Original Article

Baseline Naive CD4+ T-cell Level Predicting Immune Reconstitution in Treated HIV-infected Late Presenters

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Abstract

Background: Among HIV-infected patients initiating antiretroviral therapy (ART), early changes in CD4+ T-cell subsets are well described. However, HIV-infected late presenters initiating treatment present with a suboptimal CD4+ T-cell reconstitution and remain at a higher risk for AIDS and non-AIDS events. Therefore, factors associated with CD4+ T-cell reconstitution need to be determined in this population, which will allow designing effective immunotherapeutic strategies.

Methods: Thirty-one adult patients with baseline CD4+ T-cell count <350 cells/mm^3 exhibiting viral suppression after ART initiation were followed in the HIV/AIDS research center of Peking Union Medical College Hospital in Beijing, China, from October 2002 to September 2013. Changes in T-cell subsets and associated determinants were measured.

Results: Median baseline CD4+ T-cell count was 70 cells/mm^3. We found a biphasic reconstitution of T-cell subsets and immune activation: a rapid change during the first 6 months followed by a more gradual change over the subsequent 8 years. Baseline CD4+ T-cell count >200 cells/mm^3 in comparison to CD4+ T-cell count ≤200 cells/mm^3 was associated with more complete immune Reconstitution (77.8% vs. 27.3% respectively; $P = 0.017$) and normalized CD4/CD8 ratio. We showed that the baseline percentage of naive CD4+ T-cell was a predictive marker for complete immune reconstitution (area under receiver operating characteristic curve 0.907), and 12.4% as cutoff value had a sensitivity of 84.6% and a specificity of 88.2%.

Conclusions: Baseline naive CD4+ T-cell percentage may serve as a predictive marker for optimal immune reconstitution during long-term therapy. Such study findings suggest that increasing thymic output should represent an avenue to improve patients who are diagnosed late in the course of infection.

Key words: Antiretroviral Therapy; HIV; Immune Activation; Naive CD4+ T-cell; Thymic Function

Introduction

Antiretroviral therapy (ART) has transformed the lives of millions of persons infected with HIV by gradually increasing their CD4+ T-cell counts over a decade. However, CD4+ T-cell reconstitution with ART is characterized by biphasic pattern of T-cell subsets characterized by an initial phase of expansion and redistribution of CD4+ memory T-cells, followed by the second phase where reconstitution of CD4+ naive T-cells occurs with a reduction of T-cell immune activation.[1-5] Unfortunately, approximately 20% of patients may experience immune nonresponse even after suppressive ART, and this is more commonly seen in late presenters (patients with baseline CD4+ T-cell count <350 cells/mm^3 at the time of ART initiation).[6] Such population remains at a higher risk for the development of AIDS and non-AIDS events few years after having initiated ART. Therefore, information on the dynamics of CD4+ T-cell reconstitution in the late presenters remains an important issue to address. Elevation of immune activation, increase of

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memory T-cells with late differentiation, has been reported.[7] However, limited data exist on long-term outcome of patients initiating ART late in the course of infection. Some groups have defined late presenter generally as patients initiating ART below 350 cell/mm^3 CD4+ T-cells.[8,9] This may result in limited disease outcomes in ART-treated patients.[10-13]

Data on late presenters and CD4+ T-cell reconstitution are limited owing to short-term study duration and limitation of the analyses to total CD4 T-cells but not their subsets.[1,3,5,14] It remains elusive whether baseline characteristics and T-cell subset profiles could predict long-term treatment responses. To this end, we analyzed data from our outpatient cohort with late presenter participants treated with ART for over 8 years. We determined the long-term T-cell subset trajectory after ART initiation and attempted to identify prognostic factors of complete immune reconstitution.

Methods

Patients

A prospective study from October 2002 to September 2013 was conducted at the clinics of the Department of Infectious Disease in Peking Union Medical College Hospital (PUMCH), a large tertiary care hospital in Beijing, China. HIV diagnosis was determined by standard serum enzyme-linked immunosorbent assays and confirmed by Western blot analyses. Patients were considered for enrollment in this study if they were treated with ART for at least 8 years with regular follow-ups in the absence of treatment interruptions.

Thirty-one patients participated in this study. Prospective blood samples were collected during the patients' routine visits, and the patient characteristics were retrieved from the medical records. All patients gave written informed consent, and the study was approved by the Institutional review board of PUMCH. Patients received ART continuously since the inclusion; however, the drug combinations changed over the years due to new drug availability and tolerance. Control groups were established for comparison and characterized by 51 healthy volunteers recruited from the Chinese Blood Donor Corps.[15]

Patients were routinely followed up every 3 months for the first 6 months, and then every 6 months for at least 8 years. At each visit, a clinical assessment was recorded, and samples were collected for laboratory assessment, including T-cell subsets and plasma HIV RNA quantification.

Patients were classified into two groups: one group including patients with baseline CD4+ T-cell count >200 cells/mm^3, and the other group with CD4+ T-cell count ≤ 200 cells/mm^3.

T-cell subsets and viral load

For immunofluorescent surface staining and flow cytometric analysis, three-color flow cytometry (Beckman-Coulter, Brea, CA, USA) was performed using peripheral blood mononuclear cells. Immune subsets were defined using the following markers: naive CD4+ T-cells defined by CD4+, CD45RA+, and CD62L+; memory CD4+ T-cells defined by CD4+, CD45RO+, and CD45RA-; functional CD4+ T-cells defined by CD28+ and CD4+; activated CD8+ cells defined by CD8+, CD38+, or HLA-DR+. All monoclonal antibodies were purchased from Beckman-Coulter and Immunotech (Brea, CA, USA).

For viral load (VL) measurements, plasma was separated from whole blood by centrifugation within 4 h of collection and stored at −80°C until tested. The Cobas AmpliPrep/Cobas TaqMan real-time RT-PCR Assay (Roche, CA, USA) was performed according to the manufacturer's instructions.

Statistical analyses

Quantitative data were compared among two groups using the Mann–Whitney *U*-test and were described as medians with interquartile ranges (IQRs) unless otherwise stated. Qualitative data were compared among groups using Chi-square tests. Multivariate models by generalized estimating equations were utilized to analyze the following covariates: age at ART initiation, gender, transmission route, development of clinical AIDS prior to ART, time from diagnosis of HIV infection to treatment initiation, baseline VL, nadir pre-ART CD4+ T-cell counts, duration of ART, and delayed viral suppression. Clinical AIDS was defined as the presence of a clinical disease (not CD4+ T-cell count) meeting the 1993 Centers for Disease Control AIDS case definition.[16] Since there is no consensus definition of normalized CD4/CD8 ratio, we used the criteria from our institution, which are at least two CD4/CD8 ratios over 0.95. We used a Poisson regression model with a robust error variance to calculate relative risks of reaching complete immune reconstitution (CD4+ T-cells ≥500 cells/mm^3).[17,18] In the multivariate Poisson regression model, we entered all factors except for age with a $P < 0.20$ in univariate analysis, and entered age as a continuous factor, since age might affect naive CD4+ T-cell percentage. We used Cox regression analysis to model the time from ART initiation to the development of complete immune reconstitution, which was defined as the midpoint between the last CD4+ T-cell <500 cells/mm^3 and the first CD4+ T-cells ≥500 cells/mm^3. We also used receiver operating characteristic (ROC) curves to determine the diagnostic potency of different indices at baseline. Sensitivity and specificity were calculated to evaluate diagnostic performance for the complete immune reconstitution (CD4+ T-cells ≥500 cells/mm^3) at 8-year ART.[19] Statistical analyses were performed using SPSS version 20.0 (SPSS Inc., USA) and Stata version 11.0 (Stata Corp., USA) considering $P < 0.05$ statistically significant.

Results

Baseline characteristics

The characteristics of the patients are summarized in Table 1. These patients had been diagnosed with HIV for a median of 0.1 year (IQR: 0–0.8 years) before ART initiation and had a median duration of ART of 10.2 years (IQR: 9.5–10.6 years).

Table 1: Characteristics of the participants

Characteristics	Results
Age, median years (IQR)	33.8 (30.1–40.7)
Male, *n* (%)	15 (48.4)
Route of transmission, *n* (%)	
Sexual	12 (38.7)
Blood	15 (48.4)
Other	4 (12.9)
Centers for Disease Control clinical stage, *n* (%)	
A	8 (25.8)
B	4 (12.9)
C	19 (61.3)
Time from diagnosis to treatment, median years (IQR)	0.1 (0–0.8)
AIDS-defining disease, *n* (%)	14 (45.2)
HBsAg+, *n* (%)	3 (9.7)
Anti-HCV+, *n* (%)	5 (16.1)
CD4+ T-cell count, median (IQR), cells/mm^3	70 (12–223)
≤200, *n* (%)	22 (71.0)
<350, *n* (%)	9 (29.0)
CD4/CD8 ratio (IQR)	0.11 (0.02–0.26)

IQR: Interquartile range; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus.

The majority of patients were infected via blood transfusion. Fourteen patients (45.2%) had experienced AIDS-defining events, and eight patients (25.8%) had a history of infection with hepatitis B or hepatitis C virus. All patients were selected as late presenters based on CD4+ T-cell counts <350 cells/mm^3. Baseline median CD4+ T-cell count was 70 (IQR: 12–223) cells/mm^3 and median VL was 4.7 (IQR: 4.3–5.3) lg copies/ml. Of 31 patients, 30 had baseline memory and naive cell profiles available. In 22 patients with CD4+ T-cell count ≤200 cells/mm^3, naive CD4+ T-cell percentage was also lower (6.6%, IQR 4.1–12.3%) than that in 9 patients with CD4+ T-cells over 200 cells/mm^3 (27.5%, IQR 26.0–41.4%, $P < 0.001$).

Virologic suppression

During 8 years of treatment, 24 patients achieved virologic suppression within half a year of treatment initiation, whereas seven patients had virologic failure or rebound, and were switched to second-line regimens (tenofovir + lamivudine + ritonavir-boosted lopinavir). The 24 patients who demonstrated stable viral suppression were receiving zidovudine, stavudine, didanosine, and lamivudine-based regimens, which were first-line ART at the time.

Incomplete recovery of CD4+ T-cell subsets

In comparison with the reference ranges, our HIV-infected patients had lower CD4+ T-cell and naive CD4+ T-cell counts and proportions, as well as higher proportions and lower levels of memory CD4+ T-cells during 8-year ART [Figures 1 and 2, Supplementary Figure S1a and S1b]. At year 8, there were 13 patients (41.9%) with CD4+ T-cells over 500 cells/mm^3. The group with baseline CD4+ T-cell counts over 200 cells/mm^3 had a higher rate of complete immune reconstitution than that with baseline CD4+ T-cell counts ≤200 cells/mm^3 (77.8% vs. 27.3%, $P = 0.017$). Most patients with baseline CD4+ T-cell counts ≤200 cells/mm^3 did not exhibit complete immune reconstitution after 8 years of treatment [Supplementary Figure S2].

In Cox regression analysis, no clinical AIDS events, baseline CD4+ T-cell >200 cells/mm^3, and high naive CD4+ T-cell percentage were associated with complete immune reconstitution.

There was a biphasic reconstitution of CD4+ T-cells: a rapid increase during the first 6 months followed by a more gradual increase over the subsequent 8 years [Figure 1a and 1b]. Memory and naive cell recovery followed similar patterns [Figure 1c and 1d and Supplementary Figure S1a and S1b]. In multivariate analysis, clinical AIDS events and lower baseline VL were associated with a less robust CD4+ T-cell recovery [Supplementary Table S1].

Naive CD4+ T-cell percentage after 8 years of ART differed significantly between patients with complete immune reconstitution (median percentage 31.1%, IQR 21.0–41.3%) and those without complete immune reconstitution (20.1%, IQR 8.9–29.0%, $P = 0.028$). Older age, baseline CD4+ T-cell ≤200 cells/mm^3, and lower baseline VL were associated with poorer recovery of naive CD4+ T-cells [Supplementary Table S1].

In addition, most patients did not exhibit normalized CD4/CD8 ratios after 8 years of treatment [Supplementary Figure S3a-S3c]. Baseline CD4+ T-cell count over 200 cells/mm^3 was associated with better CD4/CD8 recovery since in this group, 66.7% (6/9) of patients achieved CD4/CD8 ratio exceeding 0.95 between year 7 and year 8, in comparison with 9.1% (2/22) in patients with baseline CD4+ T-cell count ≤200 cells/mm^3 ($P = 0.003$, by Fisher's exact test). We also correlated baseline naive CD4+ T-cell percentage with normalization of CD4/CD8 ratio by adding it to univariate Cox proportional model and discovered that the hazard ratio was 1.09 per 1% increase in baseline naive CD4+ T-cell percentage (95% confidence interval [*CI*] 1.04–1.15, $P = 0.001$).

Higher immune activation levels at baseline and normalization of activated CD8+ T-cells

The HIV-infected patients had higher levels of CD8+CD38+ and HLA-DR+ percentage than the normal ranges at baseline [Figure 1e and 1f]. We also observed a biphasic recovery for immune activation: a rapid decrease during the first 6 months was followed by a plateau throughout 8 years [Figure 1e and 1f]. Of note, the percentage of CD8+CD38+ cells from day 0 to year 2 was significantly higher than the controls, similar to the controls from year 2 to year 4, and significantly lower than the controls from year 4 to year 8 [Figure 1e]. During 8-year ART, the greatest decreases of the percentage of CD8+CD38+ cells were noted within the first 6 months after ART initiation. In multivariate regression analysis, male sex, viral hepatitis, and higher VL were

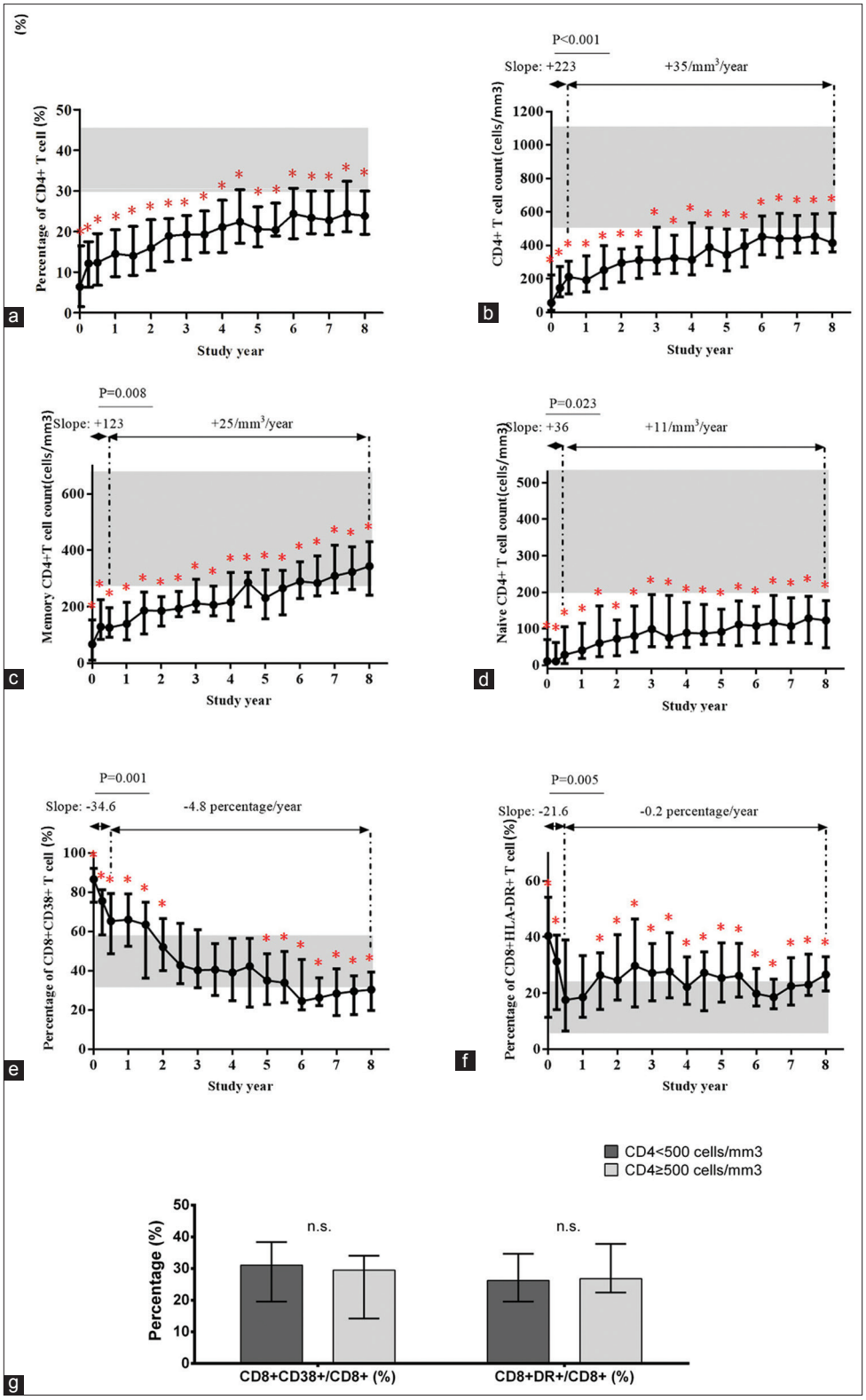

Figure 1: Medians and interquartile ranges (error bars) of CD4+ T-cell percentage and count (a and b), memory CD4+ T-cell count (c) and naive CD4+ T-cell count (d) during eight-year ART. (e and f) demonstrate medians and interquartile ranges of CD8+CD38+ T-cell percentage and CD8+HLA-DR+ T-cell percentage during 8-year ART, respectively. (g) Immune activation after 8-year ART, grouped by CD4+ T-cell count at year 8. The shaded bands in (a-f) reflect the reference ranges in HIV-negative population. The slopes in (a-f) were calculated by a linear model regression. The Wilcoxon nonparametric test was used to compare slopes in different phases. *$P < 0.05$, and NS denotes "not significant."

associated with a more robust response of the percentage of CD8+CD38+ cells during 8-year ART [Supplementary Table S2]. We also summarized CD8+CD38+/CD8+ percentage during 8 years of follow-up in each patient [Figure 2].

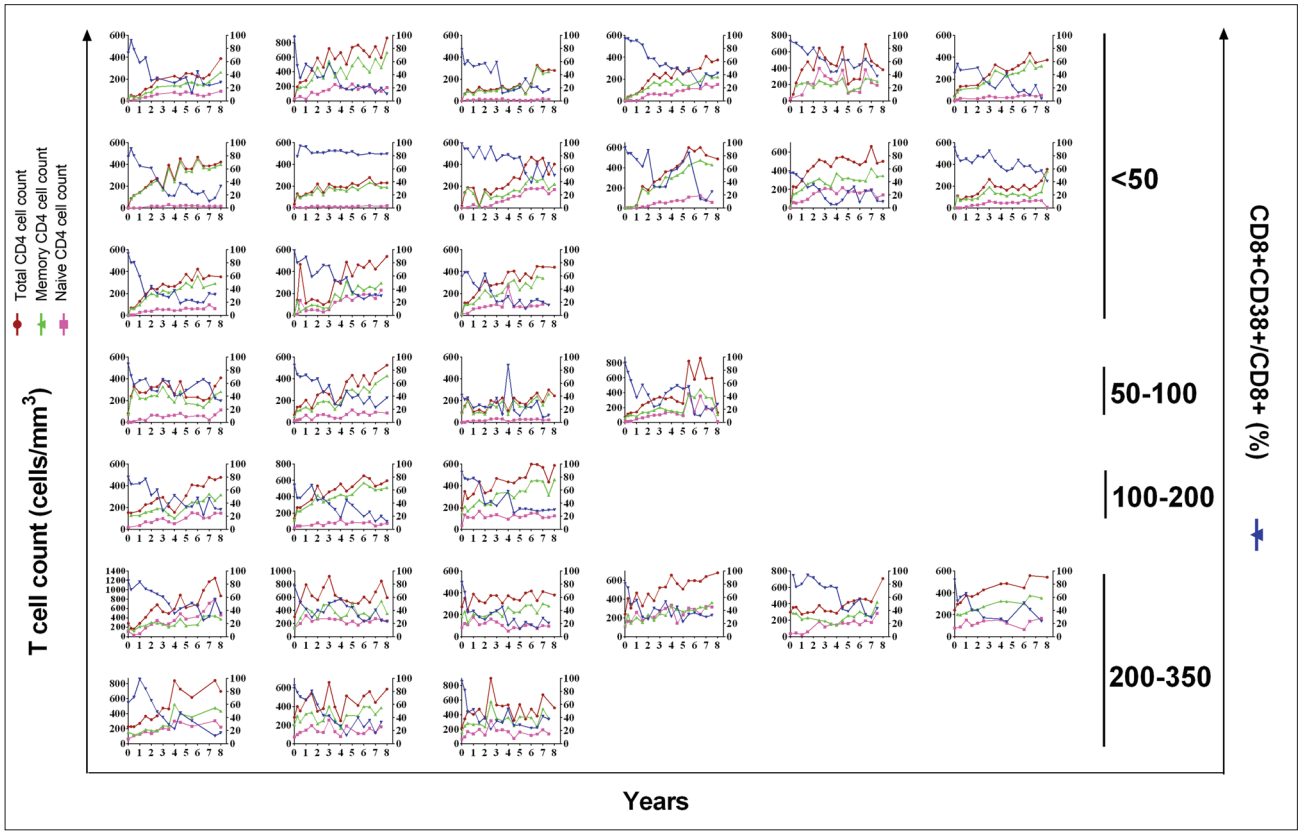

Figure 2: Total, memory, and naive CD4+ T-cell as well as CD8+CD38+/CD8+ percentage during 8-year follow-up in each patient. Patients were categorized by their baseline total CD4+ T-cell count (<50, 50–100, 100–200, and 200–350 cells/mm^3).

Correlation of immune reconstitution and immune activation after 8 years of antiretroviral therapy

We next needed to determine whether immune activation might be associated with immune reconstitution after long-term therapy. After 8 years of ART, immune activation levels were comparable between patients who achieved complete immune reconstitution and patients who did not [Figure 1g].

The predictive factors for complete immune reconstitution after 8 years of antiretroviral therapy

To determine factors associated with complete immune reconstitution in individuals demonstrating CD4+ T-cell count ≥500 cells/mm^3 after 8-year treatment, we first determined relative risks for each factor. The percentage of naive CD4+ T-cell at baseline was strongly associated with complete immune reconstitution, with a relative risk (*RR*) of 1.08 (per 1% increase in naive CD4+ T-cell percentage, 95% *CI* 1.02–1.44; $P = 0.006$) independent of baseline CD4+ count [Table 2]. We then used ROC curves to estimate the prognostic values of baseline naive CD4+ T-cell percentage. The AUC of the ROC curve was 0.907 (95% *CI:* 0.792–1.000, $P < 0.001$) for baseline naive CD4+ T-cell percentage [Figure 3]. We also compared ROC curves of potential prognostic factors such as baseline total CD4+ T-cell count or naive CD4+ T-cell count, and baseline naive CD4+ T-cell percentage, and discovered that baseline naive CD4+ T-cell percentage predicts immune reconstitution better than baseline total CD4+ T-cell count or naive CD4+ T-cell count [Figure 3].

Based on ROC curve analysis, the cutoff value for the diagnosis of complete immune reconstitution at 8-year ART was 12.4% for baseline naive CD4+ T-cells, with a sensitivity of 84.6% (95% *CI*: 53.7–97.3%), a specificity of 88.2% (95% *CI:* 62.2–97.9%), and an accuracy of 86.7%. The positive predictive value was 84.6% (95% *CI:* 53.7–97.3%) and negative predictive value was 88.2% (95% *CI*: 62.2–97.9%). We then substituted baseline naive CD4+ T-cell percentage (continuous values) with categorical values (<12.4% vs. over 12.4%) into the regression model and discovered that baseline naive CD4+ percentage over 12.4% had *RR*: 10.2 (95% *CI*: 2.8–36.5, $P < 0.001$) in multivariate analysis.

Discussion

This study described T-cell dynamics during 8-year treatment and identified prognostic factors for immune reconstitution in late presenters. We showed that in late presenters, CD4+ T-cells did not reach the normal range after long-term treatment. In fact, the percentage of CD8+CD38+, as a marker of immune activation, continued to decrease and reached a level even lower than the normal range after 8 years of ART. More importantly, it was shown at baseline that the percentage of naive CD4+ T-cell, rather than CD4+ T-cell count, could be a better prognostic factor for immune reconstitution in long-term ART.

Table 2: Relative risks of demographic and clinical characteristics associated with CD4+ T cell >500 cells/μl (*n* = 30)

Covariate	Univariate model		Multivariate model	
	RR (95% *CI*)	*P*	*RR* (95% *CI*)	*P*
Age (years)	0.99 (0.95–1.03)	0.753	1.00 (0.94–1.07)	0.914
Sex				
Male	0.47 (0.18–1.24)	0.127	0.38 (0.15–0.98)	0.046
Female	Reference		Reference	
Transmission routes				
Sex	Reference			
Blood transfusion	0.67 (0.26–1.69)	0.391		
Others/unknown	1.00 (0.32–3.16)	>0.999		
Hepatitis	1.03 (0.38–2.78)	0.956		
Clinical AIDS events prior to cART initiation	0.42 (0.14–1.24)	0.115	0.91 (0.26–3.17)	0.881
Time from diagnosis of HIV infection to treatment (years)	1.14 (1.05–1.25)	0.003	1.00 (0.88–1.13)	0.950
Baseline CD4+ T-cells (cells/mm^3)				
0–200	Reference		Reference	
>200	2.85 (1.31–6.22)	0.008	0.44 (0.08–2.47)	0.348
Baseline naïve CD4+ T-cell percentage	1.05 (1.03–1.07)	<0.001	1.08 (1.02–1.14)	0.006
Baseline VL (lg copies/ml)	1.26 (0.73–2.17)	0.415		
Delayed virologic suppression				
No	Reference			
Yes	0.62 (0.18-2.22)	0.466		

In the multivariate model, we only included factors with $P<0.20$ in univariate model. VL: Viral load; cART: Combination antiretroviral therapy; *RR*: Relative risk; *CI*: Confidence interval.

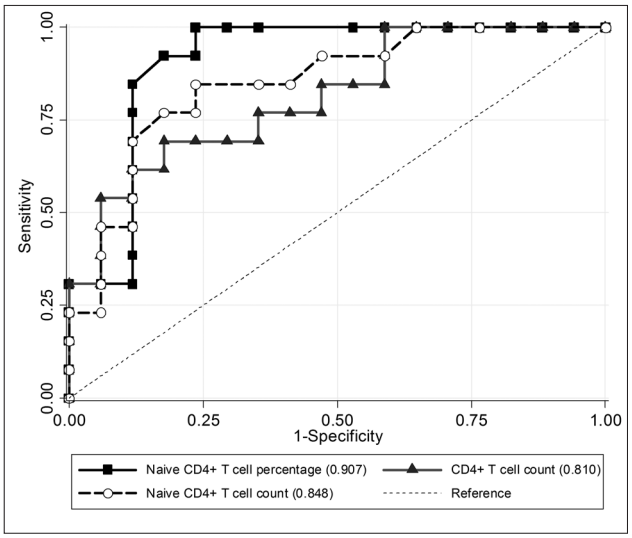

Figure 3: Receiver operating characteristic curves of different predictors for immune reconstitution. Areas under the curves were included in the parentheses.

Although some successfully treated HIV-infected patients have normalized their CD4+ T-cell count, CD4+ T-cells and naive CD4+ T-cells remained below reference ranges throughout 8 years, which is consistent with previous studies.[3,12,20-23] As shown by Robbins *et al*., in patients who initiated ART with CD4+ T-cell counts <350 cells/mm^3, the CD4+ T-cell count is less likely to return to reference range after 3-year ART.[3] In our study, all patients initiated ART with CD4+ T-cell counts <350 cells/mm^3, and CD4+ T-cell counts could not reach reference range even after 8-year ART in most patients. These results also justify the need to initiate ART when CD4+ T-cell count is higher than 350 cells/mm^3, in a hope to achieve better immunologic responses. Furthermore, CD4/CD8 ratio was not normalized in most patients with baseline CD4+ T-cell count <200 cells/mm^3 after 8 years of treatment. This index is particularly associated with mortality, immunosenescent phenotype, and non-AIDS morbidity and mortality.[24-26]

Of note, younger age was associated with higher naive CD4+ T-cell count during long-term ART [1 year younger is associated with three more naive CD4+ T-cells/mm^3, Supplementary Table S1], which could be due to better thymic output in young patients.[10,27,28]

In terms of immune activation, expression of the T-cell activation marker (CD38+) on CD8+ T-cells returned to normal levels a few years after initiation of ART and even reached a level lower than normal range after 4 years of treatment. Some long-term studies demonstrated normalization in activated CD8+ T-cells in HIV-infected patients after long-term ART[12,23] while other short-term studies showed persistent residual activation in CD8+ compartments.[3,7,11] All of these studies are consistent with our findings that normalization of immune activation might be achieved after at least 4 years of ART. Surprisingly, after long-term treatment, immune activation was not associated with CD4+ recovery [Figure 1g], suggesting that immune activation after long-term ART might not be the culprit behind limited immune reconstitution. On the other hand, CD38 is one of the many immune activation markers and may not recapitulate the whole picture of immune activation during long-term therapy. Monocyte activation, as measured by the expression of CD16 or by soluble CD14, interleukin 6, kynurenine to tryptophan ratio, etc., may remain

elevated even after treatment;[29] therefore, measurement of T-cell activation may not fully depict the immune activation picture in long-term ART.

On the other hand, could low naive CD4+ T-cell levels be responsible for the limited immune reconstitution during long-term ART need to be determined. We found baseline naive CD4+ T-cell count as a robust diagnostic approach for complete immune reconstitution at 8-year ART. Based on our study, baseline naive percentage over 12.4 may be a good predictive index for complete immune reconstitution in long-term treatment. To our surprise, baseline total CD4+ T-cell count seemed to be a less reliable predictor for complete immune reconstitution in comparison with baseline naive cell percentage, as is demonstrated in Table 2 and Figure 3. These findings suggest that depletion of naive CD4+ T-cells before ART initiation could lead to failure in immune recovery. A previous study by Schacker *et al*. demonstrated that higher pretreatment naive CD4+ T-cell percentage was associated with better immune reconstitution during 2-year ART in patients with baseline CD4+ T-cell count between 200 and 500 cells/mm^3.[30] This study supports our findings; however, it did not show this correlation in long-term treatment. In comparison, our study extended the duration of observation to 8 years and gave a cutoff value for prediction, making baseline naive CD4+ percentage not only a predictor for short-term immune reconstitution[19] but also a long-term predictor. Of note, since our patients are late presenters, whether this prognostic factor could be applied to patients with baseline CD4+ count over 350 cells/mm^3 remains to be determined.

We would like to synthesize our findings and previous reports to depict the relationship between immune reconstitution and immune activation. Before ART initiation, HIV infection and immune activation may lead to CD4+ T-cell depletion, lymphoid tissue fibrosis, and decreased thymic output,[10,19,31-33] and all of these factors may lead to a decrease in naive CD4+ T-cell reservoir. Importantly, lymphoid tissue fibrosis might also cause irreversible disruption of lymphoid structure, leading to a permanent decrease in naive cell reservoir.[34,35] At the early stage of ART, HIV replication can be suppressed, making redistribution of memory CD4+ T-cells and dampening of immune activation possible.[19] After this early stage, naive CD4+ T-cell expansion becomes the driving force behind CD4+ T-cell recovery. Should the naive reservoir have been jeopardized due to destruction of the lymphoid microenvironment during the pretreatment stage, patients may suffer from incomplete immune reconstitution. This incomplete immune reconstitution may occur despite the fact that immune activation has been quenched after ART use. This led us to determine that baseline naive CD4+ T-cells rather than immune activation levels could predict immune reconstitution in long-term ART. In line with previous studies,[32,35] we also suggest that early initiation of ART might be warranted to rescue lymphoid tissue from fibrosis and to conserve the naive cell reservoir. We also suggest that early initiation of anti-inflammatory agents might preserve naive CD4+ T-cell reservoir. On the other hand, thymopoiesis is also associated with effective CD4+ T-cell recovery by recovering naive CD4+ T-cells,[36] and previous studies with recombinant human interleukin 7 (rhIL-7) demonstrate that rhIL-7 boosts thymic output and promotes naive and central memory CD4+ T-cells in a dose-dependent manner.[37]

We followed patients for 8 years, and such long period of time enabled us to observe continuous increase in CD4+ T-cell and decrease in CD8+ cell activation. In addition, it is becoming rare to have late presenters in the developed countries, whereas such presentation is common in less-developed areas; therefore, predicting the immune reconstitution for late presenters is of great importance for the initiation of ART. Our study has some limitations as follows: (1) our sample size is still limited and further long-term study with a larger sample size is warranted to evaluate T-cell dynamics during long-term treatment. (2) T-cell comparison and reconstitution in lymphoid tissue may not be reflected in circulating cells.[38] (3) We did not measure the stem-cell like central memory CD4+ T-cell, which may also have predictive values and may also reflect the size of reservoir.[39] (4) In terms of immune activation markers, we did not obtain the proportions of CD38 and HLA-DR coexpression during our follow-up (CD4+CD38+HLA-DR+ and CD8CD38+HLA-DR+ cells).

In conclusion, our results demonstrated the persistence of T-cell imbalance throughout 8 years of ART. Baseline percentage of naive CD4+ T-cells could predict complete immune reconstitution at 8-year ART, and an optimal cutoff value was estimated at 12.4%. Promoting thymus function by immunotherapeutic agents such as rhIL-7 may be promising in the recovery of CD4+ T-cell count in late presenters.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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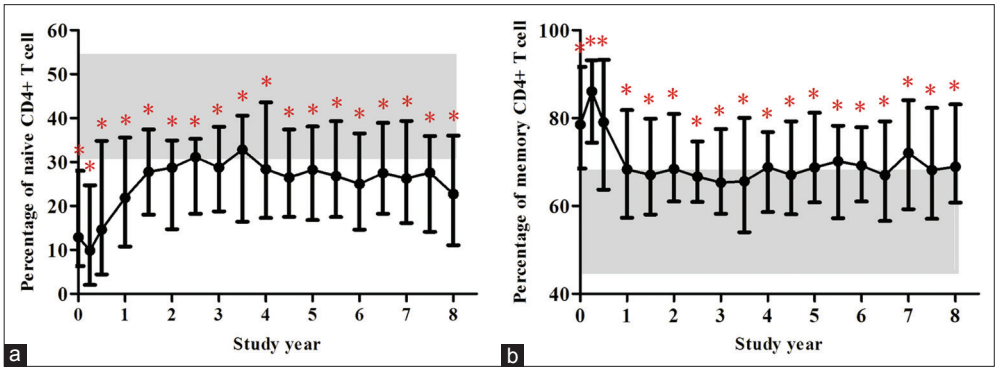
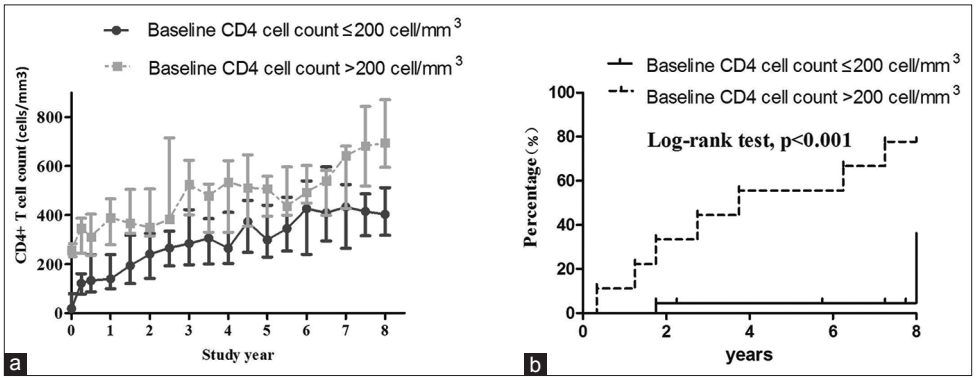
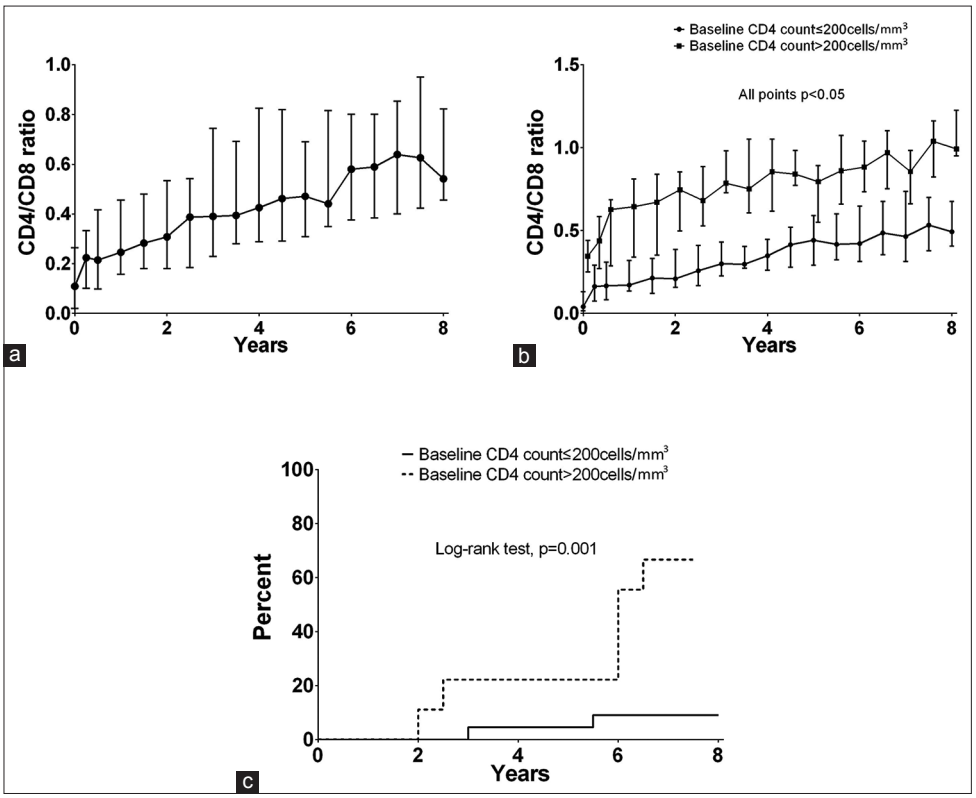


Supplementary Figure S1: Medians and interquartile ranges of memory CD4+ T cell percentage (a) and naive CD4+ T cell percentage (b) during 8 year ART. The shaded band reflects the reference ranges in HIV negative population, $*P < 0.05$.



Supplementary Figure S2: Medians and interquartile ranges (error bars) of CD4+ T cell in subgroups. (b) Kaplan–Meier graph of patients achieving complete immune reconstitution.



Supplementary Figure S3: Normalization of CD4/CD8 ratio. Medians and interquartile ranges (error bars) of CD4/CD8 ratio in the whole group (a) and in subgroups (b). Kaplan–Meier graph of patients achieving normalized CD4/CD8 ratio (c).

Supplementary Table S1: Multivariate analysis of demographic and clinical characteristics associated with CD4+ T-cell subsets response

Covariate	CD4+ T-cell		Naïve CD4+ T-cell		Memory CD4+ T-cell		CD4/CD8 ratio	
	Estimate (95% *CI*)	*P*	Estimate (95% *CI*)	*P*	Estimate (95% *CI*)	*P*	Estimate (95% *CI*)	*P*
Age (per 1 year)	−4.1 (−8.2–0.01)	0.050	−3.0 (−5.2–−0.7)	0.010	−1.4 (−4.1–1.3)	0.319	0.01 (0.002–0.02)	0.013
Sex								
Male	−63.6 (−134.2–7.0)	0.077	−1.5 (−36.5–33.5)	0.933	−61.3 (−107.7–−15.0)	0.010	−0.07 (−0.18–−0.04)	0.203
Female	Reference		Reference		Reference		Reference	
Transmission routes								
Sex	32.6 (−113.5–178.7)	0.662	−14.5 (−87.1–58.2)	0.696	35.9 (−42–113.9)	0.366	0.30 (0.10−0.49)	0.003
Blood transfusion	12.3 (−149.3–174.0)	0.881	−32.2 (−110.9–46.5)	0.423	43.7 (−39.5–126.8)	0.304	0.32 (0.13–0.52)	0.001
Others/unknown	Reference		Reference		Reference		Reference	
Viral hepatitis								
No	22.1 (−79.3–123.6)	0.669	−18.7 (−66.0–28.6)	0.439	47.7 (−20.5–115.9)	0.170	−0.10 (−0.27–0.08)	0.275
Yes	Reference		Reference		Reference		Reference	
AIDS events prior to cART								
No	74.0 (9.7–138.3)	0.024	21.2 (−8.3–50.8)	0.159	53.1 (10.8–95.4)	0.014	0.24 (0.08–0.39)	0.003
Yes	Reference		Reference		Reference		Reference	
Time from diagnosis of HIV infection to treatment (per 1 year)	26.1 (−4.4–56.5)	0.093	−0.8 (−14.6–13.1)	0.916	26.7 (6.9–46.5)	0.008	0.03 (−0.01–0.07)	0.188
Baseline CD4+ T cells (cells/mm^3)								
≤200 cells/mm^3	−75.9 (−166.7–14.8)	0.101	−107.9 (−158.2–−57.6)	<0.001	34.4 (−30.6–99.4)	0.299	−0.26 (−0.45–−0.07)	0.008
>200 but <350 cells/mm^3	Reference		Reference		Reference		Reference	
Baseline VL (per one lg copies/ml)	45.3 (0.5–90.0)	0.047	28.6 (3.0–54.3)	0.029	17.3 (−14.5–49.0)	0.286	0.04 (−0.03–0.11)	0.236
Duration of cART (years)	41.4 (33.2–49.6)	<0.001	12.9 (7.4–18.4)	<0.001	25.5 (20.4–30.6)	<0.001	0.06 (0.05–0.08)	<0.001
Delayed virologic suppression								
No	52.9 (−7.04–112.9)	0.084	−10.1 (−45.7–25.5)	0.577	68.7 (29.9–107.6)	0.001	−0.2 (−0.37–−0.03)	0.022
Yes	Reference		Reference		Reference		Reference	

VL: Viral load; cART: Combination antiretroviral therapy; *CI*: Confidence interval.

Supplementary Table S2: Multivariate analysis of demographic and clinical characteristics associated with CD8+ T-cell subsets response

Covariate	CD8+ T-cell		CD8+ CD38+ T-cell percentage		CD8+ HLA-DR+ T-cell percentage	
	Estimate (95% *CI*)	*P*	Estimate (95% *CI*)	*P*	Estimate (95% *CI*)	*P*
Age (years)	−37.2 (−57.4–−16.9)	<0.001	0.5 (−0.25–1.20)	0.198	0.3 (−0.2–0.8)	0.263
Sex						
Male	154.7 (−96.9–406.3)	0.228	10.5 (3.3–17.7)	0.004	−1.2 (−8.5–6.1)	0.746
Female	Reference		Reference		Reference	
Transmission routes						
Sex	−577.9 (−1137.6–−18.1)	0.043	2.6 (−14.4–19.5)	0.765	−8.3 (−20.6–3.9)	0.183
Blood transfusion	−624.6 (−1229.9–−19.3)	0.043	3.9 (−10.7–18.5)	0.598	−4.7 (−16.5–7.1)	0.439
Others/unknown	Reference		Reference		Reference	
Viral hepatitis						
No	377.6 (107.8–647.5)	0.006	−24.4 (−32.4–−16.5)	<0.001	7.1 (−0.2–14.4)	0.057
Yes	Reference		Reference		Reference	
AIDS events prior to cART						
No	−120.4 (−351.8–110.9)	0.308	−3.0 (−11.5–5.5)	0.484	−0.7 (−7.6–6.3)	0.852
Yes	Reference		Reference		Reference	
Time from diagnosis of HIV infection to treatment (years)	−10.4 (−95.4–74.6)	0.810	−4.7 (−7.8–−1.7)	0.002	0.2 (−2.3–2.8)	0.864
Baseline CD4+ T-cells (cells/mm^3)						
≤200	219.6 (−78.8–518.0)	0.149	−15.6 (−26.5–−4.6)	0.005	−1.9 (−11.2–7.3)	0.682
>200 but <350	Reference		Reference		Reference	
Baseline VL (lg copies/ml)	−53.8 (−197.9–90.3)	0.464	6.0 (2.5–9.5)	0.001	−0.2 (−3.6–3.1)	0.884
Duration of cART (years)	4.2 (−14.2–22.5)	0.656	−5.8 (−6.5–−5.2)	<0.001	−0.6 (−1.5–0.205)	0.139
Delayed virologic suppression						
No	189.0 (−71.4–449.5)	0.155	−12.9 (−21.5–−4.2)	0.004	−13.5 (−21.3–−5.7)	0.001
Yes	Reference		Reference		Reference	

HLA-DR: Human leukocyte antigen-D related; VL: Viral load; cART: Combination antiretroviral therapy; *CI*: Confidence interval.