

ORIGINAL RESEARCH

Factors associated with a reduced CD4 lymphocyte count response to HAART despite full viral suppression in the EuroSIDA study

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Objectives

To describe the prevalence and risk factors of poor CD4 count rise despite a good virological response on highly active antiretroviral treatment (HAART).

Methods

The patients from the EuroSIDA study who started HAART with a baseline CD4 count of <350 cells/μL and where all viral load (pVL) measures remained below 500 HIV-1 RNA copies/mL between 6 and 12 months after the start of HAART were included. The risk factors for poor CD4 count rise were analyzed by multiple regression.

Results

Seven hundred and eighty patients were included. A low CD4 count response was observed in 225 patients (29%). The risk factors for this condition were older age, lower CD4 count at baseline, higher increase from the nadir to baseline CD4 count and lower pVL at baseline. Patients taking $\geq$one drug from each of the three antiviral classes were more likely to have a good CD4 response but a minority of the study participants was taking this treatment regimen (3.1%) and the confidence interval was large.

Conclusions

A poor immune reconstitution despite a good virological control is frequent after initiation of HAART among patients with a baseline CD4 count of <350 cells/μL. The underlying mechanisms leading to this condition seems mainly driven by the age and the baseline immunological and virological status of the patients.

Keywords: CD4-lymphocyte count, cohort studies, highly active antiretroviral therapy, HIV infection, immune reconstitution

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Introduction

Several patterns of response after initiation of highly active antiretroviral treatment (HAART) have been observed in persons with HIV infection. Apart from treatment success and failure a minority of patients will present a so-called 'paradoxical response', defined as a discrepancy between the plasma viral load (pVL) and the CD4 count. The first situation occurs in 7–15% of the patients [1]. The CD4 count rises despite a persistently detectable pVL, which might be explained by the selection of mutant virus with decreased fitness compared with wild-type virus [2]. Furthermore, protease inhibitors (PI) seem to inhibit lymphocyte apoptosis independently of their antiviral effect [3, 4]. The second type of paradoxical response is where the CD4 count does not rise despite a fully suppressed viral growth has been far less studied. This

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Table 1 Overview of previous studies describing HIV-positive patients under HAART with a good viral control and a poor immunological response

	Patients *n* (% ART naive)	Study type	Treatment regimen	Duration of follow-up	Criteria for pVL and CD4 + response	Low CD4/ low pVL *n* (%)	Risk factors
Barreiro [8]	288 (?) first HAART	Retrospective cohort monocentric	1PI + 2 NRTIs	6 month	↓pVL > 1 Log or < 500 copies/mL ↑CD4 < 60 cells/μL	77 (26.9%)	Not studied
Grabar [19]	2236 (22.7%) PI naive	Prospective cohort multicentric	1PI + 2 NRTIs	6 month	↓pVL > 1 Log or < 1000 copies/mL ↑CD4 < 50 cells/μL	387 (17.3%)	Baseline pVL and CD4 count, transmission group, previous ARV treatment, first PI prescribed
Piketty [25]	162 (100%) PI naive	Prospective cohort monocentric	IDV + 2 NRTIs	12 month	↓pVL > 1 Log or < 500 copies/mL ↑CD4 < 100 cells/μL	17 (10.5%)	No significant risk factors, but the studied subgroups were small
Renaud [9]	317 (3%) PI naive	Prospective cohort monocentric	1PI + 2 NRTIs	24 month	↓pVL > 1 Log 'lack of CD4 increase'	14 (8%)	Pre-treatment rate of T-cell depletion.

HAART, highly active antiretroviral treatment; ART, antiretroviral treatment; PI, protease Inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; pVL, plasma viral load.

phenomenon seems to occur in 5–15% of the patients treated with HAART (Table 1) [5–8].

Studies on these poor responders may provide valuable insights on the immune reconstitution after the initiation of HAART. The purpose of this study is to describe the prevalence and risk factors of poor CD4 count rise despite a good virological response on HAART among patients included in the EuroSIDA study.

Patients and methods

The EuroSIDA study is a prospective observational cohort study of more than 8500 patients followed in 63 hospitals of 20 European countries. The main objective of the study is to assess the impact of antiretroviral drugs on the general population of HIV-infected patients living in Europe. The initial four cohorts of the study were included in the present analysis (cohort I identified in summer of 1994 [$n = 3118$]; cohort II in winter 1995/96 [$n = 1367$]; cohort III in spring of 1997 [$n = 2844$]; cohort IV in spring of 1999 [$n = 1227$]). The patients in the four cohorts are consecutive patients seen in the clinic after a given fixed date, up to a preset limit they had a confirmed HIV infection and were at inclusion at least 18 years old and (except for cohort IV) had a CD4 count of < 500 cells/μL. Until now, a total of over 27 000 person-years of patient experience has been collected. The general methodology of the study has been described elsewhere [9].

Patients for this study were all those who started HAART with moderate immunosuppression, with a baseline CD4 count of below 350 cells/μL, and in whom pVL at baseline (measured within the previous 6 months at most) was known and was below 500 HIV-1 RNA copies/mL by 6 months after the start of HAART. All subsequent pVL values within 1 year of HAART (of which there had to be at least one measurement between months 6 and 12) had to be below 500 copies/mL. This value was chosen due to the different pVL assays used in the different treatment centres. HAART was defined as a combination of at least three products from at least two different antiretroviral classes. Patients who used PIs or non-nucleoside analogues (NNRTIs) prior to HAART were excluded. HAART was required to have started by June 2000 to allow potential for 1 year of follow-up.

The CD4 response was assessed on the basis of the first CD4 count made after month 6 and is referred as the 'response-defining lymphocytes count'. Those with no CD4 count between month 6 and month 12 were excluded. The definition of low (or paradoxical) CD4 count response depended on the month that the blood samples were obtained. If the response-defining CD4 count was taken in month 7, then an increase from a baseline CD4 count of

below 50 cells/μL was called 'low'. For month 8 the cut-off was 55 cells/μL; month 9: 60 cells/μL; month 10: 65 cells/μL; month 11: 70 cells/μL; and month 12: 75 cells/μL. Those values were considered as the lowest normal CD4 count response after initiation of HAART [8,10]. The group of patients with a low CD4 count response despite an undetectable pVL was compared to patients with a good virological response (pVL < 500 copies/mL) and a stable or increasing CD4 count response.

Statistical methods

Associations between risk factors potentially associated with a low CD4 count response were assessed by using a logistic regression model and expressed as odds ratios, with 95% confidence intervals. The risk factors taken into account for low CD4 count were: demographic factors (age, sex ratio, ethnic origin, HIV transmission group, region), antiretroviral treatment factors (number of previous NRTI used before HAART, start date of HAART, type of HAART, HAART duration), immunological factors (nadir and baseline CD4 count, increase from CD4 nadir at baseline, month of response defining CD4 count) virological factors (highest pVL ever, baseline pVL, time to reach a pVL below 500 copies/mL) and clinical factors (previous AIDS, positive HCV antibody, positive HBs antigen, previous episode of opportunistic infection). A two-sided *P*-value below 0.05 was considered significant. Univariable (fitting the risk factor [covariate] of interest alone) and multivariable logistic regression models were used to assess the effects of each potential risk factor before and after adjustment for other risk factors, respectively. The multivariable model was selected by including covariates that were significant ($P < 0.05$) in the univariable model. The exception was that nadir CD4 count, CD4 count change from nadir to baseline and CD4 count at baseline were not included in the same model, as there is a linear dependency between them. The choice of exclusion of nadir CD4 count or change from nadir CD4 count was arbitrary. The effect of adding back in all the covariates one by one was assessed, to arrive at a final multivariable model. For the covariates in this final model, the multivariable odds ratios presented were those from this model itself. For those covariates not in the final model the multivariable odds ratios were those obtained from adding the covariate to the final model. All statistical analyses were performed using SAS statistical software, version 6.12 (SAS Institute Inc, Cary, NC, USA).

Results

Of the 8556 patients, 59 patients were excluded due to missing CD4 count between 6 and 12 months of HAART and a total of 780 patients satisfied the eligibility criteria for this analysis. The baseline characteristics of the study population are presented in Table 2. The time to reach pVL below 500 copies/mL was not significantly different between the low CD4 count group and the other group; median 3 months (interquartile range 1–3) vs. 2 months (1–4), respectively. The patients were mainly distributed across Western Europe, as East European patients have only recently been included in the study. Ninety-eight patients (12.7%) had a viral load below the detection level at baseline. The median month of the response-defining CD4 count was month 8 (interquartile range IQR 7; 10) and this was the same for the patients with low CD4 count and for those with a good response. A low CD4 count response was observed in 225 persons (29%). For those defined as having a low CD4 count response the median difference from baseline of the response-defining was a CD4 count of 22 cells/μL (IQR −8; 41). The equivalent value for those with a good response was 154 cells/μL (IQR 103; 245).

Table 3 shows the univariable and multivariable associations with the odds of a low CD4 count response. Several factors were independently associated with a poor immunological reconstitution after initiation of HAART in our population of patients with low CD4 count at baseline and a good virological control on HAART. Patients had 50% increased odds of a low CD4 count increase after start of HAART for each 10 years older. Additionally, they had 23% lower odds of low CD4 count increase after start HAART for each 100 cells/μL higher on baseline CD4 count and those who increased their CD4 count before having started HAART had a 92% higher odds of a low CD4 count increase on HAART for each 100 cells/μL higher. Finally, a lower baseline viral load was associated with 30% decreased odds of developing a low CD4 count response on HAART for each Log lower. A previous episode of Kaposi's sarcoma was also of borderline significance. The number of other relevant opportunistic infections (atypical mycobacteria and cytomegalovirus) and of malignancies (non-Hodgkin lymphomas) was too small to be included in the analysis. Other variables, such as ethnic origin, body mass index, time since first positive HIV test and time to reach a viral load below detection level were not significantly associated with a low CD4 count increase in a logistic regression model. The patients were studied for a period of 4 years, during which time the availability of antiretroviral products has increased and the treatment guidelines were modified several times. The moment when HAART was started seemed to have had no influence on the outcome variable since the start date of HAART was not significantly associated with a poor immune reconstitution. No specific antiretroviral agent had a significant influence on the poor immune reconstitution (data not shown). The

Table 2 Baseline characteristics of the study population

		CD4 lymphocyte response	
	Total	**Low**	**Not low**
n	780	225	555
Age [years] (median; IQR)	38 (34–46)	41 (36–49)	37 (33–45)
Sex ratio (M:F)	4.31	4.63	4.19
Ethnic origin *n* (%)			
caucasian	663 (87%)	195 (88%)	468 (87%)
others	99 (13%)	27 (12%)	70 (13%)
HIV transmission group *n* (%)			
homosexual men	383 (49%)	106 (47%)	277 (50%)
IDU	146 (19%)	50 (22%)	96 (17%)
heterosexual	196 (25%)	48 (21%)	148 (27%)
other	55 (7%)	21 (9%)	34 (6%)
Region *n* (%)			
south	154 (20%)	42 (19%)	112 (20%)
central	238 (31%)	76 (34%)	162 (29%)
north	376 (48%)	102 (45%)	274 (49%)
east	12 (2%)	5 (2%)	7 (1%)
Number of previous NRTIs *n* (%)			
0 (i.e. naive)	302 (39%)	68 (30%)	234 (42%)
1	25 (3%)	7 (3%)	18 (3%)
2	238 (30%)	74 (33%)	164 (30%)
3	114 (15%)	41 (18%)	73 (13%)
$\geq$4	101 (13%)	35 (16%)	66 (12%)
Duration of ART [month] (median; IQR)	11 (0–41)	16 (0–52)	7 (0–35)
CD4 nadir [cells/μL] (median; IQR)	150 (80–228)	130 (74–197)	158 (81–237)
CD4 at HAART [cells/μL] (median; IQR)	195 (118–274)	190 (124–272)	200 (111–274)
Increase from CD4 nadir at HAART [cells/μL] (median; IQR)	6 (0–60)	31 (0–85)	0 (0–47)
Highest pVL ever [log] (median; IQR)	4.7 (4.1–5.3)	4.5 (3.6–5.1)	4.8 (4.2–5.3)
pVL at HAART [log] (median; IQR)	4.4 (3.4–5.1)	3.9 (3.0–4.8)	4.5 (3.7–5.2)
Previous AIDS *n* (%)	166 (21%)	55 (24%)	111 (20%)
Start HAART *n* (%)			
before 1997	127 (16.3%)	41 (18%)	86 (16%)
1997	368 (47.2%)	105 (47%)	263 (47%)
1998	287 (23.9%)	56 (25%)	131 (24%)
1999 or after	98 (12.6%)	23 (10%)	75 (14%)
Type of HAART *n* (%)			
NNRTI no PI	84 (10.8%)	28 (12%)	56 (10%)
PI no NNRTI	672 (86.2%)	195 (87%)	477 (86%)
NNRTI and PI	24 (3.1%)	2 (1%)	22 (4%)
Number of drugs prescribed at baseline *n* (%)			
3	642 (82.3%)	185 (82%)	457 (82%)
>3	138 (17.7%)	40 (18%)	98 (18%)

If not specified, values are expressed as *n* (%) *n*, number of observations; IQR, interquartile range; IDU, injecting drug user; NRTI, nucleoside reverse transcriptase inhibitor, ART, antiretroviral treatment; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase Inhibitors, PI, protease inhibitors.

type of HAART prescribed influenced the CD4 count response, patients who were receiving a combination of at least one product from each class were significantly less likely to have a low response than patients on other HAART regimens. No other patient characteristics, HIV-related or treatment parameters were independently associated with a paradoxical response.

Discussion

Risk factors for a low increase of the CD4 count, despite a good virological control, are not fully described and the physiopathology remains unknown. Several hypotheses have been raised:

Prolonged damages to the immune system of patients in an advanced stage of the disease may lead to qualitative (cellular dysfunction) and or quantitative (lack of CD4 cell increase) defects [11]. The impaired immunological recovery following HAART may be related to a reduced thymic function such as the thymic involution observed with age [12].

A low level of viral replication under treatment would be responsible for a continuous damage on the immune system, thereby preventing a reconstitution of the CD4

Table 3 Univariable and multivariable odd ratios of a low (paradoxical) CD4+lymphocyte count response

	Odds ratio (95% CI);	*P*-value	Univariable	Multivariable
*Age (per 10 years older)	1.52 (1.29–1.79)	$P<0.0001$	1.49 (1.26–1.78)	$<$**0.0001**
Female	0.91 (0.61–1.35)	$P=0.63$	0.87 (0.57–1.34)	$P=0.53$
HIV exposure				
homosexual men	1.00		1.00	
IDU	1.36 (0.91–2.05)	$P=0.14$	1.38 (0.89–2.14)	$P=0.15$
heterosexual	0.85 (0.57–1.26)	$P=0.41$	0.77 (0.51–1.18)	$P=0.25$
other	1.61 (0.90–2.91)	$P=0.11$	1.37 (0.73–2.58)	$P=0.33$
Naive at start of HAART	0.59 (0.43–0.83)	$P=0.002$	1.08 (0.73–1.61)	$P=0.69$
Number of NRTI previously experienced (per extra drug)	1.19 (1.07–1.31)	$P=0.001$	0.99 (0.87–1.12)	$P=0.84$
Time from start of ART (per year)	1.12 (1.05–1.19)	$P=0.0003$	1.04 (0.97–1.11)	$P=0.30$
CD4 nadir (per 100 cells/μL higher)	0.78 (0.66–0.93)	$P<0.0001$	0.52 (0.38–0.72)	$P<$**0.0001**
* CD4 at HAART (per 100 cells/μL higher)	0.99 (0.85–1.16)	$P=0.92$	0.77 (0.63–0.93)	$P=$**0.006**
* Increase from CD4 nadir at HAART (per 100 cells/μL higher)	1.91 (1.44–2.52)	$P<0.0001$	1.92 (1.39–2.67)	$P<$**0.0001**
Viral load max ever (per 1 log higher)	0.70 (0.60–0.83)	$P<0.0001$	0.87 (0.68–1.11)	$P=0.26$
*Viral load at HAART (per 1 log higher)	0.68 (0.58–0.80)	$P<0.0001$	0.70 (0.60–0.83)	$P<$**0.0001**
Previous AIDS	1.29 (0.90–1.87)	$P=0.17$	1.22 (0.80–1.84)	$P=0.36$
Date of start of HAART (per year more recent)	0.89 (0.75–1.06)	$P=0.19$	0.92 (0.76–1.10)	$P=0.35$
Month of response-defining CD4 count (per month later)	0.97 (0.87–1.07)	$P=0.53$	0.98 (0.88–1.10)	$P=0.77$
Type of HAART				
PI and NNRTI	0.22 (0.05–0.96)	$P=0.04$	0.15 (0.03–0.70)	$P=$**0.02**
NNRTI no PI	1.22 (0.75–0.98)	$P=0.41$	1.21 (0.72–2.02)	$P=0.48$
PI no NNRTI	1.00		1.00	
Positive HCV antibody	1.10 (0.68–1.79)	$P=0.70$	1.10 (0.66–1.85)	$P=0.71$
Positive HBs Ag	1.08 (0.60–1.94)	$P=0.79$	1.26 (0.69–2.30)	$P=0.46$
Previous PCP	0.57 (0.27–1.19)	$P=0.13$	0.48 (0.22–1.06)	$P=0.07$
Previous KS	2.35 (1.05–5.22)	$P=0.04$	2.48 (1.01–6.41)	$P=$**0.05**

95% CI, 95% confidence interval; IDU, intravenous drug user; NRTI, nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral treatment. PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; HCV, hepatitis C virus; PCP, *pneumocystis carinii* pneumonia; KS, Kaposi's sarcoma. Factors marked with * are included in all multivariable models. Choice of inclusion of increase from CD4 nadir rather than CD4 nadir was arbitrary. Multivariable odds ratio for CD4 nadir does not include adjustment for increase in CD4 count from nadir.

lymphocyte pool [13]. It remains unclear whether patients with an undetectable pVL under the lowest level of detection still have an ongoing viral replication, either in the blood or in the immune organs, able to overcome the turnover of new CD4 lymphocytes.

Antiretroviral drugs may be toxic for lymphocytes. Nucleoside analogues (NRTI) act as a chain breaker and interfere with DNA synthesis. For some of them (zidovudine and zalcitabine) an immune toxicity has been documented in a murine model and on blood from healthy donors [14, 15]. Few studies were performed on blood from HIV-seropositive persons [16] and only one study has tested the potential toxicity of PIs [17] . All those studies were performed *in vitro*.

The prevalence of poor immune restoration despite a good virological control varies widely between studies (Table 1). This, however, may be due to differences in follow-up duration or in study design (including inclusion criteria, time and definition used for immune restoration). In the EuroSIDA cohort nearly 30% of the patients starting HAART with a moderate immunosuppression (i.e. at a CD4 count of below 350 cells/μL) had a poor immunological outcome despite a good virological response. Our results might have been biased by the fact that at least one CD4 count had to be available between months 6 and 12 to allow inclusion in the study; however, the number of patients excluded due to the absence of CD4 count was low.

The following risk factors have been associated with a poor immune reconstitution during HAART in other studies: older age [12, 18–20], HIV transmission group [18], hepatitis C virus (HCV) coinfection [21], pretreatment with NRTI [22], type of PI prescribed [22], low CD4 count and a high pVL at baseline [22].

The only factors found to be independently related to the odds of low CD4 count response in this study were older age, lower CD4 count at baseline, higher increase from the nadir to baseline CD4 counts and lower pVL at baseline.

The association between baseline CD4 count and the presence of a paradoxical response will be influenced by regression to the mean, since the baseline measure was used to calculate the change from baseline. This is likely to bias the association such that we may have underestimated the strength of the relationship between low baseline CD4 count and greater probability of a low CD4 count response [23].

In addition, the lack of CD4 count increase, to some extent, could be due to NRTI pre-exposure. In some

patients, HIV replication may already have been suppressed somewhat as shown by the association with the lower pVL at baseline. The positive effect of antiretroviral treatment on CD4 count response may already have occurred as shown by the high odds ratio associated with the CD4 count increase from nadir to baseline. For those reasons some CD4 response is likely to have occurred before start of HAART in several patients.

Interestingly, a previous history of AIDS, opportunistic infections or HCV infection was not associated with a low CD4 response. A previous history of Kaposi's sarcoma was slightly associated with a poorer CD4 response and this may have been caused by the specific treatment received (chemotherapy, corticosteroids or irradiation). Information on hepatitis C serology was missing in 56% (436/780) of the patients and the overall prevalence of HCV antibody carrier was 25% (108/436), while other investigators who found a significant association between those two parameters reported a much higher prevalence (37.5%, 1157/3111, $P<0.0001$, compared to our study) [22].

A qualitative evaluation of the immune function was not possible in this setting. Nevertheless, qualitative assays of the immune function such as the determination of activation markers (e.g. the percentage of CD8 CD38 cells) or the evaluation of T-cell function by the expression of CD28, among others, should be assessed to detect any qualitative defect in this population. For practical reasons, a pVL level of 500 copies/mL has been used as lowest detection level in this study. At this level, it is almost certain that a certain amount of viral replication is still occurring, the association between residual viral replication and low CD4 count should, therefore, be assessed by finer monitoring tools. Despite their potential cytotoxic effects, nucleoside analogues were not associated with a poorer immunological outcome. Despite a beneficial *in vitro* effect of the PI on the immune system [3,4], PI use was not associated with a better immunological outcome in this setting. Patients who received more potent antiretroviral combinations composed of at least one product of each class experienced a better CD4 response, but those data should be interpreted with caution as a minority of study participants (3.1%) were on triple class therapy and as the confidence interval was large. Those antiretroviral combinations might, nevertheless, enhance the suppression of HIV replication and allow a better immune reconstitution [24].

In two studies, half of the patients with a poor immunological response at 6 months presented a significant increase in CD4 count at 12 months [22] and at 30 months [25], which would imply that those patients experienced only a delay in immune reconstitution. On the other hand, poor immune reconstitution is a risk factor for disease progression at 30 months [22] compared to full responders, even when the virus was fully suppressed.

In conclusion, a poor immune reconstitution despite a good virological control seems frequent after initiation of HAART in patients with a baseline CD4 count of <350 cells/μL. Those presenting with this condition should be monitored closely until their immunity is sufficiently restored. The underlying mechanisms leading to this condition are unknown. It seems mainly driven by the age and the baseline immunological and virological status of the patients, and is an argument to encourage the initiation of HAART when the immunity has not yet been too damaged. At last two main theories remain plausible. First, this condition is caused by a lack of regeneration capacity of the immune system. In this case, the patients may benefit from an adjuvant therapy such as interleukin-2 [26]. Secondly, a residual viral replication prevents immunological reconstitution. In this case other monitoring tools, such as pVL with a very low detection level, proviral DNA measurement [27] or the measure of viral replication in other compartments than blood, such as in the lymphoid organs, should be evaluated.

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