we selected only the two main subtypes, CRF01_AE and CRF07_BC. Patients were stratified according to sex, route of infection, education, marital status, BMI, AST, ALT and SCR. According to the WHO definition of obesity in Asian populations,[23] the BMI cut-off value was 25 kg/m^2. Other numerical variables were determined based on the median values. In addition, the joint association between HIV subtypes and ART regimens on immunological response was estimated. All tests were two-sided, and difference with P<0.05 were considered to be statically significant. All data management and statistical analyses were performed using Stata/MP version 15.1 for windows (Copyright 1985-2017 Stata Crop LLC, College Station, Texas 77845 USA) and SAS version 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA.) was completed.

Results

Description of baseline characteristics

A flow chart of participants is shown in Figure 1. A total of 326 PLWHA were included in the present study, with an mean follow-up of about 1 year. The distribution of HIV subtypes was as follows :CRF01_AE (n=100); CRF07_BC (n = 157); Other (n=69). The mean age was 41.9±15.0 years, with 20% of participants < 42 years of age. The median (quartile 1, quartile 3) baseline CD4 cell and CD8 cell counts were 279.5 (175.0-382.0) and 657.75 (481.0-913.0) cells/uL, respectively. In addition, the median (quartile 1, quartile 3) baseline WBC counts were 5.6 (4.5- 6.7)× 10^9/L. Characteristics of the study participants according to immunological responses are shown in Table 1. Compared with patients without immunological response, those with immunological response were more likely to exhibit higher baseline CD4, CD8, and WBC levels. They also tended to have a shorter time between HIV diagnosis and treatment. In addition, the majority of study participants were in HIV-infected at the time of ART and did not transition to AIDS status. Furthermore, gender, age, marital status and history of STIs were not significantly different between the 2 groups.

Association between immunological response and different HIV subtypes in PLWHA

The associations between immunological responses and the different subtypes of HIV are summarized in Table 2. The proportion of positive immunological responses in PLWHA with CRF01_AE was obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs 65.6% vs 69.6%). Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other subtypes: aOR (95%CI) =1.0(0.5,2.0)], in which adjusted for the infection status, WBC, time from diagnosis to treatment and CD8. After removing patients with CRF01_AE, there was also no significant difference in the immunological response between the 2 remaining subtypes [other subtypes: OR (95% CI) =1.2(0.6,2.3)].

Subgroup analysis

Results of subgroup analysis revealed no difference in immunological response between patients with the CRF07_BC versus CRF01_AE subtype in any subgroup(Figure 2).

Joint association between HIV subtypes and ART regimens on immunological response

Due to the large heterogeneity of patients with other subtypes, only 2 other subtypes were retained. The joint associations between the two subtypes and the three ART regimens are summarized in Table S2. Among PLWHA with CRF01_AE, the effectiveness of ART for PLWHA receiving 3TC+EFV+TDF increased by 24% for those receiving 3TC+AZT+EFV [P = 0.052, OR (95% CI) = 3.4(1.0,11.8)]. But this effect disappeared after adjusting for infection status, WBC, CD8 and time from diagnosis to treatment. None of the other associations were significant.

Discussion

The global distribution of the HIV-1 genotypes is highly heterogeneous and varies geographically.[24] In addition, the distribution of HIV-1 may be related to different routes of infection[25] and forms of population mobility, etc. Various aspects of disease progression, all-cause mortality, viral suppression status, and immune recovery status following ART treatment in PLWHA may also be influenced by the diversity of HIV-1 genotypes. Therefore, it is important to understand the epidemiological characteristics of HIV-1 subtypes in a given region[26] to enable more targeted treatment and control of the epidemics. Results of the present study demonstrated that CRF01_AE (30.7%) was the predominant HIV-1 genotype in Huzhou, followed by CRF07_BC (17.2%). This is similar to findings reported in previous studies examining HIV-1 subtype diversity in Shanghai, Jiangsu, and Guangxi provinces.[27] However, there are also studies reporting different results, such as a survey of HIV-1 in Yunnan Province, which found CRF08_BC to be the most common subtype.[28] Previous studies have shown that the prevalence of CRF01_AE is significantly higher in the southern provinces of China.[29] This is consistent with its location of Huzhou. The difference in distribution may be related to the route of transmission. In our study, CRF07_BC was the most common genotype in the population with homosexual transmission. In fact, it has been officially reported by the state that heterosexual transmission has become a major risk factor for PLWHA in China.

China has one of the highest numbers of HIV-1 genotypes, with 10 circulating recombinant forms (CRFs) identified for the first time in this country (CRF01_AE, CRF07_BC, CRF08_BC, CRF55_01B, CRF57_BC, CRF59_01B, CRF61_BC, CRF62_BC, CRF64_BC and CRF65_cpx). A previous studies by Taylor BS et al. reported that HIV-1 subtype diversity is associated with the response to ART.[30] A comprehensive assessment of the effect of HIV-1 subtype diversity on long-term clinical outcomes during ART can help inform planning recommendations. Our study found that PLWHA with CRF07_BC had significantly higher baseline CD4 cell counts than those with CRF01_AE. Previous studies reported that PLWHA with CRF01_AE experience a faster rate of CD4 cell decline and faster progression to HIV/AIDS in natural infection.[31-33] This phenomenon indicates that patients with CRF01_AE may benefit more from ART. However, we did not find any differences in the immunological responses of the different subtypes among PLWHA. We hypothesize that this may be related to the short follow-up period. In the future, it will be necessary for us to continue to follow the CD4 records of this cohort to test our hypotheses.

Our study had several limitations, the first of which were its small sample size and short follow-up

period. However, identification of HIV-1 genotyping is not a routine practice in testing programs in China, and the subtyping of this group of patients was performed in a pilot study in Huzhou. Second, HIV-1 subtypes are regionally relevant and sampling bias is inevitable. Third, there was a large amount of missing data regarding VL, but we chose immunological response as the study endpoint. Although we are currently in the era of "total treatment" for HIV, long-term serial CD4 cell counts are necessary to determine disease progression and changes in immune status to assess th effectiveness of treatment. Finally, although the study population was a longitudinal cohort, we were unable to determine an accurate survival time because our outcome was an immunological response, so we constructed a multifactorial logistic regression model. Nevertheless, this is the first study from East China to analyze the association between HIV subtypes and immunological responses, considering the joint association between subtypes and ART regimens. Therefore, our findings must to be validated in cohorts with larger samples sizes.

Conclusion

In summary, we found no evidence of an association between HIV-1 subtypes and immunological responses, suggesting that currently widely used antiretroviral drugs have similar effectiveness in the subtypes that predominate in the Huzhou area.

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Author Contributions

J.M.H., L.J., L.X.Q. and Y.Z.R.: Conceptualization, funding acquisition, supervision, draft and editing. W.Y.N.: Conceptualization, data management, data analysis, methodology, draft and editing. T.Z.W., L.X.F., W.Z.Q., R.F.L. and Z.X.J.: Conducting a research and investigation process, data management, data collection. M.G.Y.: Conceptualization, data management, data analysis, methodology, writing-review and editing, supervision. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics Statement

The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease Control and Prevention (CDC) , and for which the batch number is HZ2021001.

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FIGURE LEGENDS

Figure1. A flowchart of PLWHA with different subtypes in Huzhou , 2018-2020

Figure2. Forest plot of subgroup analysis

Adjusted for infection status, white blood cell count, time from diagnosis to treatment, and CD8 lymphocytes cell counts (when grouped by sex, route of infection, education, marital status, BMI, ALT, AST, SCR).

Abbreviations: OR=Odds ratio; CI=confidence interval; MSM=men who have sex with men; HR=Heterosexual transmission; BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Numerical variables in the subgroup analysis were grouped according to cut-off values (BMI) determined in previous studies or according to the median values of the indicators taken in the current study (ALT, AST, SCR).

Table1. Comparison of demographic and laboratory characteristics of PLWHA in different immunological response status

Variables	Without immunological response (n=101)	With immunological response (n=225)	P
Categorical variables			
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.267
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.189
Farmers	33 (32.7)	84 (37.3)	
Service industry	11(10.9)	30(13.3)	
Worker category	35 (34.7)	49(21.8)	
To be employed	7 (6.9)	20(8.9)	
Other	15 (14.9)	42(18.7)	
Education level			0.227
Primary school or under	35 (34.7)	62 (27.6)	
Middle and high school	53 (52.5)	119 (52.9)	
College or above	13(12.9)	44(19.6)	
History of venereal disease			0.576
None	84 (83.2)	182 (80.9)	
Yes	16(15.8)	42(18.7)	
Not available	1(1.0)	1(0.4)	
Route of infection			0.505
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.359
History of men who have sex with men	37 (36.6)	73(32.4)	
Sexual contact occurring out of wedlock	60 (59.4)	134(59.6)	
Sexual contact with a partner	4(4.0)	18(8.0)	
Marital status			0.368
Unmarried	31(30.7)	70 (31.1)	
Married or with a spouse	46(45.5)	116(51.6)	
Divorced or widowed	24 (23.8)	39(17.3)	
Regimens			0.876
3TC+AZT+EFV	18 (17.8)	35(15.6)	
3TC+EFV+TDF	77 (76.2)	176 (78.2)	
Other	6(5.9)	14(6.2)	
Infection status			0.002
AIDS	21 (20.8)	86(38.2)	

HIV	80 (79.2)	139(61.8)	
Sample source			0.964
Pre-operative testing	24 (23.8)	53(23.6)	
Testing Consultancy	20 (19.8)	50 (22.2)	
Other attendee testing	25 (24.8)	52(23.1)	
Other	32 (31.7)	70 (31.1)	
WHO Clinical Classification			0.228
I or II	99 (98.0)	224 (99.6)	
III or IV	2(2.0)	1(0.4)	
Continuous variables			
Age (years)	43.0 (30.0,56.0)	40.0 (29.0,52.0)	0.368
BMI (kg/m^2)	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.392
First CD4 cell counts(pcs/uL)	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.001
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.027
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.844
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.495
SCR (μmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.539
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.240
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.287
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.015
Time from diagnosis to start of treatment (days)	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.059

Note: Information on continuous variables: none followed a normal distribution, statistical description using median (lower quartile, upper quartile) and Mann-Whitney test to compare differences between groups. Information on categorical variables: statistical description using frequency (composition ratio), chi-square test on R x C scale or Fisher's exact probability method to compare differences between groups.

Abbreviations: PLWHA=people living with HIV/AIDS; MSM=men who have sex with men; HR=Heterosexual transmission;BMI=body mass index; CD4=CD4lymphocyte; CD8=CD8 lymphocyte; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine; 3TC+AZT+EFV=zidovudine+efavirenz+lamivudine; 3TC+EFV+TDF=lamivudine + efavirenz + tenofovir.

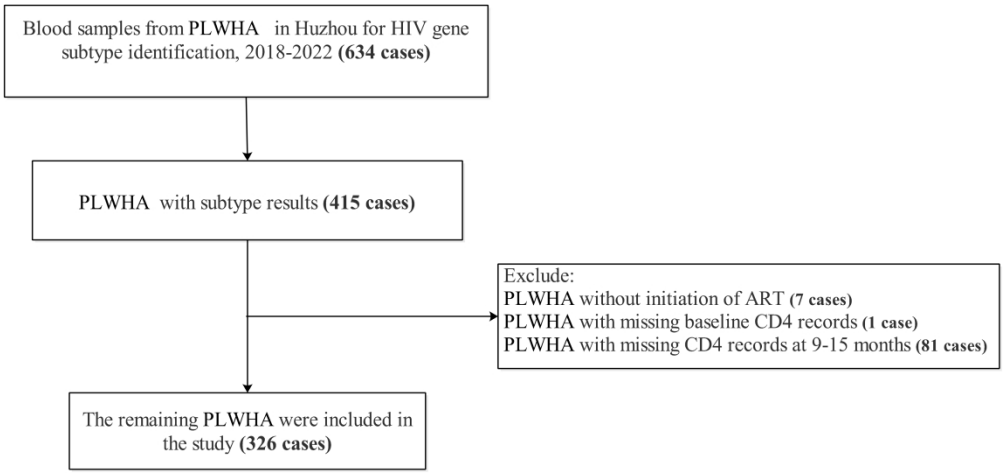
Table2. Association of immunological response status with viral subtypes in PLWHA

			Crude		Adjusted	
Variables	**N**	**# (%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Three subtypes						
CRF01_AE	100	74(74.00)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF07_BC	157	103 (65.6)	0.7(0.4,1.2)	0.158	0.8(0.4,1.4)	0.405
Other	69	48(69.6)	0.8(0.4,1.6)	0.527	1.0(0.5,2.0)	0.955
Two subtypes						
CRF07_BC	157	103 (65.6)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
Other	69	48(69.6)	1.2 (0.7,2.2)	0.561	1.2 (0.6,2.3)	0.545

Note: Three subtypes are compared using the CRF01_AE as the reference and two subtypes are compared using the CRF07_BC as the reference.

Adjusted for Infection status, WBC, time from diagnosis to treatment and CD8.

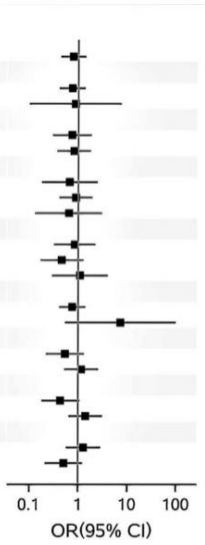
Abbreviations: OR=Odds ratio; CI=confidence interval



A flowchart of PLWHA with different subtypes in Huzhou , 2018-2020

207x97mm (600 x 600 DPI)

Subgroup	CRF01_AE	CRF07_BC	OR(95% CI)	P-Value
Total	74(28.79)	103(40.08)	0.79(0.44-1.41)	0.4320
Gender				
Male	67(73.63)	87(63.50)	0.75(0.41-1.38)	0.3500
Female	7(77.78)	16(80.00)	0.87(0.10-7.47)	0.9020
Infection pathway				
MSM	34(75.56)	40(60.61)	0.74(0.30-1.83)	0.5090
HR	40(72.73)	63(69.23)	0.82(0.37-1.78)	0.6070
Education				
primary school or under	69(75.00)	89(67.42)	0.66(0.18-2.47)	0.5370
middle and high school	5(62.50)	14(56.00)	0.89(0.41-1.91)	0.7660
college or above	43(72.88)	54(65.85)	0.64(0.13-3.01)	0.5680
Marital status				
Unmarried	19(82.61)	18(69.23)	0.85(0.32-2.24)	0.7440
Married	12(66.67)	31(63.27)	0.46(0.17-1.27)	0.1350
Divorced or widowed	12(66.67)	20(66.67)	1.08(0.29-4.01)	0.9040
BMI				
BMI<25	30(69.77)	31(67.39)	0.75(0.41-1.40)	0.3670
BMI ≥ 25	32(82.05)	52(64.20)	7.27(0.54-98.42)	0.1360
ALT				
ALT<25.1	32(78.05)	53(61.63)	0.54(0.22-1.32)	0.1740
ALT ≥ 25.1	42(71.19)	50(70.42)	1.16(0.52-2.57)	0.7210
AST				
AST<24.3	39(81.25)	47(58.75)	0.43(0.18-1.07)	0.0700
AST ≥ 24.3	35(67.31)	56(72.73)	1.41(0.64-3.12)	0.3930
SCR				
SCR<71.5	34(68.00)	54(69.23)	1.27(0.57-2.85)	0.5610
SCR ≥ 71.5	40(80.00)	49(62.03)	0.51(0.21-1.22)	0.1290



Forest plot of subgroup analysis

381x213mm (72 x 72 DPI)

TableS1. Sensitivity analysis of missing values

Variables with missing values	Before filling	After filling	P-value
BMI (kg/m^2)	22.0 (20.0,24.2)	22.0 (20.2,23.9)	0.931
AST (U/L)	23.0 (19.0,29.0)	24.2 (19.1,30.3)	0.236
SCR (μmol/L)	70.6 (61.0,79.5)	70.9 (60.9,80.6)	0.735
HB (g/L)	149.0 (135.0,158.0)	149.0 (135.8,157.0)	0.861
PLT (10^9/L)	206.0 (170.0,241.0)	203.0 (170.0,237.0)	0.798
WBC (10^9/L)	5.6 (4.5,6.7)	5.7 (4.5,6.7)	0.918
ALT (U/L)	25.0 (17.0,38.0)	25.1 (17.4,39.9)	0.570
CD8 cell counts (pcs/uL)	657.8 (481.0,913.0)	664.5 (479.0,919.0)	0.894

Abbreviations: BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Table S2. Joint associations between HIV subtypes and ART regimens on immunological response

				Crude		Adjusted	
Subtype	**Treatment**	**n**	**#(%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
CRF01_AE	3TC+AZT+EFV	12	6(50.0)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF01_AE	3TC+EFV+TDF	84	65 (77.4)	3.4(1.0,11.8)	0.052	2.8 (0.7,10.4)	0.127
CRF01_AE	Others	4	3 (75.0)	3.0(0.2,37.7)	0.395	3.3(0.2,44.1)	0.368
CRF07_BC	3TC+AZT+EFV	27	19(70.4)	2.4(0.6,9.6)	0.226	2.3(0.5,10.1)	0.261
CRF07_BC	3TC+EFV+TDF	121	79 (65.3)	1.9(0.6,6.2)	0.299	1.9 (0.5,6.8)	0.315
CRF07_BC	Others	9	5 (55.6)	1.3(0.2,7.1)	0.801	1.1(0.2,6.9)	0.906

Note: Adjusted for infection status, WBC, CD8, time from diagnosis to treatment

Abbreviations: OR=Odds ratio; CI=confidence interval.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,line 24	a historical cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1,lines 39-45	Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01_AE
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 2, lines 70-80	CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses[15], which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and suboptimal CD4 restoration during ART[18].
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 2, lines 85	Therefore，we hypothesized that the effectiveness of ART differs among HIV-1 subtypes.
Methods				
Study design	4	Present key elements of study design early in the paper	Page 3, line 93	The present investigation was a retrospective cohort study.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 3, lines 93-95	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-

				PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 3, lines 99	The Inclusion criteria for the study were as follows:
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Page 4, lines 151-152	The distribution of HIV subtypes was as follows :CRF01_AE (n=100); CRF07_BC (n = 157); Other (n=69).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 3, lines 119-120	Immunological response was defined as an increase in CD4 cell counts > 30% from baseline at 12 months after initiation of ART.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 3, lines 93-95	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Bias	9	Describe any efforts to address potential sources of bias		
Study size	10	Explain how the study size was arrived at	Page 3, lines 93-94	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control

	Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 3, lines 12-13	Information on laboratory tests was obtained from the Huzhou CDC or a local hospital.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 4, lines 36-39	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response
		(*b*) Describe any methods used to examine subgroups and interactions	Page 4, lines 44-46	Subgroup analysis was used to explore the impact of various demographic characteristics and different laboratory investigation results on this association.
		(*c*) Explain how missing data were addressed	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*e*) Describe any sensitivity analyses	Page 4, lines 31-32	Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (see Table S1).
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed		

		(b) Give reasons for non-participation at each stage		
		(c) Consider use of a flow diagram	Page 4, line 154	A flow chart of participants is shown in Figure 1.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 4 line 158-159	Characteristics of the study participants according to immunological responses are shown in Table 1.
		(b) Indicate number of participants with missing data for each variable of interest		
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 4, line 154-155	A total of 326 PLWHA were included in the present study, with an mean follow-up of about 1 year.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 4, line 168-169	The proportion of positive immunological responses in PLWHA with CRF01_AE was obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs 65.6% vs 69.6%).
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 8 4-5, Lines 170-175	Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other subtypes: aOR (95%CI) =1.0(0.5,2.0)], in which adjusted for the infection status, WBC, time from diagnosis to treatment and CD8.
		(*b*) Report category boundaries when continuous variables were categorized		

	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Analysis of the immunological response to antiviral therapy in patients with different subtypes of HIV/AIDS: a retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072597.R3
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Analysis of the immunological response to antiviral therapy in patients with different subtypes of HIV/AIDS: a retrospective cohort study

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Abstract

Objective: To evaluate the effectiveness of standardised antiretroviral therapy (ART) among different HIV subtypes in people living with HIV/AIDS (PLWHA), and to screen the best ART regimen for this patient population.

Design: A retrospective cohort study was performed, and PLWHA residing in Huzhou, China, between 2018 and 2020, were enrolled.

Setting and participants: Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.

Analysis and outcome measures: Data regarding demographic characteristics and laboratory investigation results were collected. Immune system recovery was used to assess the effectiveness of ART, and an increased percentage of $CD4^+$ T lymphocyte (CD4) counts> 30% after receiving ART for > 1 year was determined as immunopositive. A multiple logistic regression model was used to comprehensively quantify the association between PLWHA immunological response status and virus subtype. In addition, the joint association between different subtypes and treatment regimens on immunological response status was investigated.

Results: Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate logistic regression models, there was no difference in the immunological response between patients with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy [CRF07_BC: aOR (95%CI) = 0.8 (0.4, 1.4); other subtypes: aOR (95%CI) = 1.2 (0.6, 2.3)]. There was no evidence of an obvious joint association between HIV subtypes and ART regimens on immunological

response.

Conclusions: Standardised ART was beneficial to all PLWHA, regardless of HIV subtypes, although it was more effective, to some extent, in PLWHA with CRF01_AE.

Strengths and limitations of this study

- The present study was based on a population-based cohort rather than case-control or descriptive investigations.
- The study analysed the association between HIV subtypes and immunological responses, considering the joint association of subtypes and ART regimens.
- HIV-1 subtypes were regionally relevant and sampling bias was inevitable.
- This study had a small sample size because HIV-1 genotyping is not routinely performed in China.
- Data regarding viral load was missing from a large number of records.

INTRODUCTION

AIDS is a major public health problem.[1] As of 2019, 38 million individuals worldwide are living with HIV, and 690,000 have died from HIV-related illnesses.[2] HIV harms human health primarily by infecting the body and destroying immune cells, and individuals living with HIV often live as asymptomatic carriers for decades or more before eventually developing AIDS and secondary comorbidities.[3] In China, the HIV epidemic has generally stabilised from an annual increase at the beginning of the 21st century.[4] However, there is a wide variation in the type of epidemic and geographical spread, which poses a great challenge to the prevention and control of AIDS in China.[5]

Although there is still no effective cure for AIDS, previous studies have shown that antiretroviral therapy (ART)is widely used to control HIV/AIDS, with good results.[6] Since 2016, all people living with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell levels, have been eligible to receive free-state ART and regular follow-up visits by health service staff.[7]In 2019, the world has entered an era of universal access to ART, and many developing countries, including China, have in large part achieved full coverage of ART, with an increasing number of PLWHA receiving free ART and achieving viral control.[8] However, the HIV epidemic in China has shown no signs of slowing down.[9]

HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate, which may produce biological variability that affects treatment outcomes.[10] There are 2 major HIV types— HIV type 1 (HIV-1) and HIV type 2 (HIV-2)， and PLWHA with HIV-2 tend to have a lower viral load than those with HIV-1. HIV-1 is by far the most prevalent type, with almost 95% of global HIV infections are of type 1, and HIV-1 is further divided into four groups, including groups M, O, N and P. The Group M is the world's major epidemic pathogen of HIV,[11] and is further divided into 10 subtypes (A,B,C,D,F,G,F,J,K, and L), a series of circulating recombinant forms (CRFs)[12] and unique recombinant forms(URFs). Currently, CRFs formed by recombination among subtypes B,C, and CRF01_AE are the most common in China.. HIV-1 CRF01_AE and CRF07_BC account for 36.2% and 40.8% of the population reported to be infected in 2018, respectively, according to the results of the 2018 China Molecular Epidemiology Survey,[13] and these 2 branches have become the most predominant CRFs of HIV in China.[14]CRF01_AE was reported to harbour a high prevalence of CXCR4 viruses,[15] which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and suboptimal CD4 restoration during ART.[18] Studies investigating the effectiveness of ART in patients

with major HIV-1 subtypes have been inconclusive. An analysis of the impact of HIV-1 subtype diversity on long-term clinical outcomes of ART in Guangxi Province suggested that patients with CRF01_AE may benefit more from immediate ART than those with CRF07_BC.[19]However, studies in southern Nigeria and the UK did not report an association between HIV-1 subtypes and immunological or virological responses after treatment.[20, 21]Therefore, we hypothesised that the effectiveness of ART differs among HIV-1 subtypes. Accordingly, we aimed to analyse the association between patients with HIV/AIDS with different subtypes and immunological responses after ART in Huzhou, China, between 2018 and 2021, and to further explore whether different ART regimens have a modifying effect on the association between different HIV subtypes and immunological responses.

METHODS

Study design and participants

The present investigation was a retrospective cohort study. Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed. This study was approved by the Ethics Committee of the Centre for Disease Control and Prevention (CDC) in Huzhou. All participants identified in the AIDS-PCIS receive a combination antiretroviral regimen containing at least three antiretroviral drugs and sign an informed consent form at the time of initiation of ART, allowing the use of clinical records in future epidemiological studies. The Inclusion criteria for the study were as follows: 1) complete laboratory blood tests before receiving ART; 2) living in Huzhou area including temporary residents; 3) starting ART between January 1, 2018, and December 31, 2020; 4) having a complete record of CD4 cell counts to 9-15 months after receiving ART. Ultimately, data from 326 PLWHA were included in the present study.

Demographic characteristics and laboratory information

Data on the demographic and clinical characteristics of the study participants were collected at the time of their registration in a face-to-face survey interview or extracted from their medical records using a structured questionnaire designed specifically for AIDS-PCIS. Information collected included age, sex, height, weight, marital status, occupation, history of sexually transmitted infections (STIs), disease status, sample source, clinical staging by the World Health Organization (WHO), and route of infection. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Information on laboratory tests was obtained from the Huzhou CDC or a local hospital. Tests included $CD4^+$ T lymphocytes (CD4) , $CD8^+$ T lymphocytes (CD8), viral load (VL), white blood cell (WBC), platelets, haemoglobin , serum creatinine (SCR), triglyceride, total cholesterol, fasting plasma glucose , aminotransferase (ALT), aspartate (AST), and total bilirubin. All laboratory parameters were assessed at the local hospital or central laboratory of the Huzhou CDC by trained technicians in strict accordance with clinical guidelines.

Study outcomes

Immunological response was defined as an increase in CD4 cell counts >30% from baseline at 12 months after initiation of ART. For those patients who did not undergo CD4 cell count testing 12 months after starting ART treatment, test results obtained at 9-15 months were selected for analysis, which was the closest estimate to 12 months.[22].

Statistical analysis

Because missing values introduced some bias in the results, all variables with missing ratios >30% were eliminated from the final working dataset. Otherwise, the missing values were filled using a 5-fold

multiple imputation approach. Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (see Table S1). We described continuous variables using mean ± standard deviation (SD) or median and interquartile range (IQR), and the Student's t-test or Wilcoxon rank sum test was used to compare the differences of patients with and without immunological responses. Categorical variables were shown as proportions, and chi-square or Fisher's exact tests were used for their comparisons. The association between immunological response and participants characteristics was estimated using univariable logistic regression models. A multivariable logistic regression was performed including all variables that were associated with immunological response in the univariable analysis with a p-value of < 0.05. And the multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response, adjusting for potential confounders, including WBC count, CD8, infection status, and time from diagnosis to treatment. Given the large heterogeneity in the other subtypes, we selected only the two main subtypes, CRF01_AE and CRF07_BC. According to the WHO definition of obesity in Asian populations,[23] the BMI cut-off value was 25 kg/m^2. Other numerical variables were determined based on the median values. In addition, the joint association between HIV subtypes and ART regimens on immunological response was estimated.

All tests were two-sided, and difference was with P<0.05 were considered to be statically significant. All data management and statistical analyses were performed using Stata/MP version 15.1 for windows (Stata Corp LLC, College Station, Texas, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was completed.

Patient and public involvement

None.

RESULTS

Description of baseline characteristics

A flow chart of participants is shown in Figure 1. A total of 326 PLWHA were included in the present study, with a mean follow-up of about 1 year. The distribution of HIV subtypes was as follows: CRF01_AE (n=100); CRF07_BC (n = 157); Other (n=69). The mean age was 41.9±15.0 years, with 20% of participants < 42 years of age. The median (quartile 1, quartile 3) baseline CD4 cell and CD8 cell counts were 279.5 (175.0-382.0) and 657.75 (481.0-913.0) cells/uL, respectively. In addition, the median (quartile 1, quartile 3) baseline WBC counts were 5.6 (4.5- 6.7)× 10^9/L. Characteristics of the study participants according to immunological responses were shown in Table 1. Compared with patients without immunological response, those with immunological response were more likely to exhibit higher baseline CD4, CD8, and WBC levels. They also tended to have a shorter time between HIV diagnosis and treatment. In addition, the majority of study participants were in HIV-infected at the time of ART and did not transition to AIDS status. Furthermore, gender, age, marital status and history of STIs were not significantly different between the 2 groups.

Association between immunological response and different HIV subtypes in PLWHA

The associations between immunological responses and the different subtypes of HIV are summarised in Table 2. The proportion of positive immunological responses in PLWHA with CRF01_AE was obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs. 65.6% vs. 69.6%). Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8 (0.4, 1.4); other subtypes: aOR (95%CI) = 1.0 (0.5, 2.0)], in which adjusted for the infection status, WBC, time from

diagnosis to treatment and CD8. After removing patients with CRF01_AE, there was also no significant difference in the immunological response between the 2 remaining subtypes [other subtypes: aOR (95% CI) = 1.2 (0.6, 2.3)].

Joint association between HIV subtypes and ART regimens on immunological response

Due to the large heterogeneity of patients with other subtypes, only 2 other subtypes were retained. The joint associations between the two subtypes and the three ART regimens are summarised in Table S2. Among PLWHA with CRF01_AE, the effectiveness of ART for PLWHA receiving 3TC+EFV+TDF increased by 24% for those receiving 3TC+AZT+EFV [P = 0.052, OR (95% CI) = 3.4 (1.0, 11.8)]. But this effect disappeared after adjusting for infection status, WBC, CD8 and time from diagnosis to treatment. None of the other associations were significant.

DISCUSSION

The global distribution of the HIV-1 genotypes is highly heterogeneous and varies geographically.[24] In addition, the distribution of HIV-1 may be related to different routes of infection[25] and forms of population mobility, etc. Various aspects of disease progression, all-cause mortality, viral suppression status, and immune recovery status following ART treatment in PLWHA may also be influenced by the diversity of HIV-1 genotypes. Therefore, it is important to understand the epidemiological characteristics of HIV-1 subtypes in a given region [26] to enable more targeted treatment and control of the epidemics. Results of the present study demonstrated that CRF01_AE (30.7%) was the predominant HIV-1 genotype in Huzhou, followed by CRF07_BC (17.2%). This is similar to findings reported in previous studies examining HIV-1 subtype diversity in Shanghai, Jiangsu, and Guangxi provinces.[27] However, there are also studies reporting different results, such as a survey of HIV-1 in Yunnan Province, which found CRF08_BC to be the most common subtype.[28] Previous studies have shown that the prevalence of CRF01_AE is significantly higher in the southern provinces of China.[29] This is consistent with its location of Huzhou. The difference in distribution may be related to the route of transmission. In our study, CRF07_BC was the most common genotype in the population with homosexual transmission. In fact, it has been officially reported by the state that heterosexual transmission has become a major risk factor for PLWHA in China.

China has one of the highest numbers of HIV-1 genotypes, with 10 circulating recombinant forms (CRFs) identified for the first time in this country (CRF01_AE, CRF07_BC, CRF08_BC, CRF55_01B, CRF57_BC, CRF59_01B, CRF61_BC, CRF62_BC, CRF64_BC and CRF65_cpx). A previous studies by Taylor BS et al. reported that HIV-1 subtype diversity is associated with the response to ART.[30] A comprehensive assessment of the effect of HIV-1 subtype diversity on long-term clinical outcomes during ART can help inform planning recommendations. Our study found that PLWHA with CRF07_BC had significantly higher baseline CD4 cell counts than those with CRF01_AE. Previous studies reported that PLWHA with CRF01_AE experience a faster rate of CD4 cell decline and faster progression to HIV/AIDS in natural infection.[31-33] This phenomenon indicates that patients with CRF01_AE may benefit more from ART. However, we did not find any differences in the immunological responses of the different subtypes among PLWHA. We hypothesise that this may be related to the short follow-up period. In the future, it will be necessary for us to continue to follow the CD4 records of this cohort to test our hypotheses.

Our study had several limitations, the first of which were its small sample size and short follow-up period. However, identification of HIV-1 genotyping is not a routine practice in testing programs in

China, and the subtyping of this group of patients was performed in a pilot study in Huzhou. Second, HIV-1 subtypes are regionally relevant and sampling bias is inevitable. Third, there was a large amount of missing data regarding VL, but we chose immunological response as the study endpoint. Although we are currently in the era of "total treatment" for HIV, long-term serial CD4 cell counts are necessary to determine disease progression and changes in immune status to assess the effectiveness of treatment. Finally, although the study population was a longitudinal cohort, we were unable to determine an accurate survival time because our outcome was an immunological response, so we constructed a multifactorial logistic regression model. Nevertheless, this is the first study from East China to analyse the association between HIV subtypes and immunological responses, considering the joint association between subtypes and ART regimens. Therefore, our findings must to be validated in cohorts with larger samples sizes.

CONCLUSION

In summary, we found no evidence of an association between HIV-1 subtypes and immunological responses, suggesting that currently widely used antiretroviral drugs have similar effectiveness in the subtypes that predominate in the Huzhou area.

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Contributors

J.M.H., L.J., L.X.Q. and Y.Z.R.: Conceptualisation, funding acquisition, supervision, draft and editing. W.Y.N.: Conceptualisation, data management, data analysis, methodology, draft and editing. T.Z.W., L.X.F., W.Z.Q., R.F.L. and Z.X.J.: Conducting a research and investigation process, data management, data collection. M.G.Y.: Conceptualisation, data management, data analysis, methodology, writing-review and editing, supervision. All authors reviewed and approved the final manuscript.

Competing interests

The authors declare no conflict of interest.

Data availability statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Ethics approval

The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease Control and Prevention (CDC) (batch number HZ2021001).

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FIGURE TITLE

Figure 1. Flowchart of PLWHA with different subtypes in Huzhou, 2018-2020

Table 1. Comparison of demographic and laboratory characteristics of PLWHA in different immunological response status

Variables	Without immunological response (n=101)	With immunological response (n=225)	P
Categorical variables			
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.267
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.189
Farmers	33 (32.7)	84 (37.3)	
Service industry	11(10.9)	30(13.3)	
Worker category	35 (34.7)	49(21.8)	
To be employed	7 (6.9)	20(8.9)	
Other	15 (14.9)	42(18.7)	
Education level			0.227
Primary school or under	35 (34.7)	62 (27.6)	
Middle and high school	53 (52.5)	119 (52.9)	
College or above	13(12.9)	44(19.6)	
History of venereal disease			0.576
None	84 (83.2)	182 (80.9)	
Yes	16(15.8)	42(18.7)	
Not available	1(1.0)	1(0.4)	
Route of infection			0.505
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.359
History of men who have sex with men	37 (36.6)	73(32.4)	
Sexual contact occurring out of wedlock	60 (59.4)	134(59.6)	
Sexual contact with a partner	4(4.0)	18(8.0)	
Marital status			0.368
Unmarried	31(30.7)	70 (31.1)	
Married or with a spouse	46(45.5)	116(51.6)	
Divorced or widowed	24 (23.8)	39(17.3)	
Regimens			0.876
3TC+AZT+EFV	18 (17.8)	35(15.6)	
3TC+EFV+TDF	77 (76.2)	176 (78.2)	
Other	6(5.9)	14(6.2)	
Infection status			0.002
AIDS	21 (20.8)	86(38.2)	

HIV	80 (79.2)	139(61.8)	
Sample source			0.964
Pre-operative testing	24 (23.8)	53(23.6)	
Testing Consultancy	20 (19.8)	50 (22.2)	
Other attendee testing	25 (24.8)	52(23.1)	
Other	32 (31.7)	70 (31.1)	
WHO Clinical Classification			0.228
I or II	99 (98.0)	224 (99.6)	
III or IV	2(2.0)	1(0.4)	
Continuous variables			
Age (years)	43.0 (30.0,56.0)	40.0 (29.0,52.0)	0.368
BMI (kg/m^2)	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.392
First CD4 cell counts(pcs/uL)	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.001
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.027
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.844
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.495
SCR (μmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.539
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.240
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.287
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.015
Timefrom diagnosis to start of treatment (days)	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.059

Note: Information on continuous variables: none followed a normal distribution, statistical description using median (lower quartile, upper quartile) and Mann-Whitney test to compare differences between groups. Information on categorical variables: statistical description using frequency (composition ratio), chi-square test on R x C scale or Fisher's exact probability method to compare differences between groups.

Abbreviations: PLWHA=people living with HIV/AIDS; MSM=men who have sex with men; HR=Heterosexual transmission; BMI=body mass index; CD4=CD4lymphocyte; CD8=CD8 lymphocyte; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine; 3TC+AZT+EFV=zidovudine+efavirenz+lamivudine; 3TC+EFV+TDF=lamivudine + efavirenz + tenofovir.

Table 2. Association of immunological response status with viral subtypes in PLWHA

			Crude		Adjusted	
Variables	**N**	**# (%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Three subtypes						
CRF01_AE	100	74(74.00)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF07_BC	157	103 (65.6)	0.7(0.4,1.2)	0.158	0.8(0.4,1.4)	0.405
Other	69	48(69.6)	0.8(0.4,1.6)	0.527	1.0(0.5,2.0)	0.955
Two subtypes						
CRF07_BC	157	103 (65.6)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
Other	69	48(69.6)	1.2 (0.7,2.2)	0.561	1.2 (0.6,2.3)	0.545

Note: Three subtypes are compared using the CRF01_AE as the reference and two subtypes are compared using the CRF07_BC as the reference.

Adjusted for infection status, WBC, time from diagnosis to treatment and CD8.

Abbreviations: OR=Odds ratio; CI=confidence interval.

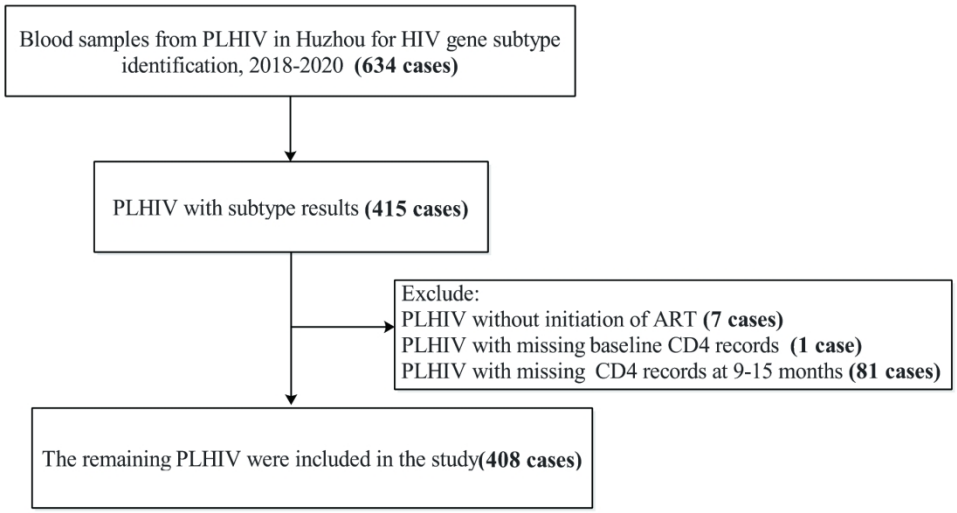

Figure 1. A flowchart of PLWHA with different subtypes in Huzhou , 2018-2020

170x96mm (300 x 300 DPI)

TableS1. Sensitivity analysis of missing values

Variables with missing values	Before filling	After filling	P-value
BMI (kg/m^2)	22.0 (20.0,24.2)	22.0 (20.2,23.9)	0.931
AST (U/L)	23.0 (19.0,29.0)	24.2 (19.1,30.3)	0.236
SCR (μmol/L)	70.6 (61.0,79.5)	70.9 (60.9,80.6)	0.735
HB (g/L)	149.0 (135.0,158.0)	149.0 (135.8,157.0)	0.861
PLT (10^9/L)	206.0 (170.0,241.0)	203.0 (170.0,237.0)	0.798
WBC (10^9/L)	5.6 (4.5,6.7)	5.7 (4.5,6.7)	0.918
ALT (U/L)	25.0 (17.0,38.0)	25.1 (17.4,39.9)	0.570
CD8 cell counts (pcs/uL)	657.8 (481.0,913.0)	664.5 (479.0,919.0)	0.894

Abbreviations: BMI=body mass index; ALT=alanine aminotransferase; AST=aspartate aminotransferase; PLT=platelets; HB=haemoglobin; WBC=white blood cells; SCR=serum creatinine.

Table S2. Joint associations between HIV subtypes and ART regimens on immunological response

				Crude		Adjusted	
Subtype	**Treatment**	**n**	**#(%)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
CRF01_AE	3TC+AZT+EFV	12	6(50.0)	1.0(1.0,1.0)	Ref.	1.0(1.0,1.0)	Ref.
CRF01_AE	3TC+EFV+TDF	84	65 (77.4)	3.4(1.0,11.8)	0.052	2.8 (0.7,10.4)	0.127
CRF01_AE	Others	4	3 (75.0)	3.0(0.2,37.7)	0.395	3.3(0.2,44.1)	0.368
CRF07_BC	3TC+AZT+EFV	27	19(70.4)	2.4(0.6,9.6)	0.226	2.3(0.5,10.1)	0.261
CRF07_BC	3TC+EFV+TDF	121	79 (65.3)	1.9(0.6,6.2)	0.299	1.9 (0.5,6.8)	0.315
CRF07_BC	Others	9	5 (55.6)	1.3(0.2,7.1)	0.801	1.1(0.2,6.9)	0.906

Note: Adjusted for infection status, WBC, CD8, time from diagnosis to treatment

Abbreviations: OR=Odds ratio; CI=confidence interval.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1,line 24	a historical cohort study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1,lines 39-45	Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01_AE
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 2, lines 70-80	CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses[15], which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and suboptimal CD4 restoration during ART[18].
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 2, lines 85	Therefore，we hypothesized that the effectiveness of ART differs among HIV-1 subtypes.
Methods				
Study design	4	Present key elements of study design early in the paper	Page 3, line 93	The present investigation was a retrospective cohort study.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 3, lines 93-95	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-

				PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 3, lines 99	The Inclusion criteria for the study were as follows:
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Page 4, lines 151-152	The distribution of HIV subtypes was as follows :CRF01_AE (n=100); CRF07_BC (n = 157); Other (n=69).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 3, lines 119-120	Immunological response was defined as an increase in CD4 cell counts > 30% from baseline at 12 months after initiation of ART.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 3, lines 93-95	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Bias	9	Describe any efforts to address potential sources of bias		
Study size	10	Explain how the study size was arrived at	Page 3, lines 93-94	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control

	Information System (the AIDS-PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 3, lines 12-13	Information on laboratory tests was obtained from the Huzhou CDC or a local hospital.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 4, lines 36-39	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response
		(*b*) Describe any methods used to examine subgroups and interactions	Page 4, lines 44-46	Subgroup analysis was used to explore the impact of various demographic characteristics and different laboratory investigation results on this association.
		(*c*) Explain how missing data were addressed	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Page 4, lines 29-32	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		(*e*) Describe any sensitivity analyses	Page 4, lines 31-32	Subsequently, a sensitivity analysis of the comparison of pre-and post-imputation was additionally applied to validate the stability of the imputations (see Table S1).
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed		

		(b) Give reasons for non-participation at each stage		
		(c) Consider use of a flow diagram	Page 4, line 154	A flow chart of participants is shown in Figure 1.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 4 line 158-159	Characteristics of the study participants according to immunological responses are shown in Table 1.
		(b) Indicate number of participants with missing data for each variable of interest		
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 4, line 154-155	A total of 326 PLWHA were included in the present study, with an mean follow-up of about 1 year.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 4, line 168-169	The proportion of positive immunological responses in PLWHA with CRF01_AE was obviously higher than that in PLWHA with CRF07_BC and other subtypes (74.0% vs 65.6% vs 69.6%).
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 8 4-5, Lines 170-175	Compared to patients with CRF01_AE, the possibility of an immune response for those with CRF07_BC and other subtypes was not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other subtypes: aOR (95%CI) =1.0(0.5,2.0)], in which adjusted for the infection status, WBC, time from diagnosis to treatment and CD8.
		(*b*) Report category boundaries when continuous variables were categorized		

	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.