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Factors influencing the normalization of CD4+ T-cell count, percentage and CD4+/CD8+ T-cell ratio in HIV-infected patients on long-term suppressive antiretroviral therapy

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Abstract

We evaluated factors associated with normalization of the absolute CD4+ T-cell counts, per cent CD4+ T cells and CD4+/CD8+ T-cell ratio. A multicentre observational study was carried out in patients with sustained HIV-RNA <50 copies/mL. Outcomes were: CD4-count >500/mm^3 and multiple T-cell marker recovery (MTMR), defined as CD4+ T cells >500/mm^3 *plus* %CD4 T cells >29% *plus* CD4+/CD8+ T-cell ratio >1. Kaplan–Meier survival analysis and Cox regression analyses to predict odds for achieving outcomes were performed. Three hundred and fifty-two patients were included and followed-up for a median of 4.1 (IQR 2.1–5.9) years, 270 (76.7%) achieving a CD4+ T-cell count >500 cells/mm^3 and 197 (56%) achieving MTMR. Using three separate Cox models for both outcomes we demonstrated that independent predictors were: both absolute CD4+ and CD8+ T-cell counts, %CD4+ T cells, a higher CD4+/CD8+ T-cell ratio, and age. A likelihood-ratio test showed significant improvements in fitness for the prediction of either CD4+ >500/mm^3 or MTMR by multivariable analysis when the other immune markers at baseline, besides the absolute CD4+ count alone, were considered. In addition to baseline absolute CD4+ T-cell counts, pretreatment %CD4+ T cells and the CD4+/CD8+ T-cell ratio influence recovery of T-cell markers, and their consideration should influence the decision to start antiretroviral therapy. However, owing to the small sample size, further studies are needed to confirm these results in relation to clinical endpoints.

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Introduction

Following HIV infection, CD4+ and CD8+ T-lymphocyte homeostasis is regulated on the basis of absolute T-lympho-cyte counts (CD8+ counts increase as CD4+ counts decline) until advanced stages of disease when the CD8+ count also declines. [1]. Many patients receiving effective antiretroviral therapy (ARV) (those who maintain undetectable plasma HIV RNA levels) experience significant increases in absolute CD4-counts [2,3]. The attainment of a CD4+ T-cell count greater than 500 cells/mm^3 has been associated with a reduction in mortality rates to the level of the general population [4]. Although a significant proportion of patients starting therapy with CD4+ T-cell counts <350 cells/mm^3 achieves this goal [5–7], about 25–30% of patients do not [8]. Host factors, including pretreatment CD4+ T cells, are important predictors of immunological recovery [5–7,9–11]. More recently, attention has focused on levels of immune activation as a correlate of blunted CD4+ T-cell recovery

[12] and an independent predictor of mortality [13], often from non-AIDS related conditions [14].

Most studies focused on CD4+ T-cell count increases as the principal sign of immunological recovery after initiation of antiretroviral therapy, but other T-cell markers may have important prognostic value. Castagna et al.[15] found that the percentage of CD4+ T cells (%CD4+) predicts the absolute magnitude of CD4+ T-cell count recovery over a short period of follow-up, and both %CD4+ T cells and the CD4+/CD8+ T-cell ratio predict risk for AIDS-related [16] and non-AIDS related morbidities [17–19]. To what extent the %CD4+ T cells and CD4+/CD8+ T-cell ratio normalize after prolonged periods of effective antiretroviral therapy has yet to be described.

Therefore, we undertook an analysis of a large cohort of patients experiencing suppressive antiretroviral therapy to characterize the recovery of multiple T-lymphocyte parameters after prolonged periods of suppression of virus replication. We describe the proportions of patients who achieve increases in absolute CD4+ T-cell counts to a value >500 cells/mm^3, as well as a multiparametric measure of T-cell recovery (multiple T-cell marker recovery, MTMR), defined as absolute CD4+ T-cell counts, %CD4+ T cells and CD4+/CD8+ T-cell ratio above normal levels.

Methods

Design of the study and cohort
We conducted a retrospective analysis of patients enrolled in the Italian MASTER (Management of Standardized Evaluation of Retroviral HIV Infection) study, a longitudinal multi-centre cohort in nine referral centres throughout Italy (http://www.mastercohort.it). The distinguishing characteristic of this cohort is that data are compiled in a common electronic chart (Health & Notes 3.5®, Healthware S.p.A., Naples, Italy) in use in the participating centres. Data are recorded over a standardized time-scale every 3 months, with merging and data cleaning performed at a single centre every 6 months.

Inclusion criteria and data collection
Patients included in this study commenced antiretroviral therapy between 2000 and 2005, with regimens consisting of two nucleoside/tide reverse transcriptase inhibitors (NRTI) *plus*: (i) a protease inhibitor (PI) ± low-dose ritonavir (PI/r) as a booster, (ii) a non-nucleoside reverse transcriptase inhibitor (NNRTI) or (iii) abacavir. Patients had to achieve an HIV-1 RNA level <50 copies/mL within the first 12 months and maintain a viral load below this threshold for ≥2 years.

Patients had to have at least two HIV-1 RNA determinations per year. Absolute CD4+ and CD8+ T-cell counts, %CD4+ and %CD8+ T cells and CD4+/CD8+ T-cell ratios were collected at pretreatment [baseline, i.e. within 6 months before highly active antiretroviral therapy (HAART) initiation] and follow-up time-points. Subjects gave written informed consent for participation in the observational cohort, and each site obtained approval by their Ethics Committee.

Study outcomes and additional definitions of immune status
We selected two different primary immunological outcomes: the achievement of a CD4+ T-cell count >500 cells/mm^3 and the achievement of MTMR, comprising a CD4+ T-cell count >500 cells/mm^3 *plus* a %CD4+ T cells >29% *plus* a CD4+/CD8+ T-cell ratio >1. A patient's follow-up was censored at death, at loss to follow-up, or at an increase in HIV-1 RNA >50 copies/mL, whichever occurred first.

To evaluate whether immune markers below a certain degree of immune impairment could predict the outcomes and to evaluate discordance in defining a certain stage of immune suppression, the following cut-offs were defined: [1,14]: (i) CD4+ T-cell count ≤200 cells/mm^3 and %CD4+ T cells ≤14% and CD4+/CD8+ T-cell ratio ≤0.3; (ii) CD4+ T-cell count ≤350 cells/mm^3 and %CD4+ T cells ≤20% and CD4+/CD8+ T-cell ratio ≤0.5; and (iii) CD4+ T-cell count ≤500 cells/mm^3 and %CD4+ T cells ≤29% and CD4+/CD8+ T-cell ratio≤1.

Statistical analysis
Predictors of immune recovery. Time to endpoints was assessed by Kaplan–Meier survival curves. Date of ARV initiation was chosen as the starting point. Univariate and multivariable Cox regression models were used to assess relative hazards of CD4+ T-cell recovery >500/mm^3 and MTMR, considering both baseline and time-updated covariates, with a robust variance computation by a grouped jackknife [20,21].

The models included baseline T-cell parameters (fitted as numeric) combined in separate multivariable models as follows: (i) absolute CD4+ and absolute CD8+ T-cell counts (model 1); (ii) %CD4 and %CD8 T cells (model 2); and (iii) CD4+/CD8+ T-cell ratio (model 3). Also, categorical absolute CD4+ T-cell counts (> or ≤350/mm^3) and CD4+/CD8+ T-cell ratio (> or ≤0.5) were tested as independent variables because 350 CD4+/mm^3, corresponding to a CD4+/CD8+ T-cell ratio of 0.5, has been indicated as a landmark threshold for starting therapy [22].

Moreover, the models included the time-fixed covariates at baseline of age, gender, mode of HIV transmission, calendar year, HCV/HBV co-infection status and HIV RNA level. Time-varying covariates included type of anchor drug (PI/r,

PI, NNRTI, abacavir), regimen number (defined as any single drug modification occurring during the follow-up), and number of AIDS-defining events or non-AIDS-related illnesses and neoplasias.

Comparison between absolute CD4+ T-cell count and composite measures of immune status at baseline for predictions of immune recovery. Absolute CD4+ T-cell counts and composite measures of T-cell status at baseline were compared for predictions of immune recovery in order to verify if the inclusion of one or more additional covariates would lead to a significant improvement in a model fit. For this analysis, likelihood ratio tests (LRTs) were executed [23]. Two base/null models were set up, one fitted with the sole baseline CD4+ T-cell count and another with the sole baseline CD4+/CD8+ T-cell ratio. More complex/alternative models considered were those fitted with the base variable plus one or more of the following variables (if not already included): CD4+ T-cell count, CD8+ T-cell count, %CD4+ T cells, %CD8 T cells and CD4+/CD8+ T-cell ratio. In addition, we executed an LRT comparing the base models against those fitted with the full set of covariates as previously listed. LRTs were assessed by considering both the complete MTMR endpoint and the achievement of a CD4+ T-cell count >500 cells/mm^3. As several comparisons were made, we adjusted the set of p-values obtained with the Bonferroni correction [24].

All p were two tailed, and values <0.05 were considered statistically significant. The mathematical programming suite *R*, with the survival library, was used to perform all statistical analyses and to generate graphs [25].

Results

Patients' characteristics

A total of 352 patients, 269 (76.4%) men, met the inclusion criteria and were followed for a median of 4.1 (IQR 2.1–5.9) years. Baseline characteristics are shown in Table 1. Among the 352 patients, only 41 (11.7%) began therapy with CD4+ T-cell counts ≥350 cells/mm^3. Each patient had at least one T-cell marker, among the absolute CD4+ T-cell count, CD4+ T-cell percentage and CD4+/CD8+ T-cell ratio, lower than normal at baseline, and 154 (43.7%) had all three of these markers in the severely low range. A slight majority of subjects (51.4%) had concordant values of the T-cell markers in one of the three classes of immune suppression. For example, among the 41 subjects with baseline CD4+ T-cell counts >350 cells/mm^3, 25 (61%) had %CD4+ T cells ≤20% or a CD4+/CD8+ T-cell ratio ≤0.5.

TABLE 1. Patients' characteristics

Demographics and risk for HIV transmission	n (%)
Age (years, mean (IQR))	39 (34.7–45.7)
Male gender	269 (76.42)
Caucasians	352 (100)
Mode of transmission	
Heterosexual	189 (53.7)
MSM	66 (18.7)
Intravenous drug use	74 (21)
Other/unknown	23 (6.5)
Viro-immunological markers and serological status	n (%)
HIV RNA (log$_{10}$ copies/mL, mean (IQR))	4.87 (4.32–5.28)
CD4+ T-cell count (cells/mm^3, mean (IQR))	190.5 (77–286)
Absolute CD4+ T-cell count	
>500/mm^3	8 (2.3)
350–500/mm^3	33 (9.4)
200–349/mm^3	130 (36.9)
<200/mm^3	181 (51.4)
CD8+ T-cell count (cells/mm^3, mean (IQR))	841.5 (541.8–1212)
Absolute CD8+ T-cell count	
>1200/mm^3	91 (25.3)
800–1200/mm^3	98 (27.8)
400–799/mm^3	116 (32.9)
<400/mm^3	47 (13.3)
%CD4+ T-cells	12.8% (6.5–18%)
%CD4+ T-cells	
>29%	12 (3.4)
20–29%	51 (14.5)
14–19%	77 (21.9)
<14%	212 (60.2)
CD4+/CD8+ T-cell ratio (mean (IQR))	0.21 (0.11–0.32)
CD4+/CD8+ T-cell ratio	
>1	1 (0.3)
0.5–1	36 (10.2)
0.3–0.49	66 (18.7)
<0.3	249 (70.7)
HBsAb status	
Positivity	75 (21.3)
Negativity	157 (44.6)
Unknown	120 (34.9)
HBsAg	
Positivity	23 (6.5)
Negativity	252 (71.6)
Unknown	77 (21.8)
HCV-Ab	
Positivity	80 (22.7)
Negativity	205 (58.2)
Unknown	67 (19.0)
Antiretroviral therapy	n (%)
Antiretroviral regimens	
NRTI *plus* NNRTI	249 (70.7)
NRTI *plus* PI	22 (6.5)
NRTI *plus* PI/r	59 (16.8)
Abacavir	22 (6.5)
Clinical events	n (%)
Clinical events occurring before baseline	
AIDS events	36 (10.2)
Non-AIDS illnesses	82 (23.3)
Non-AIDS neoplasias	3 (0.8)
Clinical events occurring during follow-up	
AIDS events	18 (5.1)
Non-AIDS illnesses	54 (15.3)
Non-AIDS neoplasias	5 (1.4)

N, number; IQR, interquartile range; NRTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, protease inhibitor boosted with ritonavir.
Among the non-AIDS-related illnesses before HAART initiation cardiovascular events were found in 10/352 patients, liver diseases in 32/352 patients, metabolic diseases in 38/352 patients and kidney diseases in one patient.
During follow-up, cardiovascular events were found in 4/352 patients, liver diseases in 26/352 patients, metabolic diseases in 9/352 patients. No patients experienced kidney diseases.
Three neoplasias were found at baseline (one cervical, one kidney and one skin neoplasia) and five during the follow-up (three skin, one non-Hodgkin lymphoma and one connective tissue neoplasia).

Recovery of T-cell markers

Among the 352 patients, an absolute CD4+ T-cell count >500 cells/mm^3 was achieved by 270 (76.7%) patients. By

TABLE 2. Predictors of achieving a CD4+ T-cell count to >500/mm^3

Time-fixed variables	Univariate HR (95% CI)	p	Multivariable 1 HR (95% CI)	p	Multivariable 2 HR (95% CI)	p	Multivariable 3 HR (95% CI)	p
Age (per 10 years older)	0.71 (0.62–0.83)	<0.001	0.79 (0.68–0.92)	0.003	0.78 (0.67–0.92)	0.002	0.78 (0.66–0.91)	0.002
Males (vs. female)	0.67 (0.51–0.88)	0.003	0.90 (0.66–1.23)	0.523	0.98 (0.70–1.39)	0.918	0.89 (0.65–1.22)	0.465
Baseline year (per 1 year more recent)	1.03 (0.96–1.11)	0.393	1.03 (0.95–1.12)	0.521	1.02 (0.93–1.11)	0.719	1.03 (0.94–1.12)	0.551
Mode of transmission								
Heterosexual	(ref.)	—	(ref.)	—	(ref.)	—	(ref.)	—
Homo/bisexual	1.0 (0.75–1.41)	0.859	1.06 (0.74–1.53)	0.748	1.04 (0.71–1.50)	0.852	1.06 (0.74–1.53)	0.740
Intravenous drug use	0.83 (0.61–1.13)	0.211	1.03 (0.95–1.55)	0.892	0.95 (0.63–1.44)	0.805	0.91 (0.61–1.35)	0.638
Other/unknown	0.50 (0.28–0.90)	0.020	0.72 (0.38–1.38)	0.327	0.64 (0.34–1.21)	0.719	0.52 (0.27–1.01)	0.053
HBV status								
HBsAb positivity (vs. negative/unknown)	1.06 (0.79–1.42)	0.675	1.12 (0.81–1.54)	0.488	1.10 (0.80–1.52)	0.546	1.12 (0.82–1.54)	0.473
HBsAg positivity (vs. negative/unknown)	0.94 (0.57–1.54)	0.827	0.78 (0.45–1.35)	0.381	1.00 (0.60–1.69)	0.989	1.10 (0.67–1.82)	0.706
HCVAb positivity (vs. negativity/unknown)	0.85 (0.64–1.14)	0.259	0.86 (0.56–1.31)	0.477	0.92 (0.59–1.43)	0.707	1.01 (0.67–1.53)	0.966
CD4+ count (per 50 cells/mm^3 higher)	1.17 (1.14–1.20)	<0.001	1.35 (1.27–1.45)	<0.001	Not entered	Not entered		
CD8+ count (per 200 cells/mm^3 higher)	1.02 (0.98–1.06)	0.219	0.94 (0.90–0.98)	<0.001	Not entered	Not entered		
%CD4 (per 10% higher)	2.12 (1.82–2.47)	<0.001	Not entered		2.23 (1.85–2.70)	<0.001		
%CD8 (per 10% higher)	2.25 (1.91–2.65)	<0.001			1.02 (0.91–1.13)	0.775		
CD4+/CD8+ T-cell ratio (per 0.3 higher)	3.25 (2.65–3.99)	<0.001	Not entered		Not entered		2.27 (1.89–2.74)	<0.001
HIV RNA (per $\log_{10}$ higher)	1.04 (0.91–1.21)	0.534	1.37 (1.15–1.62)	<0.001	1.33 (1.13–1.56)	<0.001	1.26 (1.07–1.48)	0.005
Time-varying variables								
Antiretroviral therapy								
NRTI *plus* NNRTI	(ref.)	—	(ref.)	—	(ref.)	—	(ref.)	—
NRTI *plus* PI	0.64 (0.36–1.12)	0.134	0.49 (0.19–1.25)	0.135	0.56 (0.27–1.16)	0.117	0.61 (0.32–1.18)	0.144
NRTI *plus* PI/r	0.92 (0.66–1.30)	0.666	1.12 (0.77–1.63)	0.541	1.14 (0.78–1.67)	0.493	1.10 (0.77–1.57)	0.613
Other	1.07 (0.66–1.74)	0.747	0.86 (0.53–1.40)	0.555	0.82 (0.50–1.35)	0.430	0.91 (0.56–1.46)	0.687
Therapy line	N/A[a]		N/A[a]		N/A[a]		N/A[a]	
AIDS events	0.74 (0.54–1.00)	0.031	1.03 (0.74–1.44)	0.873	0.91 (0.71–1.17)	0.453	0.83 (0.64–1.09)	0.358
Non-AIDS events	1.02 (0.85–1.23)	0.820	1.04 (0.82–1.32)	0.721	1.14 (0.92–1.41)	0.246	1.16 (0.95–1.42)	0.140
Non AIDS-defining neoplasias	0.30 (0.09–0.91)	0.036	0.03 (0–0.45)	0.011	0.39 (0.14–1.06)	0.066	0.34 (0.14–0.84)	0.018

HR, hazard ratio; 95% CI, 95% confidence interval; p, probability; NRTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, protease inhibitor boosted with ritonavir.

Multivariable model 1 considered absolute CD4+ and CD8+ T-cell counts, multivariable 2 considered %CD4+ and %CD8+ T cells and multivariable 3 considered CD4+/CD8+ T-cell ratio as independent covariate

[a] Not fitted because of singularities.

contrast, only 197 (56%) achieved MTMR. By 2 years, the estimated proportion of patients not reaching an absolute CD4+ T-cell count >500 cells/mm^3 was 0.565 (0.5157–0.619), whilst that of patients not reaching MTMR was 0.759 (0.715–0.805). By 5 years, estimated proportions were 0.282 (0.2368–0.335) and 0.526 (0.475–0.582), respectively.

Predictors of immune recovery
Predictors of CD4+ T-cell counts to >500 cells/mm^3. Table 2 shows univariate and multivariable results for the main models. The univariate analysis demonstrated a significant increased probability of achieving a CD4+ T-cell count >500 cells/mm^3 for higher baseline absolute CD4+ T-cell counts, %CD4+ T cells and CD4+/CD8+ T-cell ratio. In contrast, increasing age, male gender, non-AIDS-defining cancers and higher %CD8+ T cells were associated with a significantly reduced probability of increasing one's CD4+ T-cell count to >500 cells/mm^3. In the multivariable analyses, both higher baseline CD4+ T-cell counts and lower CD8+ T-cell counts (model 1), higher baseline %CD4+ T cells (model 2), and higher baseline CD4+/CD8+ T-cell ratio (model 3) were significantly associated with an increased probability of achieving CD4+ T-cell count to >500 cells/mm^3. Additional variables associated with this outcome were higher pretreatment HIV-1 RNA levels and younger age. In contrast, a diagnosis of a non-AIDS-defining cancer was significantly associated with a lower probability of achieving a CD4+ T-cell count to >500/mm^3.

As demonstrated in Fig. 1(a), having both a baseline CD4+ T-cell count ≤350 cells/mm^3 *and* a CD4+/CD8+ T-cell ratio ≤0.5 was associated with the lowest probability of reaching CD4+ T-cell count >500 cells/mm^3 by Kaplan–Meier survival analysis (log-rank p <0.001). If treatment was delayed until the patient's CD4+ count was ≤350 cells/mm^3 but the CD4+/CD8+ T-cell ratio was >0.5 there was a similar rate of CD4+ T-cell recovery compared with patients whose baseline CD4+ count was >350 cells/mm^3 but had a CD4+/CD8+ T-cell ratio ≤0.5.

Predictors of MTMR. Table 3 shows univariate and multivariable results for the main models. By multivariable analyses, both higher CD4+ T-cell count and lower CD8+ T-cell count at baseline (model 1), both higher %CD4+ T cells and lower %CD8+ T cells at baseline (model 2), or higher CD4+/CD8+ T-cell ratio at baseline (model 3) were significantly associated with increased probability of MTMR. Other variables significantly associated with this outcome were: HAART initiation in more recent calendar years (models 1 and 3) and higher HIV-1 RNA level at baseline (models 1 and 2). In contrast, variables significantly associated with a decreased probability of MTMR were: older age (models 1–3) and a diagnosis of non-AIDS-defining cancers (model 1).

As was the case for achieving a CD4+ T-cell count >500 cells/mm^3, the baseline CD4+ T-cell count and baseline CD4+/CD8+ T-cell ratio each contributed to the rate at which patients achieved MTMR (Fig. 1b). The group with a baseline CD4+ T-cell count ≤350 cells/mm^3 *plus* a CD4+/CD8+ T-cell ratio ≤0.5 had the lowest probability of reaching MTMR by Kaplan–Meier survival analysis (log-rank p <0.001). Although the hazard ratio (HR) for achieving an MTMR was not statistically significantly in favour of patients with baseline CD4+ T-cell count ≤350 cells/mm^3 *plus* a
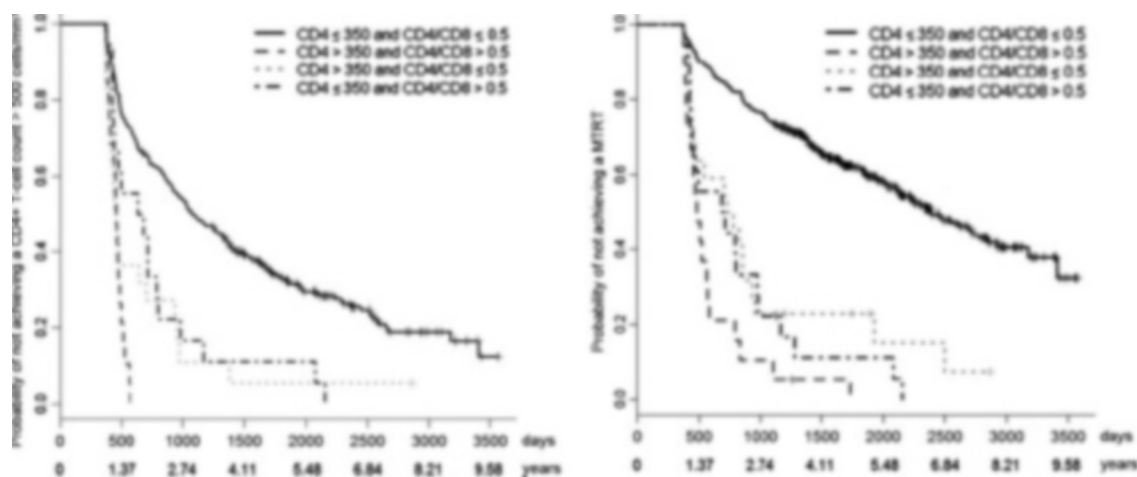

FIG. 1. Kaplan–Meier survival curve showing the proportion of patients over time not reaching a CD4+ T-cell count >500 cells/mm^3 (left panel) or achieving multiparametric T-cell marker recovery (right panel). Patients are stratified by CD4+ T-cell counts ≤ or >350 cells/mm^3 and CD4+/CD8+ T-cell ratios ≤ or >0.5 at baseline.

TABLE 3. Predictors of achieving multiple T-cell marker recovery

Time-fixed variables	Univariate		Multivariable 1		Multivariable 2		Multivariable 3	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age (per 10 years older)	0.69 (0.58–0.83)	<0.001	0.77 (0.65–0.92)	0.004	0.70 (0.58–0.85)	<0.001	0.73 (0.61–0.88)	0.001
Males (vs. Female)	0.60 (0.45–0.81)	<0.001	0.92 (0.63–1.35)	0.694	1.02 (0.69–1.54)	0.890	0.99 (0.67–1.48)	0.980
Baseline year (per 1 year more recent)	1.05 (0.96–1.16)	0.238	1.12 (1.01–1.24)	0.026	1.07 (0.97–1.18)	0.194	1.11 (1.01–1.23)	0.039
Mode of transmission								
Heterosexual	(ref.)	–	(ref.)	–	(ref.)	–	(ref.)	–
Homo/bisexual	1.00 (0.70–1.45)	0.969	0.93 (0.61–1.42)	0.745	0.98 (0.62–1.52)	0.916	1.12 (0.75–1.67)	0.578
Intravenous drug use	0.80 (0.56–1.15)	0.235	1.06 (0.68–1.65)	0.796	0.99 (0.63–1.54)	0.953	1.02 (0.67–1.58)	0.898
Other/unknown	0.58 (0.31–1.09)	0.098	0.95 (0.5–1.8)	0.876	0.83 (0.41–1.70)	0.618	0.75 (0.37–1.50)	0.412
HBV status								
HBsAb positivity (vs. negative/unknown)	0.98 (0.69–1.40)	0.924	0.86 (0.57–1.31)	0.485	1.03 (0.69–1.57)	0.865	0.96 (0.64–1.44)	0.840
HBsAg positivity (vs. negative/unknown)	0.99 (0.55–1.81)	0.986	0.85 (0.47–1.56)	0.612	0.95 (0.53–1.71)	0.877	1.03 (0.58–1.84)	0.912
HCVAb positivity (vs. negativity/unknown)	0.79 (0.56–1.11)	0.182	0.73 (0.48–1.11)	0.149	0.86 (0.55–1.35)	0.526	0.89 (0.58–1.35)	0.576
CD4+ count (per 50 cells/mm^3 higher)	1.14 (1.06–1.22)	<0.001	1.35 (1.26–1.46)	<0.001	Not entered	Not entered	Not entered	Not entered
CD8+ count (per 200 cells/mm^3 higher)	0.97 (0.92–1.02)	0.218	0.85 (0.79–0.91)	<0.001	Not entered	Not entered	Not entered	Not entered
%CD4 (per 10% higher)	3.29 (2.80–3.86)	<0.001	Not entered	Not entered	2.30 (1.85–2.86)	<0.001	Not entered	Not entered
%CD8 (per 10% higher)	0.79 (0.71–0.87)	<0.001	Not entered	Not entered	0.86 (0.76–0.98)	0.026	Not entered	Not entered
CD4+/CD8+ T-cell ratio (per 0.3 higher)	3.25 (2.65–3.99)	<0.001	Not entered	Not entered	Not entered	Not entered	2.42 (1.99–2.95)	<0.001
HIV RNA (per log$_{10}$ higher)	1.12 (0.76–1.65)	0.187	1.29 (1.08–1.55)	0.006	1.21 (1.04–1.42)	0.016	1.16 (0.93–1.44)	0.085
Time-varying variables								
Antiretroviral therapy								
NRTI *plus* NNRTI	(ref.)	–	(ref.)	–	(ref.)	–	(ref.)	–
NRTI *plus* PI	0.58 (0.28–1.20)	0.139	0.65 (0.28–1.51)	0.321	0.57 (0.26–1.23)	0.153	0.6 (0.27–1.33)	0.208
NRTI *plus* PI/r	0.84 (0.56–1.25)	0.389	0.82 (0.54–1.25)	0.362	1.01 (0.65–1.55)	0.983	0.89 (0.58–1.37)	0.613
Other	1.45 (0.93–2.27)	0.100	1.30 (0.84–1.99)	0.235	1.32 (0.84–2.08)	0.225	1.26 (0.81–1.96)	0.305
Therapy line	1.08 (0.82–1.42)	0.567	1.13 (0.84–1.51)	0.422	1.14 (0.85–1.53)	0.370	1.15 (0.86–1.53)	0.340
AIDS events	0.69 (0.45–1.04)	0.075	0.834 (0.48–1.44)	0.517	0.85 (0.56–1.30)	0.463	0.81 (0.52–1.27)	0.358
Non-AIDS events	0.96 (0.77–1.19)	0.715	1.07 (0.82–1.39)	0.611	1.18 (0.93–1.50)	0.161	1.16 (0.93–1.44)	0.176
Non AIDS-defining neoplasias	0.60 (0.22–1.61)	0.313	0.02 (0.00–0.41)	0.012	0.92 (0.43–1.99)	0.847	0.58 (0.24–1.42)	0.235

HR, hazard ratio; 95% CI, 95% confidence interval; p, probability; NRTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, protease inhibitor boosted with ritonavir.

Multivariable model 1 considered absolute CD4+ and CD8+ T-cell counts, multivariable 2 considered %CD4+ and %CD8+ T cells and multivariable 3 considered CD4+/CD8+ T-cell ratio as independent covariate.

Therapy line was defined as the number of combinations of antiretroviral drugs. Every change of drugs produced a new therapy line.

CD4+/CD8+ T-cell ratio >0.5 *versus* CD4+ T-cell count >350 cells/mm³ *plus* a CD4+/CD8+ T-cell ratio ≤0.5, by fitting a multivariable Cox regression model, with the group with a CD4+ T-cell count ≤350 cells/mm³ *plus* a CD4+/CD8+ T-cell ratio ≤0.5 as the reference condition, we found increasing HR (95% confidence interval, CI) as follows: 3.67 (2.19–6.16, p <0.001) for CD4+ T-cell count >350 cells/mm³ *plus* a CD4+/CD8+ T-cell ratio ≤0.5; 4.5 (2.83–7.16, p <0.001) for CD4+ T-cell count ≤350 cells/mm³ *plus* a CD4+/CD8+ T-cell ratio >0.5; and 8.22 (5.19–13.04, p <0.001) for CD4+ T-cell count >350/mm³ *plus* a CD4+/CD8+ T-cell ratio >0.5.

According to the sensitivity analysis, even in severely immunologically impaired patients a greater absolute CD4+ T-cell count (HR, 1.07; 95% CI, 1–1.16, p 0.048 per 10 CD4+/mm³ higher) was an independent predictor of MTMR. By the same model, absolute CD8+ T-cell count was not significantly associated with MTMR when fitted numerically. However, using a stratification of CD8+ T-cell counts <400 *versus* >1100 cells/mm³, a trend towards an increased hazard of MTMR was observed (HR, 3.60; 95% CI, 0.93–13.94; p 0.060). Moreover, in separate models, either %CD4+ T cells (HR, 1.12; 95% CI, 1.02–1.22; p 0.012 per 1% higher) or CD4+/CD8+ T-cell ratio (HR, 1.25; 95% CI 1.06–1.48; p 0.007 per 0.04 higher) were independent predictors of MTMR.

Comparison between absolute CD4+ T-cell count and composite measures of immune status at baseline for predictions of immune recovery. When executing LRTs considering different nested models (Table 4), we found in general that the addition of other laboratory markers besides the CD4+ T-cell count alone increased the model fit significantly, even after correction of the p-values with the Bonferroni procedure. With regard to the endpoint of achieving a CD4+ T-cell count >500 cells/mm³, starting from the base model with only the baseline CD4+ T-cell count variable, the addition of the baseline CD8+ T-cell count covariate did not lead to a significantly better fit, while the addition of the baseline CD4+/CD8+ T-cell ratio, or the addition of both baseline %CD4 T cells and baseline %CD8 T cells, led to a significant improvement of the model fit. Conversely, the addition of baseline CD4+ T-cell count, or baseline CD8+ T-cell count or baseline %CD4% and %CD8 T cells to the base model made by the baseline CD4+/CD8+ T-cell ratio alone always led to a significant improvement of the model fit. With respect to the MTMR endpoint, when starting with the base model made by the baseline CD4+ T-cell count alone and adding any of the other above-mentioned covariates, significant improvements in the likelihood were produced. When starting with the base model made by the baseline CD4+/CD8+ T-cell ratio alone, appreciable improvements in the model fit were found only when adding the baseline %CD4+ and %CD8+ T cells together.

Notably, the multivariable model made from the full set of covariates (including all other clinical/demographic variables besides the immunological markers) always produced a significantly better fit than any base models for both endpoints.

Discussion

To our knowledge, this is the largest analysis of immune reconstitution of multiple T-cell markers in patients on prolonged suppression of virus replication to levels <50 copies/mL. We defined an MTMR immunological endpoint that includes changes in absolute CD4+ T-cell counts, CD4+ T-cell percentage and the CD4+/CD8+ T-cell ratio. Absolute

TABLE 4. Comparison between absolute CD4+ T-cell count and composite measures of immune status at baseline for predictions of immune recovery (likelihood ratio tests).

Endpoint	Null (N)	Alternative (A)	L_N	L_A	df_N	df_A	p-value	p_B	R_N^2	R_A^2
CD4+ T-cell	CD4+	CD4+ and CD8+	−1364	−1363	1	2	0.1060	1.0000	0.20	0.21
count >500 cells/mm³		CD4+ and CD4+/CD8+	−1364	−1354	1	2	<0.0001	0.0001	0.20	0.25
		CD4+ and CD4% and CD8%	−1364	−1347	1	3	<0.0001	<0.0001	0.20	0.28
	CD4+/CD8+	CD4+/CD8+ and CD4+	−1366	−1354	1	2	<0.0001	<0.0001	0.20	0.25
		CD4+/CD8+ and CD8+	−1366	−1358	1	2	0.0001	0.0008	0.20	0.23
		CD4+/CD8+ and CD4% and CD8%	−1366	−1360	1	3	0.0013	0.0180	0.20	0.23
	CD4+	Full set of covariates	−1364	−1323	1	19	<0.0001	<0.0001	0.20	0.42
MTMR	CD4+	CD4+ and CD8+	−1020	−1011	1	2	<0.0001	0.0004	0.12	0.15
		CD4+ and CD4+/CD8+	−1020	−987	1	2	<0.0001	<0.0001	0.12	0.23
		CD4+ and CD4% and CD8%	−1020	−971	1	3	<0.0001	<0.0001	0.12	0.27
	CD4+/CD8+	CD4+/CD8+ and CD4+	−988	−987	1	2	0.1210	1.0000	0.22	0.23
		CD4+/CD8+ and CD8+	−988	−987	1	2	0.1050	1.0000	0.22	0.23
		CD4+/CD8+ and CD4% and CD8%	−988	−977	1	3	<0.0001	0.0002	0.22	0.26
	CD4+	Full set of covariates	−1020	−966	1	19	<0.0001	<0.0001	0.12	0.31

MTMR, multiple T-cell marker recovery; L, log-likelihood; d.f., degrees of freedom; pB, Bonferroni's adjusted p-value; R^2, R-squared.

CD8+ T-cell counts and the CD4+/CD8+ T-cell ratio independently predicted immune restoration defined as a CD4+ T-cell count >500 cells/mm^3, irrespective of absolute CD4+ T-cell count at baseline. This result is consistent with the findings of Castagna *et al.*[15] after 6 months of effective therapy. Each of the T-cell markers contributed independently to the composite marker. Therefore, MTMR may be a better predictor of when to initiate antiretroviral therapy, assuming one therapeutic goal is the most complete level of immune reconstitution that can be achieved.

This is one of few studies that examine changes in T-cell markers over a long period of virological suppression; patients were followed for a median of 4 years and 25% for 6 or more years on effective therapy. As has been previously reported, the majority (77%) of patients reached CD4+ T-cell count >500/mm^3 [5,6]. However, MTMR was obtained in only a fraction (56% overall), indicating that reconstitution of absolute CD4+ T-cell counts does not always reflect normalization of T-cell homeostasis. Our data suggest that normalization of T-cell markers continues throughout the period of continued antiretroviral therapy, in contrast to the plateau effect described in some studies [6,26,27], but not in others [5,7]. Interestingly, in the studies (including this report) with longer follow-up that used a more sensitive level of HIV-1 RNA for inclusion [5,7], the plateau effect was not observed. This reinforces the importance of maintaining HIV-1 RNA levels <50 copies/mL to achieve as complete an immunological reconstitution as can be achieved.

We are aware of only one other study that evaluated changes in absolute CD4+ and CD8+ T-cell counts, CD4+ T-cell percentage and the CD4+/CD8+ T-cell ratio during a similar period of follow-up to that in our study [7]. However, this report is based on only 49 patients, and a viral load of <400 HIV-1 RNA copies/mL was used to define effective therapy, a cut-off that does not exclude periods of incomplete viral suppression.

A decline in CD8+ T-cell counts is likely to be a hallmark of the most profound period of immune suppression in the life of someone with HIV [1]. The combination of a low CD4+ and CD8+ T-cell count may represent a state of immune deficiency that is difficult to reverse. This may explain why absolute CD8+ T-cell counts in patients with CD4+ T < 200 cells/mm^3 failed to predict CD4+ T-cell recovery in our sensitivity analysis.

Our study is too small to infer the clinical relevance of MTMR in contrast to absolute CD4+ T-cell count recovery alone. However, per cent CD4+ T cells has been shown to be an independent predictive factor of AIDS progression [16], and in other studies the CD4+/CD8+ T-cell ratio was a predictor of risk for Hodgkin's lymphoma [17] and myocar-dial infarction [18,19] independent from other clinical and immunological factors.

We examined other clinical factors that predicted increases of CD4+ T-cell counts to >500 cells/mm^3 and MTMR. Younger patients had a better chance of achieving these outcomes, as has been described [28]. In addition, patients with higher baseline HIV-1 RNA levels were more likely to achieve both an increase in CD4+ T-cell count to >500 cells/mm^3 and MTMR, which is similar to findings in several studies [3,29–31]. Patients with a diagnosis of non-AIDS-related malignancies were less likely to achieve these immunological thresholds, possibly due to damage by chemotherapy. Lastly, patients who started treatment in more recent years had higher probabilities of reaching MTMR.

Our study has several limitations. Because we focused only on patients with sustained virological suppression we cannot make any conclusion about the effects of intermittent increases in virus replication to measureable levels, and therefore, these data are not representative of the immunological changes in many patients cared for in clinical practice. Because of the sample size we were not able to make any observations on the impact of specific classes of antiretroviral therapy on immunological recovery, which has been shown to influence the magnitude of CD4+ T-cell recovery according to some studies [32,33], but not to others [34–36]. Moreover, other possible factors such as viral phenotype (CCR5-tropic versus CXCR4-tropic strains) or drug-resistance signature mutations at entry were not considered in this study.

In addition, we used single measurements of baseline and endpoint CD4+ T-cell counts. One might argue that a single CD4+ T-cell count measurement could be imprecise, and the usage of a linear mixed model approach might be more appropriate [37], because it has the added advantage of estimating mean and subject-specific slope estimates. However, the MTMR endpoint was a combined indicator and should be more robust as compared with a putative transient achievement of just one indicator over a certain threshold (for instance a CD4+ T-cell count >500 cells/mm^3). In addition, the usage of single baseline measurements keeps the model simpler and applicable in clinical practice. Further directions could include the comparison of our simple approach with another model based on slope estimation to evaluate whether a more complex model improves predictive power and clinical utility.

In conclusion, our results suggest for the first time that allowing any of the commonly monitored T-cell parameters, absolute CD4+ T-cell counts, CD4+ T-cell percentage and CD4+/CD8+ T-cell ratio, to fall below certain thresholds will compromise a patient's ability to achieve an absolute CD4+

T-cell count >500 cells/mm^3. Therefore, larger studies are needed to evaluate if each of these markers should be considered in deciding when to initiate antiretroviral therapy. It is important to note that to include these markers in clinical practice, the results of more studies (ideally randomized clinical trials) are mandatory. Moreover, if our results are confirmed and clinically validated, discussions of immune reconstitution in HIV patients should include normalization of these parameters as well as absolute CD4+ T-cell counts.

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Transparency Declaration

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