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## **BMJ Open**

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

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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<sup>+</sup> 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<sup>1</sup>. As of 2019, 38 million people worldwide are living with HIV and 690,000 people have died from HIV-related illnesses<sup>2</sup>. 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<sup>3</sup>. In China, the HIV epidemic has generally stabilised from a year-on-year increase at the beginning of the 21st century<sup>4</sup>. 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<sup>5</sup>.

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<sup>6</sup>. 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<sup>7</sup>. 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<sup>8</sup>. Despite this, the HIV epidemic in China shows no sign of slowing down<sup>9, 10</sup>.

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<sup>11</sup>. 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<sup>12</sup>. 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)<sup>2</sup>. 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<sup>13</sup>, and these two branches have become the most predominant HIV CRFs in China<sup>14</sup>. 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\_BC15. 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<sup>16, 17</sup>. 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)<sup>2</sup>. Information on laboratory tests was obtained from the Huzhou CDC or local hospital. Tests included CD4 , CD8<sup>+</sup> 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<sup>7</sup>. 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<sup>18</sup>. 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<sup>19</sup>. 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<sup>20</sup>. The cut-off value of 25 kg/m<sup>2</sup> 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.

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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

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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 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 multiplicative interaction = 0.27, P additive interaction = 0.79) and corrected for covariates (P multiplicative interaction = 0.37, P 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

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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<sup>21</sup>. In addition, the distribution of HIV-1 may be related to different routes of infection<sup>22</sup>, 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<sup>23</sup> 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<sup>11, 15, 24</sup>. 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<sup>25</sup>. Previous studies have shown that the prevalence of CRF01\_AE is significantly higher in southern provinces<sup>26</sup>. 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<sup>27</sup>. 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<sup>28-30</sup>. 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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**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).

	Negative immunological	Positive immunological	
Variables	response (n=101)	response (n=225)	Р
Categorical variables	1	1	
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.26
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.18
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			0.22
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			0.57
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			0.50
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.35
History of men who have	37 (36.6)	73(32.4)	
sex with men			
Sexual contact occurring	60 (59.4)	134(59.6)	
out of wedlock			
Sexual contact with a	4(4.0)	18(8.0)	
partner			
Marital status			0.36
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			0.87
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			0.00
AIDS	21 (20.8)	86(38.2)	

Table1. Comparison of demographic and laboratory characteristics of PLWHA in different

3	HIV	80 (79.2)	139(61.8)	
4 5	Sample source			0.964
6	Pre-operative testing	24 (23.8)	53(23.6)	
7	Testing Consultancy	20 (19.8)	50 (22.2)	
8 9	Other attendee testing	25 (24.8)	52(23.1)	
10	Other	32 (31.7)	70 (31.1)	
11	WHO Clinical Classification			0.228
12	I or II	99 (98.0)	224 (99.6)	
13 14	III or IV	2(2.0)	1(0.4)	
15	Continuous variables			
16	Age (years)	43.0 (30.0,56.0)	40.0 (29.0,52.0)	0.368
17 18	BMI (kg/m <sup>2</sup> )	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.392
19	First CD4 cell	382.0 (246.0,477.0)	237.0 (155.0,325.0)	< 0.001
20	counts(pcs/uL)			
21 22	CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.027
22	AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.844
24	ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.495
25	SCR (µmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.539
26 27	HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.240
28	PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.287
29	WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.015
30 31	Time from diagnosis to start	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.059
32	of treatment (days)			
33		: 11 (11	1 11	• •• 1

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.

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Table2. Association of immune response status with viral subtypes in PLWHA								
			Univariate	e logistic	Multivariable	ble logistic		
			regressio	n model	regression	model		
Variables	Ν	# (%)	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



451x240mm (72 x 72 DPI)

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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 unde	er69(75.00)	89(67.42)	0.66(0.18-2.47)	0.5370	
middle and high school	0 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	the All second A				
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	52(02.05)	02(01:20)	1.27 (0.04 50.42)	0.1000	_
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		50(70.12)		0.7	
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				0.0000	-
SCB<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	

381x213mm (72 x 72 DPI)

OR(95% CI)

TableS1. Sensitivity analysis of missing values						
Variables with missing values	Before filling	After filling	<b>P-value</b>			
BMI (kg/m <sup>2</sup> )	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.



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Table S2. Treatment effectiveness of different regimens for different subtypes and the interaction of subtypes with treatment regimens

	Po	ositive imn	nune respo	onse	Po	sitive imm	nune respo	onse	Po	ositive imn	nune respo	onse	$\mathbf{P}_{\text{multiplicative}}$	Padditive
Madala		CRF	-01_AE			CRF	07_BC			Other	subtypes		interaction	interaction
woders	Total	Option 1	Option 2	Option 3	Total	Option 1	Option 2	Option 3	Total	Option 1	Option 2	Option 3		
	[n(%)]	[n(%)]	[n(%)]	[n (%)]	[n(%)]	[n(%)]	[n(%)]	[n(%)]	[n(%)]	[n(%)]	[n(%)]	[n(%)]		
Single-factor	74/100	6/12	65/84	3/4	103/15	19/27	79/121	5/9	48/69	10/14	32/48	6/7	0.27	0.79
model	(74)	(50)	(77)	(75)	7	(70)	(65)	(56)	(70)	(71)	(67)	(86)		
					(65)									
Multiple-factor	74/100	6/12	65/84	3/4	103/15	19/27	79/121	5/9	48/69	10/14	32/48	6/7	0.37	0.80
model*	(74)	(50)	(77)	(75)	7	(70)	(65)	(56)	(70)	(71)	(67)	(86)		
					(65)									

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



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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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## **BMJ Open**

## 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 < INFECTIOUS DISEASES, PREVENTIVE MEDICINE, PUBLIC HEALTH

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1	Analysis of the immunological response to antiviral therapy in
2	patients with different subtypes of HIV/AIDS: a retrospective
3	cohort study
4 5	Yanan Wang <sup>2,3,1,†</sup> , Zhongrong Yang <sup>1,†</sup> , Jing Li <sup>1</sup> , Zhenqian Wu <sup>1</sup> , Xiaoqi Liu <sup>1</sup> , Zhaowei Tong <sup>4</sup> , Xiaofeng Li <sup>4</sup> , Feilin Ren <sup>1</sup> , Xiaojuan Zhu <sup>1</sup> , Meihua Jin <sup>1,*</sup> , Guangyun Mao <sup>2,3,5*</sup>
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17 18 19	Keywords: HIV/AIDS, ART, CD4 T lymphocyte count, CRF07_BC, CRF01_AE, immunological response
20	Abstract
21 22 23	<b>Objective:</b> 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.
24 25	<b>Methods:</b> 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
26	were collected. Immune system recovery was used to assess the curative effectiveness of ART, and an
27	increased percentage of CD4 <sup>+</sup> T lymphocyte (CD4) counts> 30% after receiving ART for > 1 year was
28	determined as immunopositive. A multiple logistic regression model was used to comprehensively
30	addition, the joint association between different subtypes and treatment regimens on immunological
31	response status was investigated.
32	<b>Results:</b> Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the
33	percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate
34	logistic regression models, there was no difference in the immunological response between patients
35	with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy
36	[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
37	evidence of an obvious joint associationbetween HIV subtypesand ART regimens on immunological
38	response.
39	Conclusion: Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it
40	was more effective, to some extent, in PLWHA with CRF01_AE.
41	
	1

#### 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<sup>4</sup>.[1] As of 2019, 38 million individuals worldwide are living with HIV, and 690,000 have died from HIV-related illnesses<sup>2</sup>.[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<sup>3</sup>.[3] In China, the HIV epidemic has generally stabilized from anannual increase at the beginning of the 21st century<sup>4</sup>.[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<sup>5</sup>.[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<sup>6</sup>.[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<sup>7</sup>.[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<sup>8</sup>.[8] However, the HIV epidemic in China has shown no signs of slowing down<sup>9</sup>.[9]

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HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate, which may produce biological variability that affects treatment outcomes<sup>10</sup>.[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<sup>14</sup>,[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<sup>13</sup>,[13] and these 2 branches have become the most predominant CRFs of HIV in China<sup>44</sup>.[14] CRF01\_AE was reported to harbor a high prevalence of CXCR4 viruses<sup>45</sup>,[15] which contributed to rapid CD4 T-lymphocyte count depletion in natural infection\_[16, 17] and suboptimal CD4 restoration during ART<sup>48</sup>.[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<sup>19</sup>.[19] However, studies

in southern Nigeria and the UK did not report an association between HIV-1 subtypes and

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immunological or virological responses after treatment<sup>20, 21</sup>. [20 21] Therefore, we hypothesized that the effectiveness of ART differs among HIV-1 subtypes. Accordingly, we aimed to analyze the assocaiation 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

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#### 93 Study design and participants

94 The present investigation was a retrospective cohort study. Data from 625 patients, who were newly 95 diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS-PCIS) in 96 Huzhou between 2018 and 2020, were reviewed. This study was approved by the Ethics Committee of 97 the Centre for Disease Control and Prevention (CDC) in Huzhou. All participants identified in the 98 AIDS-PCIS receive a combination antiretroviral regimen containing at least three antiretroviral drugs 99 and sign an informed consent form at the time of initiation of ART, allowing the use of clinical records 100 in future epidemiological studies. The Inclusion criteria for the study were as follows:1) complete 101 laboratory blood tests before ART; 2)temporary residents; 3) starting ART between January 1, 2018, and 102 December 31, 2020; 4) having a complete record of CD4 cell counts to 9-15 months after receiving ART. 103 Ultimately, data from 326 PLWHA were included in the present study.

- 104 Patient and public involvement
  - 105 None.

#### 106 Demographic characteristics and laboratory information

107 Data on the demographic and clinical characteristics of the study participants were collected at the time 108 of their registration in a face-to-face survey interview or extracted from their medical records using a 109 structured questionnaire designed specifically for AIDS-PCIS. Information collected included age, sex, 110 height, weight, marital status, occupation, history of sexually transmitted infections (STIs), disease 111 status, sample source, clinical staging by the World Health Organization (WHO), and route of infection. 112 Body mass index (BMI) was calculated as weight (kg)/height (m)<sup>2</sup>. Information on laboratory tests was 113 obtained from the Huzhou CDC or a local hospital. Tests included CD4<sup>+</sup> T lymphocytes (CD4), CD8<sup>+</sup> T 114 lymphocytes (CD8), viral load (VL), white blood cell (WBC), platelets, hemoglobin, serum creatinine 115 (SCR), triglyceride, total cholesterol, fasting plasma glucose, aminotransferase (ALT), aspartate (AST), 116 and total bilirubin. All laboratory parameters were assessed at the local hospital or central laboratory of 117 the Huzhou CDC by trained technicians in strict accordance with clinical guidelines. 118 **HIV** subtypes 119 HIV subtypes among the 326 PLWHA included in the present study were distributed as follows: 120 CRF01\_AE (n=100); CRF07\_BC (n=157); and other (n=69). 121 Study outcomes 122 Only 1 of the 326 patients with HIV/AIDS died during treatment, a large amount of VL data were-was 123 missing, and immune recovery status was chosen as the only outcome in this study. Immunological

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<sup>22</sup>.[22]

128	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. 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<sup>23</sup>, [23] the BMI cut-off value was 25 kg/m<sup>2</sup>. 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.

#### 152 Results

#### 153 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 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)× 109/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.

### 166 Association between immunological response and different HIV subtypes in PLWHA

167 The associations\_between\_immunological responses and the\_different subtypes of HIV are summarized
168 in Table 2. The proportion of positive immunological responses in PLWHA withCRF01\_AEwas
169 obviously higher than that in PLWHA with CRF07\_BC and other subtypes (74.0% vs 65.6% vs 69.6%).
170 Compared to patients with CRF01\_AE, the possibility of an immune response for those with CRF07\_BC
171 and other subtypes was not significantly different [CRF07\_BC: aOR (95%CI) = 0.8(0.4,1.4); other

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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<sub>7</sub>, 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)].

#### 177 Subgroup analysis

178 Results of subgroup analysis revealed no difference in immunological response between patients with
179 the CRF07\_BC versus CRF01\_AE subtype in any subgroup\_(Figure 2), which was consistent with the
180 main statistical analysis.

#### 182 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.

#### 190 Discussion

The global distribution of the HIV-1 genotypes is highly heterogeneous and varies geographically<sup>24</sup>.[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<sup>27</sup>.[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<sup>28</sup>.[28] Previous studies have shown that the prevalence of CRF01\_AE is significantly higher in the southern provinces of China<sup>29</sup>.[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<sup>30</sup>.[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<sup>31-33</sup>.[31-33] This phenomenon indicates that patients with CRF01\_AE may benefit more 

#### **BMJ** Open

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.

#### Acknowledgements

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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.
- Funding
- This work was supported by Medical and Health Research Project of Zhejiang Province (2022KY369),

260 Huzhou science and technology research plan project (2023GYB28), the Huzhou Medical Key 261 Supporting Discipline (Epidemiology), and the Key Laboratory of Emergency detection for Public 262 Health of Huzhou.

#### 263 **Ethics Statement**

264 The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease 265 Control and Prevention (CDC), and for which the batch number is HZ2021001.

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- Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies
- **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; BMI=body mass index; ALT=alanine aminotransferase; HR=Heterosexual transmission; 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).

	Negative immunological	Positive immunological	
Variables	response (n=101)	response (n=225)	Р
Categorical variables			
Gender			0.210
Male	89 (88.1)	186 (82.7)	
Ethnicity			0.26
Han Chinese	100 (99.0)	216 (96.0)	
Other	1(1.0)	9(4.0)	
Occupation			0.18
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			0.22
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			0.57
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			0.50
MSM	43 (42.6)	87 (38.7)	
HR	58 (57.4)	138(61.3)	
Contact history			0.35
History of men who have	37 (36.6)	73(32.4)	
sex with men			
Sexual contact occurring	60 (59.4)	134(59.6)	
out of wedlock			
Sexual contact with a	4(4.0)	18(8.0)	
partner			
Marital status			0.36
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			0.87
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			0.00
AIDS	21 (20.8)	86(38.2)	

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

HIV	80 (79.2)	139(61.8)	
Sample source			0.96
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			0.22
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)	0.3
BMI (kg/m <sup>2</sup> )	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.3
First CD4 cell	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.0
counts(pcs/uL)			
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.0
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.8
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.4
SCR (µmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.5
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.2
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.2
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.0
Timefrom diagnosis to start	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.0
of treatment (days)			

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.

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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.

			Univariate logistic		Multivariable logistic		
			regression model		regression model		
Variables	Ν	# (%)	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	

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

**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

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

161x86mm (600 x 600 DPI)

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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	(70 60)						
Male	6/(/3.63)	87(63.50)	0.75(0.41-1.38)	0.3500			
Female	/(//./8)	16(80.00)	0.87(0.10-7.47)	0.9020		-	
Ment Pathway	24/75 56)	40(60.61)	0 74(0 20 1 82)	0 5000			
	34(75.50)	40(60.61)	0.74(0.30-1.83)	0.5090			
Education	40(72.73)	03(09.23)	0.82(0.37-1.78)	0.0070			
primary school or unde	er69(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		- (())		010000	_		
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	- <b>-</b> t		
AL1 2 25.1	42(71.19)	50(70.42)	1.16(0.52-2.57)	0.7210			
AST-24.2	20(01 25)	47(59.75)	0 42/0 19 1 07)	0.0700			
AST > 24.3	35(67.21)	47(30.73) 56(72 73)	1 41(0 64 3 13)	0.0700			
SCR	33(07.31)	50(72.75)	1.41(0.04-3.12)	0.3930			
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	<b></b>		

Forest plot of subgroup analysis

OR(95% CI)

381x213mm (72 x 72 DPI)

TableS1. Sensitivity analysis of missing values				
Variables with missing values	Before filling	After filling	P-value	
BMI (kg/m <sup>2</sup> )	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.



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

				Unadjus	ited	A	djusted
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.

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STROBE Statement	t—ch	ecklist of items that should be included in reports of observational studies	023-07259 9ht, incluc	
	Item No.	Recommendation	ding of Page	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	$\mathcal{P}_{\mathcal{H}} = 1, \text{line } 25$	a historical cohort study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	ay 1,lines 40- ay 2024. Downloadec net estrelated to text anc	Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effectiv to some extent, in PLWHA w CRF01 AE
Introduction		$\mathcal{O}_{\mathcal{O}}$	l froi d dat	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	a Ritesy A	CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses <sup>15</sup> , which contributed to rapid CD4 T- lymphocyte count depletion i natural infection <sup>16, 17</sup> and suboptimal CD4 restoration during ART <sup>18</sup> .
Objectives	3	State specific objectives, including any prespecified hypotheses	nifage 3, lines 86-	Therefore , we hypothesized that the effectiveness of ART differs among HIV-1 subtype
Methods			2025 logi	
Study design	4	Present key elements of study design early in the paper	ي Pag <mark>æ</mark> 3, line 94 ي	The present investigation wa 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 Bibliographi	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AID

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		BMJ Open	mjopen-20 by copyrig	Ρ
			23-072597 o ht, including	PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Participants	6	<ul> <li>(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</li> <li>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</li> <li>Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants</li> </ul>	n ag 3, lines ag 5 May Cor Uses related to te to te	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	And data mining, Al t	HIV subtypes among the 326 PLWHA included in the presenstudy 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	and simila	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	r Page 3, lines 94- recmologies.	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	iogr	
Study size	10	Explain how the study size was arrived at	Page 3, lines 94-	Data from 625 patients, who

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Page 21	of 24	BMJ Open BMJ Open	bmjopen-2
1 2 3 4 5 6 7 8 9 10		ight, fincluding for uses relate	6 OP250 6 OP250 6 OP250 6 OP250 6 OP250 6 OP250 6 OP250 7 OP150 7 O
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41		t to text and data mining. Al training, and similar technologies.	lownloaded from http://bmjopen.bmj.com/ on June 11, 2025 at Agence Bibliographiqu
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24

		BMJ Open	bmjopen-20 I by copyrig	Pag
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	nt 23 frago 3, lines frago 23, lines dding 7 o	Information on laboratory tests was obtained from the Huzhou CDC or a local hospital.
Statistical methods	12	( <i>a</i> ) Describe all statistical methods, including those used to control for confounding	9 Dage54, lines Dage5139 Dage5139 Dar 05es related t dated t	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response
		( <i>b</i> ) Describe any methods used to examine subgroups and interactions	io text and data m text and data m	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	4, lines 29 Al training,	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		( <i>d</i> ) 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	aPage 4, lines 3290132 Similar technologie 11	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		( <u>e</u> ) Describe any sensitivity analyses	Agence B	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			blic	<u> </u>
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	ygraphique	
		<b>4</b> . For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xh	ntml –	

3 of 24		BMJ Open	mjopen-20 by copyrig	
		(b) Give reasons for non-participation at each stage	)23-0; ht, in	
		(c) Consider use of a flow diagram	Graggo 4, line	A flow chart of participants
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	ପ୍ରି <sup>34</sup> 9 ଫୁag <mark>6</mark> 4 ସ୍ଥା <b>ଲ୍ଲସ୍ଲ</b> 58-159	Characteristics of the study participants according to
			r 2024 seigne s relat	immunological responses ar shown in Table 1.
		(b) Indicate number of participants with missing data for each variable of interest	ed t	
		(c) Cohort study—Summarise follow-up time (eg, average and total amount)	Taga 4, line	A total of 326 PLWHA were included in the present stud
			ded erie and	an mean follow-up of about
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	ange 4, line	The proportion of <b>positive</b>
				immunological responses in
			ing	PLWHA with CRF01_AE w
			, ≥ <sup>1</sup>	obviously higher than that i
			ope	PLWHA with CRF07_BC an
				subtypes (74.0% vs 65.6% vs
		Case-control study-Report numbers in each exposure category, or summary measures of exposure	Ģ <u>J</u>	
		Cross-sectional study—Report numbers of outcome events or summary measures	and	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision	<b>sagos</b> 4-5,	Compared to patients with
		(eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were	at in 🛃 170-	CRF01_AE, the possibility o
		included	<b>6</b> 75 <b>6</b>	immune response for those
			:hn	CRF07_BC and other subtyp
			202 0loç	not significantly different
			yies	[CRF07 BC: aOR (95%CI) =
			, t Ac	0.8(0.4,1.4); other subtypes:
			Jeno	(95%CI) =1.0(0.5,2.0)], in wh
			е В	adjusted for the infection sta
			libli	WBC, time from diagnosis t
			ogr	treatment and CD8.
		(b) Report category boundaries when continuous variables were categorized	aph	
		(-)	iqu	
		_	e de	
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**BMJ** Open (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time 2) mining. At training: and similar tectors in the second second

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_(	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	incl	
<u> </u>	Discussion			udir	
ŀ	Key results	18	Summarise key results with reference to study objectives	on ng fr	
Ι	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss	15 I	
			both direction and magnitude of any potential bias	Nay Enses	
Ι	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of	20) reig	
			analyses, results from similar studies, and other relevant evidence	nen ate	
_(	Generalisability	21	Discuss the generalisability (external validity) of the study results	d nen ten	
(	Other informati	on		/nlo t Su	
F	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the	tar	
	C		original study on which the present article is based	nd c	
				r (A lata	
*	*Give informatio	n sep	parately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups	s in South and cross-sectio	nal studies.
c ŀ	checklist is best u http://www.annal	used i	n conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmed /, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at w	dicianitro www.itro www.itro g, and similar technologies.	nal Medicine at
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# **BMJ Open**

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

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Secondary Subject Heading:	Public health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, PREVENTIVE MEDICINE, PUBLIC HEALTH

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1	Analysis of the immunological response to antiviral therapy in
2	patients with different subtypes of HIV/AIDS: a retrospective
3	cohort study
4	Yanan Wang <sup>2,3,1,†</sup> , Zhongrong Yang <sup>1,†</sup> , Jing Li <sup>1</sup> , Zhenqian Wu <sup>1</sup> , Xiaoqi Liu <sup>1</sup> , Zhaowei Tong <sup>4</sup> ,
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17	
18	Keywords: HIV/AIDS, ART, CD4 T lymphocyte count, CRF07_BC, CRF01_AE, immunological response
19	
20	Abstract
21	Objective: To evaluate the effectiveness of standardized antiretroviral therapy (ART) among different
22	HIV subtypes in people living with HIV/AIDS (PLWHA), and to screen the best ART regimen for this
23	patient population.
24	Research design and methods: Based on a historical cohort study, PLWHA residing in Huzhou, China,
25	between 2018 and 2020, were enrolled. Data regarding demographic characteristics and laboratory
26	investigation results were collected. Immune system recovery was used to assess the effectiveness of
27	ART, and an increased percentage of CD4 <sup>+</sup> T lymphocyte (CD4) counts > 30% after receiving ART for > 1
28	year was determined as immunopositive. A multiple logistic regression model was used to
29	comprehensively quantify the association between PLWHA immunological response status and virus
30	subtype. In addition, the joint association between different subtypes and treatment regimens on
31	immunological response status was investigated.
32	Results: Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the
33	percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate
34	logistic regression models, there was no difference in the immunological response between patients
35	with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy
36	[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
37	evidence of an obvious joint association between HIV subtypes and ART regimens on immunological
38	response.
39	Conclusion: Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it
40	was more effective, to some extent, in PLWHA with CRF01_AE.
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## 42 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.

## 52 Introduction

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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]

60 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 61 with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell levels, have been eligible to 62 63 receive free-state ART and regular follow-up visits by health service staff.[7] In 2019, the world has 64 entered an era of universal access to ART, and many developing countries, including China, have, in 65 large part achieved full coverage of ART, with an increasing number of PLWHA receiving free ART 66 and achieving viral control.[8] However, the HIV epidemic in China has shown no signs of slowing 67 down.[9]

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68 HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate,69 which may produce biological variability that affects treatment outcomes.[10] There are 2 major HIV

70 types— HIV type 1 (HIV-1) and HIV type 2 (HIV-2), and PLWHA with HIV-2 tend to have a lower

71 viral load than those with HIV-1. HIV-1 is by far the most prevalent type, with almost 95% of global 72 HIV infections are of type 1, and HIV-1 is further divided into four groups, including groups M, O, N 73 and P. The Group M is the world's major epidemic pathogen of HIV,[11] and is further divided into 10 74 subtypes (A,B,C,D,F,G,F,J,K, and L), a series of circulating recombinant forms (CRFs)[12] and unique 75 recombinant forms(URFs). Currently, CRFs formed by recombination among subtypes B,C, and 76 CRF01\_AE are the most common in China.. HIV-1 CRF01\_AE and CRF07\_BC account for 36.2% and 77 40.8% of the population reported to be infected in 2018, respectively, according to the results of the 2018 78 China Molecular Epidemiology Survey,[13] and these 2 branches have become the most predominant 79 CRFs of HIV in China.[14] CRF01\_AE was reported to harbor a high prevalence of CXCR4 viruses,[15] 80 which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and 81 suboptimal CD4 restoration during ART.[18] Studies investigating the effectiveness of ART in patients 82 with major HIV-1 subtypes have been inconclusive. An analysis of the impact of HIV-1 subtype 83 diversity on long-term clinical outcomes of ART in Guangxi Province suggested that patients with 84 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 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.

## 92 Methods

## 93 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.

- 105 Patient and public involvement
  - 106 None.

## 107 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)<sup>2</sup>. Information on laboratory tests was obtained from the Huzhou CDC or a local hospital. Tests included CD4<sup>+</sup> T lymphocytes (CD4), CD8<sup>+</sup> 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 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].
- 54 124 Statistical analysis
- 55 125 Because missing values introduced some bias in the results, all variables with missing ratios > 30% were
   56
  - 126 eliminated from the final working dataset. Otherwise, the missing values were filled using a 5-fold
  - 127 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<sup>2</sup>. 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. 

## 149 Results

## 150 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<sup>9</sup>/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.

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## 163 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.

230 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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## 239 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**

246 The authors declare no conflict of interest.

## 247 Data Availability Statement

248 The datasets used and analyzed during the current study are available from the corresponding author249 on reasonable request.

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Supporting Discipline (Epidemiology), and the Key Laboratory of Emergency detection for Public
Health of Huzhou.

- 53 255 Ethics Statement
  - 256 The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease257 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; BMI=body mass index; ALT=alanine aminotransferase; HR=Heterosexual transmission; 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)
  - udies or . determined in previous studies or according to the median values of the indicators taken in the current
  - study (ALT, AST, SCR).

Without immunological With immunological					
Variables	response (n=101)	response (n=225)			
Categorical variables		<b>I I I I I</b>			
Gender					
Male	89 (88 1)	186 (82 7)			
Ethnicity					
Han Chinese	100 (99.0)	216 (96.0)			
Other	1(1.0)	9(4.0)			
Occupation	-()	(10)			
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)	<i>4</i> 2(18.7)			
Education level	15 (14.7)	42(10.7)			
Primary school or under	35 (34 7)	62 (27 6)			
Middle and high school	53 (52 5)	119 (52 9)			
College or above	12(12.9)	119 (32.9)			
History of vonercal discase	13(12.9)	44(19.0)			
Nana	(02)	197 (90.0)			
None	04 (03.2) 16(15 9)	102(00.9)			
Ies Natarritable	16(15.8)	42(16.7)			
	1(1.0)	1(0.4)			
Route of infection	10 (10 ()				
MSM	43 (42.6)	87 (38.7)			
HR	58 (57.4)	138(61.3)			
Contact history					
History of men who have	37 (36.6)	73(32.4)			
sex with men					
Sexual contact occurring	60 (59.4)	134(59.6)			
out of wedlock					
Sexual contact with a	4(4.0)	18(8.0)			
partner					
Marital status					
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					
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					
AIDS	21 (20.8)	86(38.2)			

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

HIV	80 (79 2)	139(61.8)	
Sample source	00 (7 ).2)	107(01.0)	0.0
Pro operative testing	24 (22.8)	52(22.4)	0.5
	24 (23.8)	55(25.6)	
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			0.2
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.
BMI (kg/m <sup>2</sup> )	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.3
First CD4 cell	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.
counts(pcs/uL)			
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.8
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.
SCR (µmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.2
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.1
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.0
Time from diagnosis to start	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.
of treatment (days)	· · · · ·		

**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.

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			Cru	de	Adjus	ted
Variables	Ν	# (%)	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
	1.		1 · · · · · · · · · · · · · · · · · · ·		1 ( 1	. 1.

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

**Note:** Three subtypes are compared using the CRF01\_AE as the reference and two subtypes are compared using the CRF07\_BC as the reference.

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

Abbreviations: OR=Odds ratio; CI=confidence interval

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207x97mm (600 x 600 DPI)

1						
2						
3						
1						
4						
5						
6						
7	Subgroup	CRF01_AE	CRF07_BC	OR(95% CI)	P-Value	
8	Total	74(28.79)	103(40.08)	0.79(0.44-1.41)	0.4320	
9	Gender Male Female	67(73.63) 7(77.78)	87(63.50) 16(80.00)	0.75(0.41-1.38)	0.3500	
10	Infection pathway	24/75 56)	40(60.61)	0.74(0.20.1.02)	0.5000	
11	HR	40(72.73)	63(69.23)	0.82(0.37-1.78)	0.5090	
12	primary school or und	er69(75.00)	89(67.42)	0.66(0.18-2.47)	0.5370	
13	middle and high scho college or above Marital status	43(72.88)	14(56.00) 54(65.85)	0.89(0.41-1.91) 0.64(0.13-3.01)	0.7660 0.5680	
14	Unmarried	19(82.61)	18(69.23)	0.85(0.32-2.24)	0.7440	-
15	Divorced or widowed	12(66.67)	20(66.67)	0.46(0.17-1.27) 1.08(0.29-4.01)	0.1350 0.9040	
16	BMI<25 BMI > 25	30(69.77)	31(67.39) 52(64.20)	0.75(0.41-1.40)	0.3670	-
17	ALT	52(32.05)	52(01.20)		0.1500	
18	ALT<25.1 ALT≥25.1 AST	32(78.05) 42(71.19)	53(61.63) 50(70.42)	0.54(0.22-1.32) 1.16(0.52-2.57)	0.1740 0.7210	
19	AST<24.3 AST≥24.3	39(81.25) 35(67.31)	47(58.75) 56(72.73)	0.43(0.18-1.07) 1.41(0.64-3.12)	0.0700 0.3930	
20	SCR SCR<71 5	34(68.00)	54(69.23)	1 27(0 57-2 85)	0 5610	
21	SCR≥71.5	40(80.00)	49(62.03)	0.51(0.21-1.22)	0.1290	
22						0.1 1 10 100
23						OR(95% CI)

## 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 <sup>2</sup> )	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.



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	Table S2. Joint associations between HIV subtypes and ART regimens on immunological response						
					Crude	1	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.

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nt—ch	ecklist of items that should be included in reports of observational studies	)23-07259 ht, inclu	
Item No.	Recommendation	ding of Page of 1 No.	Relevant text from manuscript
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	eSrelated to text and	Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective, to some extent, in PLWHA with CRF01 AE
		l froi eur (	
2	Explain the scientific background and rationale for the investigation being reported	An action 2, lines An action 2, lines An action of the second of the sec	CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses <sup>15</sup> , which contributed to rapid CD4 T- lymphocyte count depletion in natural infection <sup>16, 17</sup> and suboptimal CD4 restoration during ART <sup>18</sup> .
3	State specific objectives, including any prespecified hypotheses	ar technologia (1,1)	Therefore, we hypothesized that the effectiveness of ART differs among HIV-1 subtypes.
		2028 9log	
4	Present key elements of study design early in the paper	Page 3, line 93	The present investigation was a retrospective cohort study.
5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 3, lines 93- 95 Bibliogra	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control
	Item <u>No.</u> 1 2 3 3	BMJ Open         tt—checklist of items that should be included in reports of observational studies         Item       Recommendation         1       (a) Indicate the study's design with a commonly used term in the title or the abstract         (b) Provide in the abstract an informative and balanced summary of what was done and what was found         2       Explain the scientific background and rationale for the investigation being reported         3       State specific objectives, including any prespecified hypotheses         4       Present key elements of study design early in the paper         5       Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	BMU Open     Yo oppen       it—checklist of items that should be included in reports of observational studies     Including of the study's design with a commonly used term in the title or the abstract     Including of the study's design with a commonly used term in the title or the abstract       1     (a) Indicate the study's design with a commonly used term in the title or the abstract     Integration of the study's design with a commonly used term in the title or the abstract     Integration of the study's design with a commonly used term in the title or the abstract       2     Explain the scientific background and rationale for the investigation being reported     Integration of the study of th

e 19 of 23		BMJ Open	mjopen-2 ɔy copyri	
			023-072597 c ght, including	PCIS) in Huzhou between 201 and 2020, were reviewed.from 2018-2020.
Participants	6	<ul> <li>(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</li> <li>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</li> <li>Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants</li> </ul>	g Page 3, lines 99 Prage 5 May 2024. Down Enseignement to te	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	with a manual state of the second state of the	The distribution of HIV subtypes was as follows :CRF01_AE (n=100); CRF07_B0 (n = 157); Other (n=69).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, lines 19120 Al training,	Immunological response was defined as an increase in CD4 cell counts > 30% from baselin 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	and similar technologies.	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 201 and 2020, were reviewed.from 2018-2020.
Bias	9	Describe any efforts to address potential sources of bias	geno	
Study size	10	Explain how the study size was arrived at	Page 3, lines 93- 94 bii ographi	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control
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	ight, including for	Information System (the AIDS- PCIS) in Huzhou between 2018 and 2020, were reviewed.from 2018-2020.
Continued on next page	May 2024. Downloaded from http://br Enseignement Superieur (ABES) .	
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	ies.	

Page 21	of 23
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of 23		BMJ Open	omjopen-20 by copyrig	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	ht 23 mage73, lines ding o	Information on laboratory tests w obtained from the Huzhou CDC a local hospital.
Statistical methods	12	( <i>a</i> ) Describe all statistical methods, including those used to control for confounding	n ag5 №39 por 039 por	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and
		(b) Describe any methods used to examine subgroups and interactions	oowaloaded from h tortext and data m	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	4, lines 29 Japan Grant training, Al training,	Because missing values introduc some bias in the results, all variables with missing ratios > 30 were eliminated from the final working dataset.
		( <i>d</i> ) 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	and similar techn	Because missing values introduct some bias in the results, all variables with missing ratios > 30 were eliminated from the final working dataset.
		( <u>e</u> ) Describe any sensitivity analyses	Agence Bil	Subsequently, a sensitivity analy of the comparison of pre-and pos- imputation was additionally applied to validate the stability of the imputations (see Table S1).
Results			Slio	
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	graphique	
		<b>4</b> . For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xk	ntml –	

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		(b) Give reasons for non-participation at each stage	ht, in	23-07	
		(c) Consider use of a flow diagram	ctag din 54	254, line 707	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	for uses relat	5 5 5 5 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7	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	ed t	D	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	nt Superie	4, line 02155	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	ur∜A&ES). data mining, Al traini	ren 4, line 69	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%).
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	ng,	3	
		Cross-sectional study—Report numbers of outcome events or summary measures	anc	6	
Main results	16	( <i>a</i> ) 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	similar technologies.	170- 170- 170- 170- 11. 2025 at Agence Bibliogram	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	-	hi	
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vant, consider translating estimates of relativ	ve risk into absolute risk for a me	2023-072597 on 15 May 2024. Downloaded fron Enseignement Superieur (/ ight, including for uses related to text and data	
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Other analyses	1/	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	incl	0722
Discussion			udir	59 7
Key results	18	Summarise key results with reference to study objectives	)g fo	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss	or u	1 5 7
		both direction and magnitude of any potential bias	ses	Aav
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of	rela	202
		analyses, results from similar studies, and other relevant evidence	atec	<u>4</u> —
Generalisability	21	Discuss the generalisability (external validity) of the study results		80 ≪
Other informati	on		tex	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the	per t an	a de
		original study on which the present article is based	ieu d d	d fr
Note: An Explan checklist is best u http://www.annal	ation used i	and Elaboration article discusses each checklist item and gives methodological background and published n conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosme /, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at w	ng, exaampl licinae.or wwnitro	s of transparent reporting. The STROBE /, Annals of Internal Medicine at e-statement.org.
Note: An Explan checklist is best u http://www.annal	ation ised i ls.org	and Elaboration article discusses each checklist item and gives methodological background and published n conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmed /, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at w	ng, Attracinition licianity, and similar technologies.	on June 11.2025 at
# **BMJ Open**

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

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1	Analysis of the immunological response to antiviral therapy in
2	patients with different subtypes of HIV/AIDS: a retrospective
3	cohort study
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18	Keywords: HIV/AIDS, ART, CD4 T lymphocyte count, CRF07 BC, CRF01 AE, immunological response
19	
20	Abstract
21	<b>Objective:</b> To evaluate the effectiveness of standardised antiretroviral therapy (ART) among different
22	HIV subtypes in people living with HIV/AIDS (PLWHA), and to screen the best ART regimen for this
23	patient population.
24	<b>Design:</b> A retrospective cohort study was performed, and PLWHA residing in Huzhou. China, between
25	2018 and 2020, were enrolled
<u>-</u> 5 26	Setting and participants: Data from 625 patients, who were newly diagnosed with HIV/AIDS in the
20	AIDS Prevention and Control Information System (the AIDS-PCIS) in Huzbou between 2018 and 2020
27	were reviewed
20	Analyzis and outcome measures. Data regarding demographic characteristics and laboratory.
29	investigation regults were collected Immune system recovery was used to access the effectiveness of
5U 21	APT and an increased percentage of CD4t T lumphogute (CD4) counter 20% after receiving APT for 1
22	ART, and an increased percentage of $CD4^{+}$ T tymphocyte ( $CD4$ ) counts > 50% after receiving ART for > 1
32	year was determined as immunopositive. A multiple logistic regression model was used to
33	comprehensively quantify the association between PLWHA immunological response status and virus
34	subtype. In addition, the joint association between different subtypes and treatment regimens on
35	immunological response status was investigated.
36	<b>Results:</b> Among 326 enrolled PLWHA with CRF01_AE, CRF07_BC, and other HIV/AIDS subtypes, the
37	percentages of immunopositivity were 74.0%, 65.6%, and 69.6%, respectively. According to multivariate
38	logistic regression models, there was no difference in the immunological response between patients
39	with CRF01_AE, CRF07_BC, and other subtypes of HIV/AIDS who underwent antiretroviral therapy
40	[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
41	evidence of an obvious joint association between HIV subtypes and ART regimens on immunological

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response. 43 Conclusions: Standardised ART was beneficial to all PLWHA, regardless of HIV subtypes, although it 44 was more effective, to some extent, in PLWHA with CRF01\_AE.

Strengths and limitations of this study 46

- The present study was based on a population-based cohort rather than case-control or descriptive investigations.
- 49 The study analysed the association between HIV subtypes and immunological responses, 50 considering the joint association of subtypes and ART regimens.
- 51 HIV-1 subtypes were regionally relevant and sampling bias was inevitable.
- 52 This study had a small sample size because HIV-1 genotyping is not routinely performed in China.
- 53 Data regarding viral load was missing from a large number of records.

#### **INTRODUCTION** 56

AIDS is a major public health problem.[1] As of 2019, 38 million individuals worldwide are living with 57 58 HIV, and 690,000 have died from HIV-related illnesses.[2] HIV harms human health primarily by 59 infecting the body and destroying immune cells, and individuals living with HIV often live as 60 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 61 62 beginning of the 21st century.[4] However, there is a wide variation in the type of epidemic and 63 geographical spread, which poses a great challenge to the prevention and control of AIDS in China.[5]

64 Although there is still no effective cure for AIDS, previous studies have shown that antiretroviral 65 therapy (ART)is widely used to control HIV/AIDS, with good results.[6] Since 2016, all people living 66 with HIV/AIDS (PLWHA) in China, regardless of their initial CD4 cell levels, have been eligible to 67 receive free-state ART and regular follow-up visits by health service staff.[7]In 2019, the world has 68 entered an era of universal access to ART, and many developing countries, including China, have in 69 large part achieved full coverage of ART, with an increasing number of PLWHA receiving free ART and 70 achieving viral control.[8] However, the HIV epidemic in China has shown no signs of slowing 71 down.[9]

HIV, a highly diverse virus, exhibits significant genetic variability and a high viral replication rate, 72 73 which may produce biological variability that affects treatment outcomes.[10] There are 2 major HIV 74 types- HIV type 1 (HIV-1) and HIV type 2 (HIV-2), and PLWHA with HIV-2 tend to have a lower 75 viral load than those with HIV-1. HIV-1 is by far the most prevalent type, with almost 95% of global 76 HIV infections are of type 1, and HIV-1 is further divided into four groups, including groups M, O, N 77 and P. The Group M is the world's major epidemic pathogen of HIV,[11] and is further divided into 10 78 subtypes (A,B,C,D,F,G,F,J,K, and L), a series of circulating recombinant forms (CRFs)[12] and unique 79 recombinant forms(URFs). Currently, CRFs formed by recombination among subtypes B,C, and 80 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 81 82 China Molecular Epidemiology Survey,[13] and these 2 branches have become the most predominant 83 CRFs of HIV in China.[14]CRF01\_AE was reported to harbour a high prevalence of CXCR4 viruses,[15] 84 which contributed to rapid CD4 T-lymphocyte count depletion in natural infection[16, 17] and 85 suboptimal CD4 restoration during ART.[18] Studies investigating the effectiveness of ART in patients

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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.

#### 96 METHODS

#### 97 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.

#### 109 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)<sup>2</sup>. Information on laboratory tests was obtained from the Huzhou CDC or a local hospital. Tests included CD4<sup>+</sup> T lymphocytes (CD4) , CD8<sup>+</sup> 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].

#### 55 126 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

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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<sup>2</sup>. 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 Ĉ.

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)× 109/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.

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#### 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)].

#### 177 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.

#### 185 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. 

58 215 Our study had several limitations, the first of which were its small sample size and short follow-up
 59 216 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.

## 229 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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#### 238 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.

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#### 244 Competing interests

245 The authors declare no conflict of interest.

#### 246 Data availability statement

- 247 The datasets used and analysed during the current study are available from the corresponding author248 on reasonable request.
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- 254 Ethics approval
  - 255 The study protocol had been approved by the Ethics Committee of the Huzhou Center for Disease256 Control and Prevention (CDC) (batch number HZ2021001).

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	immunological response	status
	Without immunological	With immunological
Variables	response (n=101)	response (n=225)
Categorical variables		
Gender		
Male	89 (88.1)	186 (82.7)
Ethnicity		
Han Chinese	100 (99.0)	216 (96.0)
Other	1(1.0)	9(4.0)
Occupation		
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	× /	
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	()	()
None	84 (83.2)	182 (80.9)
Yes	16(15.8)	42(187)
Not available	1(1.0)	12(10.7)
Route of infection	1(1.0)	1(0.1)
MSM	43 (42 6)	87 (38 7)
HR	58(57.4)	138(61.3)
Contact history	56 (57.4)	138(01.3)
History of mon who have	27 (26 6)	72(22.4)
soy with mon	57 (50.0)	75(52.4)
Sex with men	60 (59 4)	124(50 4)
Sexual contact occurring	60 (39.4)	134(39.6)
out of wedlock	4(4.0)	10/0 0)
Sexual contact with a	4(4.0)	18(8.0)
partner		
Marital status		
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		
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		
	21(20.8)	86(38.2)

HIV	80 (79.2)	139(61.8)	
Sample source	· · ·	· · ·	0.9
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			0.2
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)	0.3
BMI (kg/m²)	22.5 (20.0,24.8)	21.9 (20.0,23.8)	0.3
First CD4 cell	382.0 (246.0,477.0)	237.0 (155.0,325.0)	<0.
counts(pcs/uL)			
CD8 cell counts(pcs/uL)	572.0 (466.2,779.0)	691.0 (487.5,975.4)	0.0
AST (U/L)	23.0 (19.4,28.0)	23.0 (18.6,29.6)	0.8
ALT (U/L)	24.8 (16.0,37.2)	25.0 (17.6,39.0)	0.4
SCR (µmol/L)	70.3 (59.0,78.1)	70.9 (62.0,80.6)	0.5
HB (g/L)	146.5 (134.5,156.0)	149.0 (135.0,159.0)	0.2
PLT (10^9/L)	199.5 (166.5,240.5)	210.0 (171.0,241.0)	0.2
WBC (10^9/L)	5.2 (4.3,6.2)	5.8 (4.7,6.9)	0.0
Timefrom diagnosis to start	13.0 (12.0,25.0)	12.0 (8.0,19.0)	0.0
of treatment (days)			

**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.

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Variables	Ν	# (%)	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

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

**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.



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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 <sup>2</sup> )	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.



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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.

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Recommendation ndicate the study's design with a commonly used term in the title or the abstract rovide in the abstract an informative and balanced summary of what was done and what wa d ain the scientific background and rationale for the investigation being reported	ding for No. 97 on Association of the second secon	Relevant text from manuscript         a historical cohort study         Standardized ART was         beneficial to all PLWHA,         regardless of HIV subtypes         although it was more effective         to some extent, in PLWHA with         CRF01_AE         CRF01_AE was reported to         harbor a high prevalence of         CXCR4 viruses <sup>15</sup> , which         contributed to rapid CD4 T-
ndicate the study's design with a commonly used term in the title or the abstract provide in the abstract an informative and balanced summary of what was done and what wa d ain the scientific background and rationale for the investigation being reported	as Sreignement Superieur (ABES). data 2, lines data mining. Al train	a historical cohort study Standardized ART was beneficial to all PLWHA, regardless of HIV subtypes although it was more effective to some extent, in PLWHA wi CRF01_AE CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses <sup>15</sup> , which contributed to rapid CD4 T-
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en	.bmj.com/ or ving, and sim	lymphocyte count depletion i natural infection <sup>16, 17</sup> and suboptimal CD4 restoration during ART <sup>18</sup> .
e specific objectives, including any prespecified hypotheses	ar techno, 2	Therefore , we hypothesized that the effectiveness of ART differs among HIV-1 subtype
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ent key elements of study design early in the paper	Pagæ3, line 93	The present investigation was retrospective cohort study.
eribe the setting, locations, and relevant dates, including periods of recruitment, exposure, w-up, and data collection	Page 3, lines 93- 95 Bibliographic	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AIDS
ent crit w-	be the setting, locations, and relevant dates, including periods of recruitment, exposure, up, and data collection	rkey elements of study design early in the paper       Prage 3, line 93         be the setting, locations, and relevant dates, including periods of recruitment, exposure,       Page 3, lines 93-         up, and data collection       95         Bio       Page 3, line 93         For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml       Page 3, line 93

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			23-072597 o ht, including	PCIS) in Huzhou between 201 and 2020, were reviewed.from 2018-2020.
Participants	6	<ul> <li>(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</li> <li>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</li> <li>Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants</li> </ul>	n 3, lines 99 Pag5 May 2024. Down Enseignement S related to te	The Inclusion criteria for the study were as follows:
		<ul> <li>(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed</li> <li>Case-control study—For matched studies, give matching criteria and the number of controls per case</li> </ul>	where the second	The distribution of HIV subtypes was as follows :CRF01_AE (n=100); CRF07_B (n = 157); Other (n=69).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	innig 3, lines 191220 Al training,	Immunological response was defined as an increase in CD4 cell counts > 30% from baselin at 12 months after initiation o 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	and smilar technologies.	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control Information System (the AID PCIS) in Huzhou between 202 and 2020, were reviewed.from 2018-2020.
Bias	9	Describe any efforts to address potential sources of bias	genc	
Study size	10	Explain how the study size was arrived at	Pag <mark>b</mark> 3, lines 93- 94 Dio grap	Data from 625 patients, who were newly diagnosed with HIV/AIDS in the AIDS Prevention and Control

Page 19 o	of 22	BMJ Open	'bmjopen-20
1 2 3 4 5 6			Information System (the AIDS-Information System (the AIDS-PCIS) in Huzhou between 2018and 2020, were reviewed.from2018-2020.
<pre>/ 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40</pre>	Continued on next page		May 2024. Downloaded from http://bmjopen.bmj.com/ on June 11, 2025 at Agence Bibliographic Enseignement Superieur (ABES) . uses related to text and data mining. Al training, and similar technologies.
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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why		Information on laboratory tests was obtained from the Huzhou CDC or a local hospital.
Statistical methods	12	( <i>a</i> ) Describe all statistical methods, including those used to control for confounding	g Paga4, lines Praga4, lines Cruses related t telated t	A multivariate logistic regression model was used to determine the statistical association between different HIV subtypes and immunological response
		( <i>b</i> ) Describe any methods used to examine subgroups and interactions	oftext and data m	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	Al training,	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset.
		( <i>d</i> ) 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	and age 4, lines 1329 J 32 Similar techn	Because missing values introduced some bias in the results, all variables with missing ratios > 30% were eliminated from the final working dataset
		( <i>e</i> ) Describe any sensitivity analyses	Grager 4, lines gi 31at 32 Agence	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			blio	
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examine for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	ed graphiq	
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.>	tie de khtml –	

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		(b) Give reasons for non-participation at each stage	2023-072 ight, inc	
		(c) Consider use of a flow diagram	<b>H</b> ag <b>g</b> 4, line <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b>	A flow chart of participant shown in Figure 1.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	for using 2024 for using 158-159 for using relat	Characteristics of the study participants according to immunological responses a shown in Table 1.
		(b) Indicate number of participants with missing data for each variable of interest	. Do eme	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	ongeneration wale by the tand add tand tand tand tand tand tand	A total of 326 PLWHA wer included in the present stud an mean follow-up of abou
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	room Tattp://bmjopen.b Jrad SEES) . data Thining, Al trainin	The proportion of positive immunological responses in PLWHA with CRF01_AE w obviously higher than that PLWHA with CRF07_BC at subtypes (74.0% vs 65.6% v
		Case-control study-Report numbers in each exposure category, or summary measures of exposure	mj. ng,	
		Cross-sectional study—Report numbers of outcome events or summary measures	con and	
Main results	16	( <i>a</i> ) 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	4-5, ag ag ag ag ag n ag n 170- 75 11, 2025 at Agence Bibliogra similar technologies.	Compared to patients with CRF01_AE, the possibility of immune response for those CRF07_BC and other subty not significantly different [CRF07_BC: aOR (95%CI) = 0.8(0.4,1.4); other subtypes: (95%CI) =1.0(0.5,2.0)], in wl adjusted for the infection st WBC, time from diagnosis treatment and CD8.
		(b) Report category boundaries when continuous variables were categorized	ap hi	
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**BMJ** Open (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time

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of 22		BMJ Open BMJ Open Copyri: 2
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses     S       S     S
Discussion		cluc
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 informat	ion	
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
checklist is best http://www.anna	used i ıls.org	in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at y/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.jtrobe-statement.org.
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