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Low CD4 count was characterized in recent HIV CRF01_AE infection and it rapidly increased to reach a peak in the first year since ART initiation

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Abstract

Background Currently, most people living with HIV (PLWH) in China have a strong awareness of diagnosis and treatment in the early stage of HIV infection. Subtype-specific virological and immunological features of recently infected PLWH have not yet been elucidated.

Methods Data including CD4 count and viral load (VL) of 1508 anti-retroviral therapy (ART) -naïve PLWH were obtained from the HIV Database and comparatively analyzed among PLWH with different HIV subtypes. The infection status of 402 newly diagnosed and ART-naïve PLWH from a cohort of men who have sex with men (MSM) in China was evaluated using diagnosis records and LAg-Avidity EIA. Based on partial *pol* genes, HIV genotypes in 120 recent, 68 long-term, and 54 chronic infections were identified. The CD4 count, CD8 count, and VL, as well as trajectories of dynamic CD4 counts during ART of local PLWH with different HIV subtypes, were compared using non-parametric tests.

Results For the HIV database, the CD4 count in PLWH with CRF01_AE was lower than that in PLWH with CRF07_BC or subtype B. For the recently infected local PLWH, CRF01_AE was the dominant HIV subtype (65.83%), followed by CRF07_BC (18.33%) and subtype B (15.83%). Recent CRF01_AE infections showed a lower baseline CD4 count than CRF07_BC infections. During ART for recently infected PLWH, the CD4 count in the CRF01_AE group rapidly increased to reach a peak at the end of the first year post-ART, while the CD4 count in the CRF07_BC group increased slowly to reach a plateau at the end of the third year. The CD4 count in the subtype B group increased significantly to reach a plateau within the first two years and then its trajectory overlapped with that of the CRF07_BC group at the end of the third year post-ART.

Conclusions CRF01_AE rapidly reduced CD4 count during the recent HIV infection. The CD4 count of the recently infected individuals with CRF01_AE increased sharply and reached its highest level of recovery within the first year

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of ART initiation. This study revealed an important time point for estimating CD4 count recovery post-ART in individuals with different HIV subtypes.

Keywords Recent HIV infection, CRF01_AE, CD4 count, Anti-retroviral therapy, Dynamic changes of CD4 count

Introduction

In recent years, the human immunodeficiency virus (HIV) CRF01_AE infection rate has increased significantly in China and is associated with faster disease progression [1–4]. CRF01_AE is prevalent not only in men who have sex with men (MSM) but also in the heterosexual population, and sexual contact has become the most common transmission route of HIV in China [1, 5–7]. The proportion of newly diagnosed HIV infections that were transmitted through homosexual behavior in China is about 25%, and that in Harbin, Northeast China, is about 84.7% [2, 8, 9]. A study that analyzed all *pol* gene sequences from China in the HIV Database showed that CRF01_AE was the most prevalent subtype in China, accounting for 39.69% of total infections, followed by CRF07_BC (20.47%), B (17.50%), CRF08_BC (6.60%), C (6.28%), CRF55_01B (2.06%) and other CRFs (1.77%) [10].

Anti-retroviral therapy (ART) can reduce the morbidity and mortality of people living with HIV (PLWH), improve patients' quality of life, prevent HIV transmission and control global HIV pandemics [11]. Based on the current situation of prevention and control of HIV infection in China, most PLWH have a strong awareness of diagnosis and treatment, and can be found and treated early. A lower CD4 count was found in PLWH with CRF01_AE than in those with other subtypes, but the subjects in some studies were newly diagnosed PLWH, not recently infected ones [3, 12]. Therefore, an understanding of the epidemiological, subtype-specific virological and immunological features of ART-naïve PLWH at the early stage of HIV infection and dynamic tracing of the trajectory of these features post-ART would help predict immune reconstitution, control disease progression, and improve the quality of life.

In the present study, recent infections were identified in ART-naïve local PLWH in an MSM cohort. Some PLWH were followed up for 5 years after ART initiation, which was defined as post-ART. The clinical indicators, including CD4 and CD8 counts, CD4/CD8 ratio, and viral load (VL) among PLWH with HIV subtypes CRF01_AE, CRF07_BC, or B, were detailed and comparatively analyzed using an HIV database [12] and our local MSM cohort. Distinct and representative trajectories of CD4 count post-ART of PLWH with different HIV subtypes were plotted. This study will provide valuable information for developing public health policies and precise programs for the treatment and prevention of HIV infection.

Methods

Data collection from the HIV database

In total, data from 117,330 PLWH records were downloaded from the HIV Database, including 67,502 CRF01_AE, 17,448 CRF07_BC, and 32,380 subtype B cases [13]. Data including CD8 count, patient age, gender, and days from infection were largely unavailable for most of these PLWH; therefore, only available information including patient ID, ART-naïve, CD4 count, and VL (>40 copies/ml) were selected for subsequent analysis. For patient ID with multiple data records, only the first record was included. Owing to the unavailability of CD8 count data for most PLWH in the database, comparisons between subtypes were made only for CD4 count and VL data.

Study participants

Blood samples were collected from 715 ART-naïve PLWH in an MSM cohort aged 18–60 years old during 2016–2020 in Harbin, Northeast China. Individuals with hepatitis B virus, hepatitis C virus, or active tuberculosis were excluded. Among them, 562 newly (≤6 months) and 153 non-newly (>6 months) diagnosed individuals were distinguished. Among the newly diagnosed individuals, 402 PLWH with CD4 counts ≥250 cells/μl were evaluated for infection status using limiting-antigen avidity enzyme immunoassay (LAg-Avidity EIA). The LAg-Avidity assay for identifying recent HIV-1 infections has been reported to have a consistent performance across different HIV subtypes [14, 15]. Based on this method, HIV infection with different subtypes (AE, BC, and B, etc.) was classified into two stages: recent infection, the mean duration of recent infection (MDRI) was 130 days (118–142 days), and long-term infection [15, 16]. A total of 120 recently infected individuals and 282 long-term infected individuals were identified. China has implemented nationwide guidelines recommending immediate initiation of free ART after HIV diagnosis since June 2016 [17], which allowed us to recruit chronically infected individuals who were ART-naïve and had been diagnosed for more than 2 years [18]. Finally, the *pol* genes from all 120 recently, 68 long-term, and 54 chronically infected individuals were amplified, and HIV genotypes were determined (Fig. 1). These PLWH were treated with

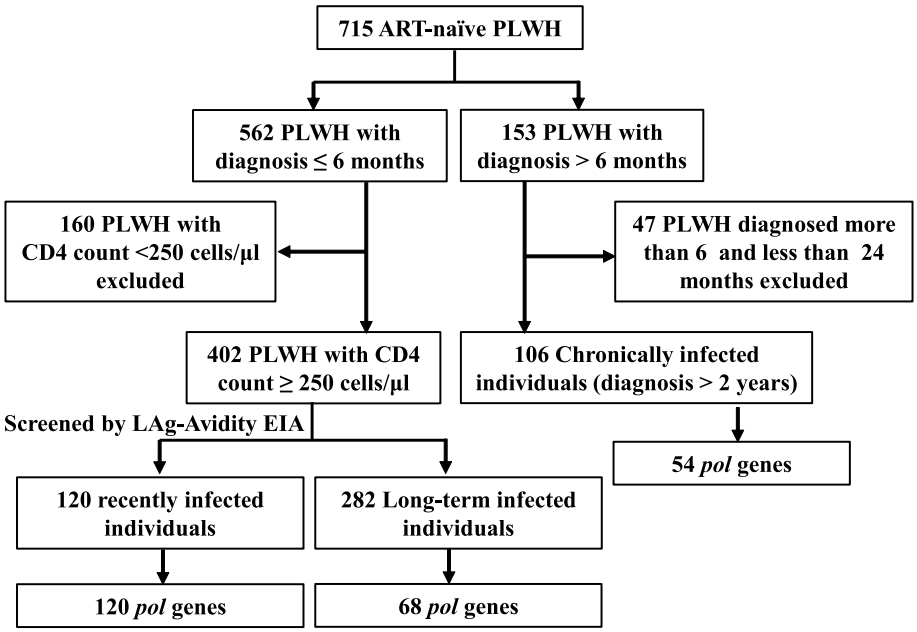

Fig. 1 The study flowchart. The ART-naïve PLWH enrolled in the study were divided into the recently, long-term and chronically infected individuals using HIV diagnosis time, CD4 count and the results of LAg-Avidity EIA. The *pol* genes of all recently infected individuals and the part of the long-term and chronically infected individuals were amplified and determined by phylogenic analysis to distinguish into the CRF01_AE, CRF07_BC, and B groups

lamivudine (3TC), tenofovir (TDF)/zidovudine (AZT), efavirenz (EFV), and their VLs were suppressed to the under-detection level at the end of the sixth-month post-ART. Some participants were followed up for more than 5 years since ART initiation. VL was detected using an m2000rt Real-Time PCR System with a detection limit of 40 copies/ml (Abbott, USA). CD4 and CD8 counts were analyzed using a BD FACS Count™ Reagent kit for flow cytometry (FACS Calibur, BD Company, USA).

This study was approved by the Institutional Review Board of the Medical Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University (2017-SCI-07), Medical Ethics Committee of Heilongjiang Provincial Hospital ([2019]092) and Medical Ethics Committee of Harbin Sixth Hospital ([2021]01). Written informed consent was obtained from all the participants.

Viral RNA exaction and amplification and phylogenetic analysis of pol genes

The sample RNA was extracted from 200 μl of plasma using a QIA ampminElute Virus Spin Kit (QIAGEN, Germany). The *pol* genes were amplified by nested RT-PCR using the PrimeScript TM One-Step RT-PCR Kit Ver.2 and Prime STAR HS DNA polymerase (Takara, Japan). The primers used for *pol* amplification were as follows: POF, 5'-TTGGAAATGTGGAAAGGAAGGAC-3'; POR, 5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3'; PIF, 5'-CAGAGCCAACAGCCCCACCA-3'; PIR, 5'-CTTCTGTATATCATTGACAGTCCAGCT-3'. Sequencing results were corrected using Chromas software and analyzed with reference sequences downloaded from HIV Databases using Clustal X software. Phylogenetic trees were constructed according to the neighbor-joining method using MEGA 5.0 software, and the genotypes of *pol* genes were determined.

Enzyme immunoassay

The PLWH who were diagnosed within 6 months and had a baseline CD4 count ≥ 250 cells/μl were evaluated using the LAg-Avidity EIA kit (Jinhao, China) to distinguish recent HIV infection from long-term HIV infection [14, 15, 19]. Briefly, 100 μl of plasma of PLWH, reference control and negative control, and calibration control from the kit with 1:101 dilutions were added to each well which was coated with recombinant HIV-specific antigen (rIDR-M) and incubated at 37 ℃ for 60 min. After washing, an acidic washing solution was added and incubated at 37 ℃ for 15 min. After washing, an enzyme working solution containing goat anti-human IgG was added. After washes, 100 μl of TMB substrate was added and the reaction was developed at 25 ℃ for 15 min. Then 100 μl of stop solution was added. The optical density was read at 450 nm with a reference wavelength of 630 nm (Bio-Rad). Samples

with an ODn value (OD value of sample/calibration) equal to or less than 2.0 were detected again in triplicate. PLWH with an ODn value between 0.4 and 1.5 (including the upper and lower limits) in the confirmatory test were identified as having recent infections, and those with an ODn value more than 1.5 were identified as having long-term infections [14, 15, 20, 21].

Statistical analysis

All statistical analyses were performed using SPSS software (version 24.0; SPSS, Chicago, Illinois, USA). The differences between two groups or among three groups were analyzed using the Mann–Whitney *U* test and Kruskal–Wallis *H* test. The trajectories of CD4 counts were drawn using the Lowess method in R language to model the variation in CD4 counts over time for the three HIV subtypes (CRF01_AE, CRF07_BC, and B). Stability was defined when the slope of the curve decreased and approached a constant value. By calculating the slope of the curve at each time point and identifying the areas where the slope changed minimally, the approximate range where the CD4 count reached a plateau was determined [22]. Correlations were analyzed using GraphPad Prism 8 software (GraphPad Inc., La Jolla, California, USA), and the Pearson correlation coefficient (*r*) is shown. The *P* value less than 0.05 was considered statistically significant.

Results

Analysis of CD4 counts and viral loads in the ART-naïve PLWH in the HIV database

A total of 117,330 records from the HIV database were screened, 1508 PLWH were included in the final analysis, including 289 PLWH with CRF01_AE, 68 with CRF07_BC and 1151 with subtype B. The CD4 count and VL were compared between the subtypes (Fig. 2). The CRF01_AE group showed a lower median CD4 count (306 cells/μl; IQR166, 457) than CRF07_BC (392 cells/μl; IQR 322, 561, $P=0.001$) or B (411 cells/μl; IQR 272, 600, $P<0.001$) group (Fig. 2A). The CRF01_AE group (5.000; IQR 4.261, 5.524) exhibited a higher log VL value than the group B (4.699; IQR 4.099, 5.269, $P<0.001$) (Fig. 2B). The comparison of VL levels between the CRF01_AE and CRF07_BC groups was not performed because of limited data in the CRF07_BC group.

Recent HIV infection in local PLWH

In this study, from 562 PLWH collected in Harbin and diagnosed within 6 months, 402 PLWH with CD4 counts ≥ 250 cells/μl were screened using EIA. In the previous study, 3 recent infections from 292 samples with CD4 count < 250 cells/μl and 364 recent infections from

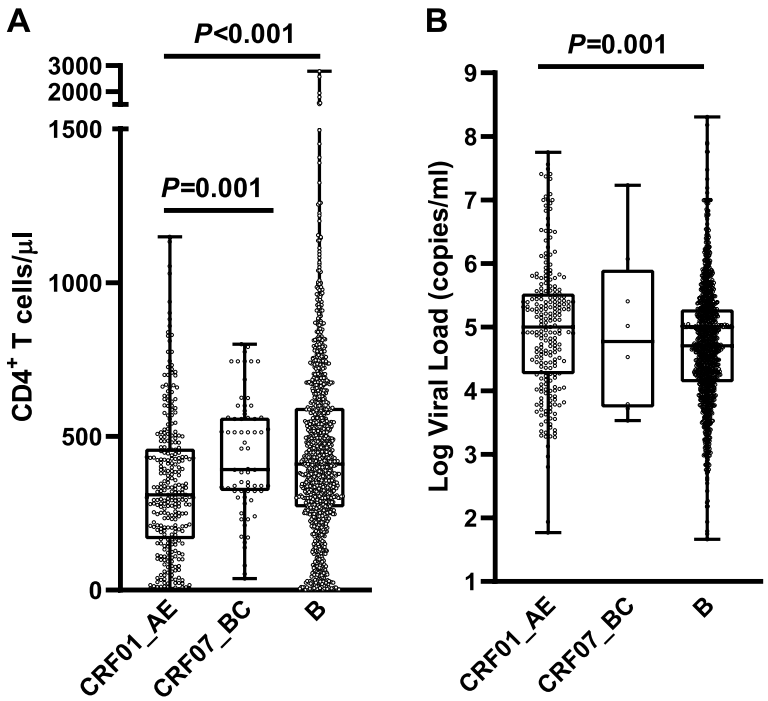

Fig. 2 Analysis of CD4 count and viral load of ART-naïve PLWH with CRF01_AE, CRF07_BC, and B from the HIV Database. **A** Baseline CD4 count (cells/μl). **B** Baseline log Viral Load (copies/ml). The median and IQR were shown as box

714 samples with CD4 count ≥ 250 cells/μl were identified, respectively [23]. That is among PLWH with CD4 counts < 250 cells/μl, the recent infection rate is approximately 1%. In this study, therefore, only the samples with CD4 counts ≥ 250 cells/μl were screened to find recent infection. Finally, 120 recent and 282 long-term infections were identified in 402 PLWH (Fig. 1).

HIV subtype distribution in the local MSM cohort

Because this study focused on the recently infected individuals, we amplified 120 *pol* genes from all screened recently infected individuals and 68 and 54 *pol* genes from long-term and chronically infected individuals, respectively, in chronological order of sample collection. Phylogenetic analysis of *pol* genes showed the HIV genotype diversity (Fig. 3). The CRF01_AE (64.88%),

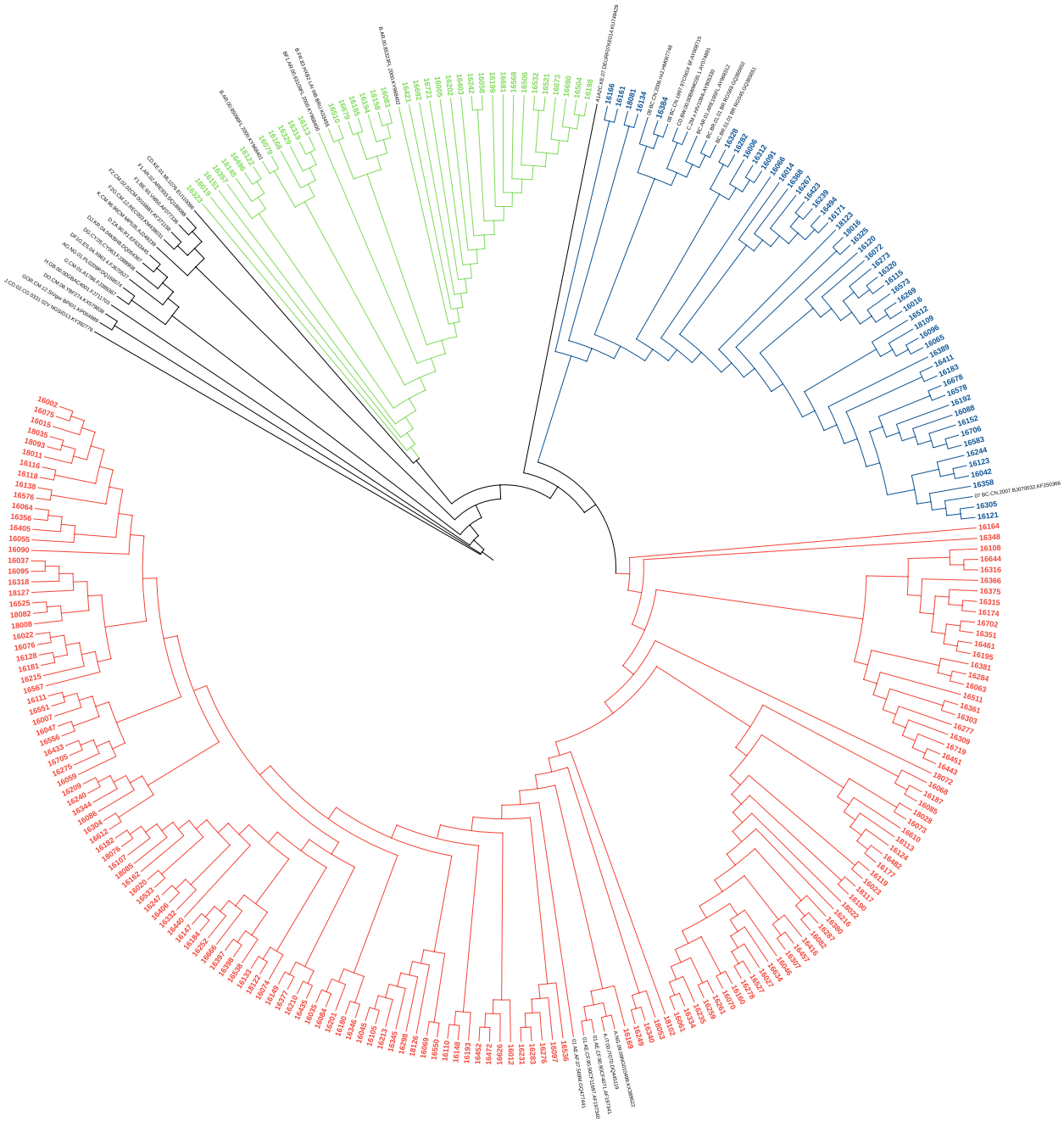

Fig. 3 Phylogenetic analysis of 242 *pol* gene segments in the recruited PLWH from MSM

CRF07_BC (19.83%), B (14.88%), and CRF08_BC (0.41%) were found in the cohort. Among the 120 recently infected individuals, CRF01_AE, CRF07_BC, and subtype B accounted for 65.83%, 18.33%, and 15.83% of the infections, respectively. Similar proportions of CRF01_AE, CRF07_BC, and subtype B were found in long-term (63.24%, 23.53%, and 13.24%) and chronically infected individuals (64.81%, 18.52%, and 14.81%). One CRF08_BC (1.85%) was identified among the chronically infected individuals (Fig. S1).

Comparisons of CD4 and CD8 counts, CD4/CD8 ratios, and viral loads among different HIV subtypes

First, 241 PLWH (excluding one chronically CRF08_BC infected individual) were divided into three groups: 157 in the CRF01_AE group, 48 in the CRF07_BC group, and 36 in the B group. There were no differences in age, CD4 count, CD8 count, CD4/CD8 ratio, or log VL at baseline and 1–2 years post-ART among the three groups (Fig. S2).

The CD4 count, CD8 count, CD4/CD8 ratio, and VL of the three groups at the three infection stages were analyzed (Fig. 4). There was no difference in the age of the PLWH among the three groups at the different infection stages (data not shown). Recently CRF01_AE infected individuals showed a lower baseline CD4 count than the CRF07_BC ($P=0.010$) or B ($P=0.088$) groups. Notably, during the long-term infection stage, although there was no significant difference in the baseline CD4 count among the three groups, the CRF07_BC group showed a higher baseline CD8 count ($P=0.046$) and a lower baseline CD4/CD8 ratio ($P=0.010$) than group B. In 1–2 years post-ART, the CRF07_BC group showed lower CD4 counts ($P=0.044$) and CD4/CD8 ratios ($P=0.002$) than group B. Meanwhile, the CRF01_AE group showed a lower post-ART CD4/CD8 ratio than group B ($P=0.026$). No differences in CD4 and CD8 counts or the CD4/CD8 ratio were found in chronically infected individuals. There was also no significant difference in the baseline VL among the three groups at the three infection stages. At the end of the sixth month after ART initiation, all 241 samples showed virological suppression (VL, <40 copies/ml).

Furthermore, the CD4 and CD8 counts and VL of recently and long-term infected individuals with the same HIV subtype infection were also compared. The recently CRF07_BC infected individuals showed a higher CD4 count ($P=0.069$, $P=0.019$), lower CD8 count ($P=0.033$, $P=0.042$), and higher CD4/CD8 ratio ($P=0.009$, $P=0.008$) at baseline and 1–2 years post-ART than the long-term infected individuals. There was no difference in the CD4 count, CD8 count, or CD4/CD8 ratio between recently and long-term CRF01_AE- or B-infected individuals. These results suggest that PLWH with different HIV subtypes may exhibit different patterns of disease progression and respond differently to ART.

The trajectories of dynamic changes of CD4 counts during five-year ART

The five-year trajectories of dynamic changes in CD4 counts of the recently CRF01_AE, CRF07_BC, and B-infected individuals since ART initiation were drawn (Fig. 5), and the loss to follow-up rate was 6.67% (8/120). Interestingly, the CD4 count of PLWH with different subtypes showed different trajectories. Since ART initiation, the CD4 count in the CRF01_AE group increased sharply and reached a peak by the end of the first year, and then it seemed to decline gradually; the CD4 count in the CRF07_BC group did not change significantly in the first year and then started increasing with a lower slope in the second year and finally reached a plateau by the end of the third year; the CD4 count in the B group increased significantly to reach a peak during the first two years and then overlapped with the trajectory of CRF07_BC at the end of the third year. From the third year post-ART, the CD4 counts in the CRF07_BC and B groups were higher than those in the CRF01_AE group.

Discussion

Nowadays, PLWH in MSM cohort have gradually become the most important group for HIV prevention and control in China [2, 5, 6]. CRF01_AE is the most prevalent HIV subtype in China, followed by CRF07_BC and B [10]. In the present study, we analyzed the CD4 count and VL in ART-naïve PLWH with CRF01_AE, CRF07_BC, and B, which were downloaded from the HIV Database. We further characterized the epidemiological, subtype-specific virological, and immunological features of the recruited PLWH in recent, long-term, and chronic infection stages in Harbin, Northeast China.

In the present study, a total of 120 recently infected individuals including 79 (65.83%) with CRF01_AE, 22 (18.33%) with CRF07_BC, and 19 (15.83%) with B were found from the recruited 715 ART-naïve PLWH. It was reported that CRF01_AE was responsible for 62.1% of HIV-infected MSM in 2009–2011 in nine cities [24] and 39.0% of PLWH, including heterosexuals, MSM, and injection drug users, in 2015 in 31 provinces of China [25]. The results of this study indicate that CRF01_AE is still the dominant subtype of PLWH among MSM in Northeast China.

The baseline CD4 count is a well-recognized predictor of immune reconstitution and disease progression [26]. According to data from ART-naïve PLWH from

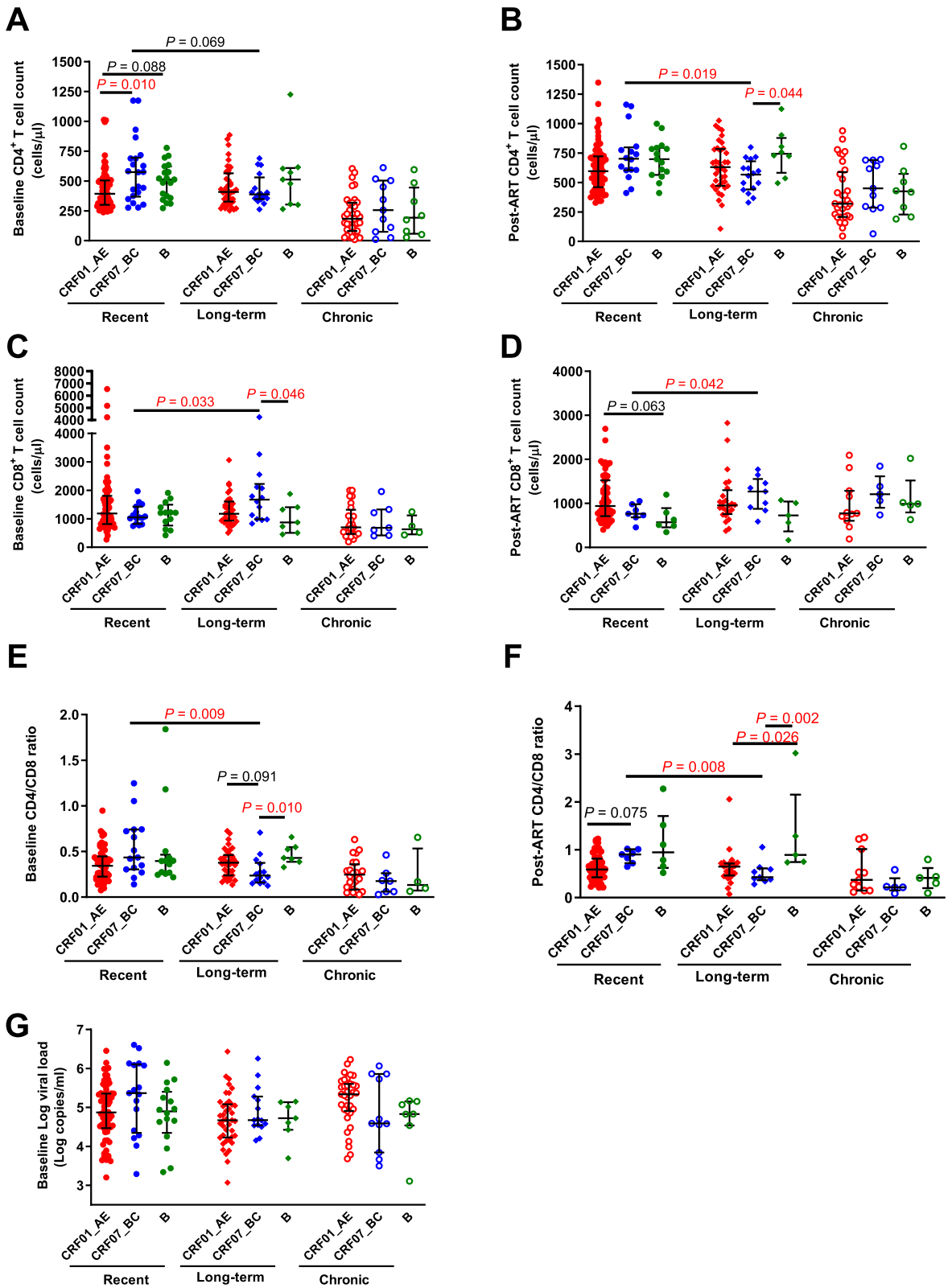

Fig. 4 Comparisons of CD4 and CD8 count, and viral load in baseline and post-ART 1 to 2 years among PLWH with three subtypes during different infection stages. The baseline and post-ART CD4 count (**A**, **B**), CD8 count (**C**, **D**), CD4/CD8 ratio (**E**, **F**), and baseline log viral load from plasma sample (**G**) were shown. The median and IQR were shown as bars

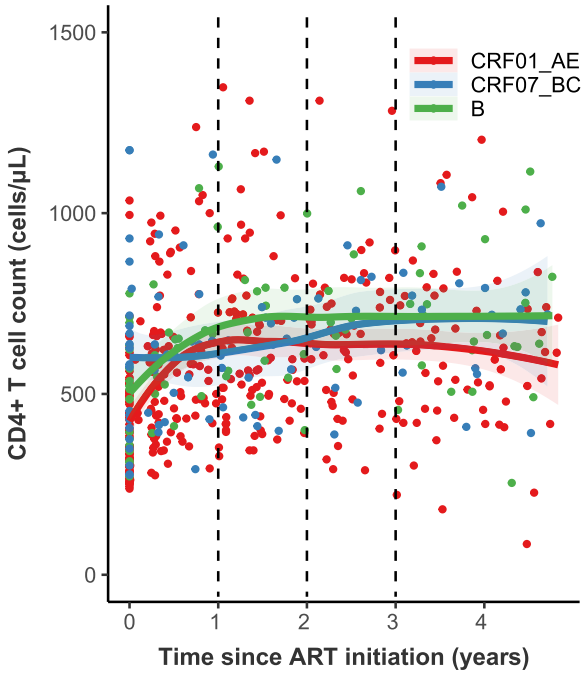

Fig. 5 The trajectories of dynamic changes of CD4 count since ART initiation. The red curve represents CRF01_AE; the blue curve represents CRF07_BC; and the green curve represents subtype B

the HIV Database, we found a lower CD4 count in the CRF01_AE group than in the CRF07_BC and B groups. However, data from the HIV Database cannot be used to distinguish the HIV infection stages. Based on *pol* genes, 120 recently infected individuals were divided into CRF01_AE, CRF07_BC, and B groups. In these recently infected individuals, although the levels of VL among the three groups were similar, the CRF01_AE group showed a lower baseline CD4 count, indicating that the virulence of HIV subtypes is different and that CRF01_AE infection could lead to rapid CD4 count reduction in the early stage of viral infection; therefore, CRF01_AE may be more virulent than CRF07_BC and subtype B. However, this difference in baseline CD4 count was not found in the corresponding long-term and chronically infected groups. The interval between HIV acquisition and ART initiation in the long-term and chronic infection groups may vary greatly, and some individuals in the long-term infection group may also be in the chronic infection stage. The difference in the duration of viral infection among different groups may complicate the baseline CD4 count at the beginning of ART and make it difficult to reflect the effect of the virus itself on the CD4 count, which further highlights the importance of recent infections in the investigation of virus pathogenicity.

The lower baseline CD4 count has been reported in the recently infected MSM with CRF01_AE in Shanghai [19, 27] and also in the PLWH with one cluster of CRF01_AE [28]; we found that the lower baseline CD4 count only in recent infection stage, instead of in long-term and chronic infection stages, suggesting that some viral factors, such as viral tropic or viral proteins may be contribute to rapid $CD4^+$ T cell loss. Some studies reported that the rapid $CD4^+$ T cell decline or poor immune recovery induced by CRF01_AE is associated with a coreceptor switch from CCR5 into CXCR4 [4, 22, 28–30]. We amplified and analyzed 31 *env* genes (22 CRF01_AE and 9 CRF07_BC or B genes) from these recently infected individuals, and no CXCR4 tropic viruses were found using two online prediction tools [31, 32] (data not shown). Therefore, we suspected that the rapid $CD4^+$ T cell loss in PLWH with CRF01_AE during the recent infection stage might have been caused by viral factors other than viral tropism switching.

It has been reported that $CD4^+$ T cell loss may be attributed to the followings: HIV kills the infected cells directly or via cell–cell fusion, apoptosis, or bystander apoptosis induced by certain viral proteins such as Env, Tat, Nef, Vpu, Vpr, and Gag, excessive immune cell activation, and pyroptosis [33–38]. We speculate that the faster $CD4^+$ T cell loss seen in CRF01_AE infection might be related to some viral gene-encoded proteins that have more activity in attacking the host immune system. A study from the Netherlands reported that the VL of a subtype B virulence mutant increased by approximately 3.5–5.5 times and the decline rate of the CD4 count was twice that of the wild-type strain [39]. One research group found significantly higher transactivation and replication of HIV CRF02_AG compared to subtype B. CRF02_AG-infected animals showed higher viremia, whereas subtype B-infected animals showed significantly more weight loss, a lower CD4 count, and a lower CD4/CD8 ratio [40]. These data support the idea that viral factors other than viremia contribute to immunosuppression and wasting syndrome in HIV/AIDS [40].

Surprisingly, during the long-term infection stage, the CRF07_BC group showed a higher baseline CD8 count, lower baseline CD4/CD8 ratio, and lower CD4 count and CD4/CD8 ratio at 1–2 years post-ART than group B. The proportion of $CD8^+/CD38^{bright}$ cells is negatively correlated with CD4 counts, independent of VL [41]. Whether lower post-ART CD4 counts and higher baseline CD8 counts are related to certain $CD8^+$ T-cell subsets should be explored further. Meanwhile, although the recently and long-term CRF07_BC infected individuals were screened from the PLWH with CD4 counts $\geq$ 250 cells/μl, the lower CD4 count and CD4/CD8 ratio and the higher CD8 count were found in the long-term CRF07_BC infected individuals, compared to the recently infected ones. This phenomenon was not observed in CRF01_AE

and B groups. This implied that ART-naïve CRF01_AE and B infections caused faster disease progression than CRF07_BC infections. Disease progression caused by HIV-1 different subtypes varies. Compared to PLWH with subtype B or CRF01_AE, PLWH with CRF07_BC had a lower probability of disease progression to immune deficiency [42].

These results prompted us to explore the responses of different subtypes of PLWH to ART. We have drawn trajectories of dynamic changes in the CD4 count of recently infected individuals over 5 years. Interestingly, the three HIV subtypes showed three distinct CD4 count trajectories. Since ART initiation, the CD4 count of PLWH with CRF01_AE rapidly increased to reach a peak in the first year, those of PLWH with CRF07_BC increased slowly to reach a plateau in the first three years, and those of subtype B increased to reach a plateau in the first two years and overlapped with the trajectories of PLWH with CRF07_BC by the end of the third year. Since the third year, the CD4 counts of CRF07_BC- and B-infected individuals were higher than those of CRF01_AE-infected individuals. These findings revealed that initiating ART in the early stage of HIV infection could rapidly restore CD4 count, especially subtypes CRF01_AE and B. These results indicate that since ART initiation, the first year for CRF01_AE, the first two years for B, and the first three years for CRF07_BC are critical for the immunological recovery of PLWH, after which the CD4 count may remain relatively stable.

The limitations of this study are the relatively low number of enrolled PLWH at different infection stages and the fact that the data, whether from the MSM cohort or the HIV database, were mainly from male PLWH; whether findings of this study can be applied to female PLWH is unclear. Due to the limited information on PLWH in the public HIV database, including the days from infection, gender, age, and coinfection status, these parameters were not analyzed. Therefore, the influence of these factors on the baseline CD4 count and VL remains unclear.

In summary, compared with CRF07_BC and subtype B, HIV CRF01_AE rapidly reduced the CD4 count in the recent infection stage, which was probably related to the virulence of CRF01_AE. After ART initiation, the CD4 count of the recently CRF01_AE-infected individuals rapidly increased from a lower baseline level to a peak in the first year and was still lower than that of CRF07_BC- and subtype B-infected individuals after three years of ART. This study revealed an important time point for estimating CD4 count recovery post-ART in individuals with different HIV subtypes.

Abbreviations

AIDS	Acquired immune deficiency syndrome
ART	Anti-retroviral therapy
CRF	Circulating recombinant form
EIA	Enzyme immunoassay
HIV	Human immunodeficiency virus
MSM	Men who have sex with men
PLWH	People living with HIV
URF	Unique recombinant form
VL	Viral load

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10799-5.

Supplementary Material 1: Fig. S1 Distribution of HIV subtypes.

Supplementary Material 2: Fig. S2 Analysis of CD4 and CD8 count, and viral load in baseline and post-ART 1 to 2 years in all enrolled PLWH. The baseline and post-ART CD4 count (A, D), CD8 count (B, E), CD4/CD8 ratio (C, F), baseline log viral load from plasma samples (G), and correlations of CD4 count (H) and CD4/CD8 ratio (I) between baseline and post ART were shown. The median and IQR were shown as box.

Supplementary Material 3: Point by point response.

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Authors' contributions

M.Z. and H.L. conceived and designed this study. X–Y.Z., Y.J., S-M H, H-R.Z., X–H.W., and Y-L.Z. performed the experiments and were responsible for data acquisition and analysis. W.L. and J-Y.W. were responsible for statistical analysis. L.W., W-J.W., T.F., X–H.C., D.W., and J-L.L. were responsible for patient recruitment and clinical management. X–Y.Z., Y.J., J-Y. W., and M.Z. wrote the article.

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Data availability

The pol gene sequences amplified and detected in the present study were uploaded into GenBank, and the serial accession numbers were PP001836 to PP002009, and PP048759 to PP048826. The data presented in this study are available on request from the corresponding authors.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board including Medical Ethics Committee of Fourth Affiliated Hospital of Harbin Medical University (2017-SCI-07), Medical Ethics Committee of Heilongjiang Provincial Hospital ([2019]092) and Medical Ethics Committee of Harbin Sixth Hospital ([2021]01). A written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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