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Drop in CD4+ counts below 200 cells/µL after reaching (or starting from) values higher than 350 cells/µL in HIV-infected patients with virological suppression.

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Abstract

Background. Aim of the study was to quantify the risk of a drop in CD4+ counts below 200 cells/μL after reaching values >350 cells/μL on antiretroviral therapy (or after starting antiretroviral therapy with CD4+ count >350 cells/μL) in the absence of virological failure.

Setting. Ambulatory care services, Italy.

Methods. Prospective cohort study of patients enrolled in the ICONA Foundation Study cohort who started ART with >350 CD4+/μL, or with ≤350 CD4+/μL and reached values >350 cells/μL after virological suppression (VS, defined by two consecutive viral loads ≤50 copies/mL). The date of CD4 count>350 was the baseline for the analysis and those with ≥1 viral load and CD4+ count after baseline were included. The primary end-point was the cumulative risk (estimated using the Kaplan-Meier method) of a CD4+ drop below 200 cells/μL over follow-up, which was censored at the date of virological failure (confirmed HIV-RNA >50 copies/mL), death or last visit.

Results: Six thousand six hundred sixty-three patients were included. A confirmed CD4+ drop below 200 cells/μL was never observed over a median follow-up of 45 (Q1: 21, Q3: 89) months, as long as VS was maintained. Upper limits of the 97.5%CI of rates of confirmed CD4+ drop below 200 cells/μL were 0.28 and 0.38/1,000 PYFU for patients with ≤350 and >350 CD4+ cells/μL at starting ART.

Conclusions: In patients who started ART in Italy with >350 CD4+ cells/μL, or reached >350 CD4+ cells/μL after VS, the risk of a CD4+ drop below 200 cells/μL in those maintaining VS was negligible.

Keywords: CD4+ cells count; CD4+ count dipping; CD4+ count monitoring; virological suppression; antiretroviral therapy.

Introduction

CD4+ T-lympocytes count is the strongest predictor of disease progression in HIV-infected patients and prophylaxis of opportunistic infection is recommended when it drops below 200 cells/µL [1-6]. Frequent CD4+ cell count monitoring has been highly recommended for many years, but the utility of monitoring has been recently debated [7, 8]. In HIV-infected people receiving antiretroviral therapy (ART), CD4+ counts tend to increase or remain stable as long as viral replication is controlled [9-12] and clinical events very infrequently occur after ART introduction [10, 13]. Furthermore, there are no treatment strategies to increase CD4+ cell count during periods of viral suppression. A CD4+ count drop below 200 cells/µL is currently seldom observed during successful antiretroviral therapy [14-17] and frequent testing may cause unnecessary anxiety in patients with clinically inconsequential fluctuations. Therefore, the utility of their monitoring has become a matter of debate [7, 8, 17]. Indeed, treatment decisions clinician can take in response to CD4+ monitoring is to start (or re-start) prophylaxis of opportunistic infections when counts drop below 200 cells/µL [1-4] or, in clinical settings were viral load testing is not routinely implemented, to prescribe viral load testing [18]. In this context, some guidelines recommend optional or stop monitoring CD4+ cell counts once virological suppressions is sustained, and the immunological status is stabilized [1,3, 18].

There are no randomized comparisons of individuals following different CD4+ count monitoring strategies as they are difficult to be performed, and, although it is well established that CD4+ cells falling below 200 cells/µL occur infrequently while viral load is suppressed [14-17, 19], robust estimates of CD4+ cells dropping below 200 cells/µL over long follow-up are limited. Furthermore, relevant differences might emerge when comparing data collected in countries with different organization of the social health system.

The aim of this analysis was to quantify the probability of a drop in CD4+ counts below 200 cells/µL after reaching values >350 cells/µL on ART (or when starting ART with CD4+ count >350 cells/µL) in the absence of virological failure (VF), in a cohort of Italian HIV-infected patients.

Methods

The ICONA Foundation Study (ICONA) is a multi-center prospective observational study of HIV-1-infected adult patients, which was set up in 1997. Eligible patients are those starting ART when they are naive to antiretrovirals, regardless of the reason. The ICONA Foundation study has been approved by IRB of all the participating centers; sensitive data from patients are seen only in aggregate form. All patients sign a consent form to participate in ICONA, in accordance with the ethical standards of the committee on human experimentation and the Helsinki Declaration (1983 revision). Demographic, clinical and laboratory data and information on therapy are collected for all participants and recorded using electronic data collection [www.icona.org].

CD4+ monitoring in cohort participants is performed at least twice yearly, according to study protocol and to Italian guidelines [2]. Antiretroviral regimens used by the studied patients were not predefined by the study protocol (which is strictly observational), but were prescribed according to current Italian guidelines [2].

Virological suppression (VS) was defined as having achieved on two consecutive occasions a viral load $\leq$50 copies/mL. Participants could be included in this analysis if they belonged to two distinct inclusion criteria group. First, participants who started ART with a CD4+ count >350 cells/µL. Second, people who started ART with ≤350 CD4+/µL, achieved VS on therapy and whose CD4+ count subsequently increased to a value >350 cells/µL. In patients who started ART with >350 CD4+/µL, baseline was defined as the date of VS after at least 6 months since initiation of ART. In those who started ART with ≤350 CD4+/µL, baseline was the date of first achieving a CD4+ value >350 CD4+/µL after VS. For both groups, we insisted on people to have at least one VL and CD4+ count assessed after baseline.

Viral load was assessed in each center according to local procedures. Yearly change in CD4+ cell counts after baseline were estimated by fitting a linear mixed model with random intercept and slope.

The primary end-point was the cumulative risk of a confirmed CD4+ drop below 200 cells/µL over follow-up, defined as two consecutive counts below 200 cells/µl (confirmed drop). We also evaluated a secondary endpoint defined using a single count below 200 cells/µl (unconfirmed drop). Participants' time at risk was censored at the date of VF (defined as a confirmed HIV-RNA >50 copies/mL), death or last clinical visit. The Kaplan Meier method was used to estimate the cumulative risk of CD4+ dropping below 200 cells/µl over time. The 97.5% upper limit of the confidence interval was calculated using a normal approximation. All analyses were repeated also using an alternative definition of viral rebound (with a cut-off of 400 copies/mL instead of 50 copies/mL).

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Six thousand six hundred sixty-three adult patients were included in the analysis (Table 1). The main differences between patients who started ART with <350 CD4+/µL and those who started ART with >350 CD4+/µL regarded gender, risk factor, nationality, CD4+ nadir, CD4+/CD8+ ratio, HCV co-infection and HIV-RNA load.

Over a median (Q1, Q3) follow-up of 45 (21, 89) months, new AIDS-defining events occurred in 124 (2%) patients, serious non-AIDS-defining [20] in 306 (5%), death in 153 (2%; 21 AIDS related, 132 non-AIDS related) and VF in 1,796 (27%). The median (Q1, Q3) CD4+ count at the time the AIDS-defining events occurred was 458 (228, 671) cells/µL.

The median (IQR) CD4+ measurements over follow-up was five (2, 12), for an average of 2.6 (2.0, 3.4) measurements/year, and the median of prospective HIV-RNA measurements was also five (2, 11), for 2.6 (2.0, 3.4) measurements/year.

Baseline CD4+ cell count was 646 (611, 682)/μL in patients who started ART with ≤350 CD4+/μL and 757 (708, 807)/μL in those who started ART with >350 CD4+/μL (difference: 110.9 [95% CI: 50.4, 171.5] cells/μL; p<.001). Estimated yearly changes in CD4+ cell counts were +27.44 (-18.2, +73.12) cells/μL in patients who started ART with ≤350 CD4+/μL and +31.99 (-8.83, +72.81) cells/μL in those who started ART with >350 CD4+/μL (difference: +4.55 [-56.5, +65.64] cells/μL; p=0.884) (supplementary figure).

A confirmed CD4+ drop below 200 cells/μL was never observed over the time of the study; unconfirmed CD4+ drops below 200 cells/μL occurred in nine patients and no clinical events were observed at the time of occurence. The Kaplan-Meier estimate of the cumulative risk of unconfirmed drop below 200 cells/μL was 0.24% (95% CI: 0.07%, 0.40%) by 4 years from baseline; no further events were observed over the following 9 years and 249 individuals were still at risk by 12 years after baseline. Upper limits of the 97.5% CI of rates of confirmed CD4+ drop below 200 cells/μL were 0.28 and 0.38/1,000 PYFU for patients with ≤350 and >350 CD4+ cells/μL at ART start, respectively. Upper limits of the 97.5% CI of rates of confirmed CD4+ drop below 200 cells/μL according to type of ART started, HCV co-infection, calendar year of baseline, baseline CD4/CD8 >0.3 or ≤0.3, time from HIV diagnosis and HIV-RNA at ART start > or ≤5 log10 copies/mL, are shown in Table 2. Of note, all these upper limits were below 2.0 per 1,000 PYFU. Only patients with calendar year baseline between 1997 and 2001 and those with a CD4+/CD8+ ≤0.3 at ART start had an upper limit which was >1 per 1000 PYFU. Results were similar when viral rebound was defined using the more conservative threshold of 400 copies/mL for the definition of VF (data not shown).

Discussion

We investigated the risk of CD4+ drop below 200 cells/μL in patients who started ART with >350 CD4+ cells/μL, or reached >350 CD4+ cells/μL after virological suppression, and no events drop were observed over a median follow-up of approximately four years, as long as virological suppression was maintained. Even in the worst case scenario, in which the true value for the incidence is equal to the upper limit of the 97.5% of the confidence interval, this incidence of a drop <200 cells/μl was between 1 and 2 per 1000

PYFU. This estimate is consistent with those previously found elsewhere. In another analysis of a cohort of 1,820 HIV-infected patients, those with HIV-1 RNA <200 copies/mL and CD4+ counts ≥300 cells/µL had a 97% probability of maintaining durable CD4+ counts ≥200 cells/µL for 4 years [14]. Similarly, in the ARTEMIS trial, only 1% of 449 patients with sustained HIV-1 RNA suppression below 400 copies/mL experienced a CD4+ count drop below 200 cells/µL on two consecutive visits [15]. In 7,250 patients in South Africa, after 10 years of ART, 93% of patients with ongoing virological suppression maintained CD4+ cell counts continuously above 200 cells/µL [19]. Furthermore, in an Asian cohort, among 1,538 patients virologically suppressed over an unreported duration of follow-up, the rate of a confirmed drop below 200 CD4+/µL was 0.77/100 person-years. There was no significant difference in the time to a confirmed drop below 200 CD4+/µL when comparing people who were monitored biannually vs. those who had annual CD4+ measurements [21]. Finally, in the PISCIS Cohort study, over a median follow-up of almost two years, CD4+ cell counts fell to <200 cells/µL in 7% of 8,695 patients [16]. However, this estimate was calculated counting also single measurements below 200 CD4+/µL.

It can be argued that experiencing a CD4 count drop below 200 cells/µL might not be clinically relevant, because clinical events typically occur also with counts of >200 cells/µL. Interestingly, nevertheless, in 39,283 HIV-infected patients with VL <1000 copies/mL or CD4+ cell counts ≥350/µL in the resource limited setting, the implementation of routine CD4+ cell count monitoring beyond 12 months after ART initiation did not seem to have an impact on long-term mortality rates [13].

It needs to be considered that frequent monitoring of CD4+ count in the setting of well controlled viral replication, might be only a source of patients' anxiety as the result of the test is unlikely to trigger treatment decision [7, 8]; reducing the frequency of CD4+ monitoring might also reduce the overall costs related to care of HIV-infected patients [8, 17]. However, knowing when CD4+ drop below 200 cells/µL is important, because in these cases (re)starting prophylaxis of opportunistic infections is recommended [1-4]. In the absence of randomized comparisons of CD4+ count monitoring strategies, our analysis provides solid estimates that should help designing such studies.

Caniglia et al, recently tried to emulated a trial comparing the effect of different frequency of CD4+ cell counts monitoring for clinical outcome using the data of a very large cohort. Most of the 39,029 cohort participants who were eligible for the comparison changed strategy during follow-up (mostly in the first six months): indeed, less than 1/10 of them did not change the initial strategy and could be maintained in the analysis over two years of follow-up, thus largely limiting the power of the study [10].

The results of our analysis confirm and expand, over a long follow-up and in a setting where there is universal access to treatment, the results of previous analyses. They also lend support to optional monitoring of CD4+ cell counts in patients with satisfactory virological and immunological response to ART.

As participants' follow-up time was censored at virological failure, the results of this study are applicable only to the population of HIV-infected individuals with current stable virological suppression. It must be noted that results also do not apply to immunological non-responders. Indeed, people who started ART with ≤350 CD4+/µL and never attained values >350 cells/µL were excluded from the analysis because less frequent CD4+ cell counts monitoring is not an option in these patients.

The main limitations of this study are the relatively small sample size and the relatively short duration of follow-up; however, all events occurred over the first three years, so it is unlikely that results will be different by repeating the analysis after waiting for further follow-up to cumulate.

In summary, in patients who started ART in Italy with >350 CD4+ cells/µL, or reached >350 CD4+ cells/µL after VS, with a stable controlled viral suppression, a confirmed CD4+ drop below 200 cells/µL was never observed over an average of four years. The results of this study support optional monitoring of CD4+ cell counts, in a setting in which viral load assessment is easily available and in patients with these characteristics; they are also useful to help designing randomized trials comparing CD4+ count monitoring strategies in virologically suppressed populations.

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Table 1. Main characteristics of patients at starting antiretroviral therapy, by baseline CD4+ count group

Characteristic	CD4+ cell count at starting antiretroviral therapy			
	>350 cells/µL	**≤350 cells/µL**	**p-value***	**Total**
	N= 2790	N= 3873		N= 6663
Age, years, Median (Q1, Q3)	39 (33, 46)	40 (34, 46)	0.092	40 (34, 46)
Female gender, n (%)	609 (21.8%)	990 (25.6%)	<.001	1599 (24.0%)
Mode of HIV transmission, n (%)			<.001	
IDU	403 (14.5%)	773 (20.0%)		1176 (17.7%)
Homosexual contacts	1239 (44.5%)	1234 (32.0%)		2473 (37.2%)
Heterosexual contacts	993 (35.6%)	1617 (41.8%)		2610 (39.2%)
Other/Unknown	149 (5.4%)	237 (6.1%)		386 (5.8%)
Non-Italian Nationality, n (%)	292 (10.5%)	523 (13.5%)	<.001	815 (12.2%)
HBsAg, n (%)			0.806	
Negative	2203 (79.0%)	3047 (78.7%)		5250 (78.8%)
Positive	111 (4.0%)	146 (3.8%)		257 (3.9%)
Not tested	476 (17.1%)	680 (17.6%)		1156 (17.3%)
HCV Ab, n (%)			<.001	
Negative	1908 (68.4%)	2469 (63.7%)		4377 (65.7%)
Positive	465 (16.7%)	802 (20.7%)		1267 (19.0%)
Not tested	417 (14.9%)	602 (15.5%)		1019 (15.3%)
Detectable CMV Ab, n (%)	1295 (46.4%)	1834 (47.4%)	0.449	3129 (47.0%)
Calendar year of baseline, Median (Q1, Q3)	2012 (2004, 2014)	2009 (2002, 2012)	<.001	2010 (2003, 2013)
AIDS diagnosis, n (%)	128 (4.6%)	760 (19.6%)	<.001	888 (13.3%)
CD4+ count nadir, cells/µL, Median (Q1, Q3)	402 (350, 484)	208 (105, 276)	<.001	284 (177, 381)
CD4+ count, cells/µL, Median (Q1, Q3)	670 (547, 848)	425 (384, 490)	<.001	490 (407, 659)

CD8+ count, cells/µL, Median (Q1, Q3)	947 (702, 1260)	940 (681, 1281)	0.669	944 (687, 1270)
CD4+/CD8+ ratio ≤0.3, n (%)	95 (3.6%)	617 (16.6%)	<.001	712 (11.2%)
HIV-RNA at ART start, log_{10} copies/mL, Median (Q1, Q3)	4.40 (3.58, 4.95)	4.82 (4.19, 5.33)	<.001	4.65 (3.96, 5.17)
Antiretroviral started, n (%)				
Zidovudine	525 (18.8%)	1003 (25.9%)	<.001	1528 (22.9%)
Lamivudine	985 (35.3%)	1698 (43.8%)	<.001	2683 (40.3%)
Abacavir	339 (12.2%)	402 (10.4%)	0.023	741 (11.1%)
Tenofovir	1688 (60.5%)	1927 (49.8%)	<.001	3615 (54.3%)
Emtricitabine	1577 (56.5%)	1764 (45.5%)	<.001	3341 (50.1%)
Efavirenz	697 (25.0%)	910 (23.5%)	0.162	1607 (24.1%)
Nevirapine	203 (7.3%)	286 (7.4%)	0.867	489 (7.3%)
Rilpivirine	397 (14.2%)	159 (4.1%)	<.001	556 (8.3%)
Lopinavir/ritonavir	189 (6.8%)	443 (11.4%)	<.001	632 (9.5%)
Atazanavir/ritonavir	379 (13.6%)	521 (13.5%)	0.876	900 (13.5%)
Darunavir/ritonavir	330 (11.8%)	470 (12.1%)	0.703	800 (12.0%)
Raltegravir	122 (4.4%)	179 (4.6%)	0.629	301 (4.5%)
Antiretroviral therapy type, n (%)			<.001	
2 NRTIs+ NNRTI	1404 (50.3%)	1390 (35.9%)		2794 (41.9%)
2 NRTIs+ PI	450 (16.1%)	924 (23.9%)		1374 (20.6%)
2 NRTIs+ PI/r	564 (20.2%)	861 (22.2%)		1425 (21.4%)
2 NRTIs+ InSTI	137 (4.9%)	119 (3.1%)		256 (3.8%)
Other	509 (18.2%)	817 (21.1%)		1326 (19.9%)

*Chi-square or Kruskal-Wallis test as appropriate

HBsAg: Hepatitis B surface antigen; HCV Ab: antibodies anti-Hepatitis C virus; CMV Ab: antibodies anti-Cytomegalovirus; ART: antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-

nucleoside reverse transcriptase inhibitor; PI: unboosted protease inhibitor; PI/r: protease inhibitor boosted with ritonavir; InSTI: integrase strand transfer inhibitor.

Table 2. CD4+ cell drop rates (per 1000 PYFU) and respective one-sided upper limit of 97.5% confidence interval.

		CD4+ drop rates (per 1000 PYFU)	
Stratification factors	**No. drops**	**PYFU**	**upper limit of 97.5% confidence interval**
CD4+ cell count at ART start			
0-350/µL	0	13306	0.28
>350/µL	0	9721	0.38
Type of ART			
PI-based ART	0	5135	0.72
NNRTI-based ART	0	9297	0.40
Other ART	0	4752	0.78
HCV co-infection			
Absent	0	4734	0.78
Present	0	15145	0.24
Year of baseline			
1997-1999	0	2059	1.80
2000-2001	0	2318	1.59

2002-2006	0	6768	0.55
2007+	0	11883	0.31
CD4+/CD8+			
0-0.3	0	2537	1.45
>0.3	0	19766	0.19
HIV-RNA at ART start, log_{10} copies/mL			
0-5	0	15640	0.24
>5	0	6921	0.53
Total	0	22561	0.16

ART: antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; HCV: Hepatitis C virus.