

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

(Low CD4 in HAART treated patients with undetectable viral load)

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**Abstract:** (177 words)

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 baseline CD4 count <350/ $\mu$ l and where all viral load (pVL) measures remained undetectable between 6 and 12 months after the start of HAART were included. The risk factors for poor CD4 count rise were analysed by multiple regression.

Results:

780 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  $\geq 1$  product from each of the 3 antiviral classes were less likely to have a low response

Conclusions:

A poor immune reconstitution despite a good virological control is frequent during the first year of HAART. 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, HIV infection, cohort studies, highly active antiretroviral therapy, immune reconstitution.

## Introduction:

Several patterns of response after initiation of highly active antiretroviral treatment (HAART) have been observed in persons with HIV infection [1]. Many patients will have a sustained treatment response with a plasma viral load (pVL) below detection limit and a rising CD4+ lymphocyte count. Some patients however will sooner or later face a treatment failure. The most important risk factors for treatment failure are bad adherence, pre-existent resistance and advanced disease stage [2]. A minority of patients will present a so-called "paradoxical response", defined as a discrepancy between the pVL and the CD4+ lymphocyte count. The first situation, where the CD4+ lymphocyte count rises, despite a persistently detectable pVL might be explained by the selection of mutant virus with decreased fitness compared with wild type virus [3]. Furthermore, protease inhibitors (PI) seem to inhibit lymphocyte apoptosis independently of their antiviral effect [4,5]. The second type of paradoxical response where the CD4+ lymphocyte does not rise despite a fully suppressed viral growth has been far less studied. This phenomenon seems to occur in 5 to 15% of the patients treated with HAART (table 1) [6,7,8,9].

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+ lymphocyte 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 study has enrolled four cohorts of adult patients [cohort I identified in summer of 1994 (n=3.118); cohort II in winter 1995/96 (n=1.367); cohort III in spring of 1997 (n=2.844); cohort IV in spring of 1999 (n=1.227)]. The patients included in the four cohorts are consecutive patients seen in

the clinic after a given fixed date, up to a pre-set limit they had a confirmed HIV infection and were at inclusion at least 18 years old and (except for cohort IV) had a CD4+ lymphocyte count  $<500/\mu\text{l}$ . Until now, a total of over 27.000 person-years of patient experience have been collected. The general methodology of the study has been described elsewhere [10].

Patients for this study were all those who started HAART with baseline CD4+ lymphocyte count below  $350/\text{mm}^3$  and in whom pVL at baseline (measured within the previous 6 months at most) was known and was below 500 copies/ml by 6 months after the start of HAART. All subsequent pVL values within one 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 protease inhibitors (Pis) or non-nucleoside analogues (NNRTIs) prior to HAART were excluded. HAART was required to have started by June 2000 to allow potential for one year of follow-up.

The CD4+ lymphocyte response was assessed on the basis of the first CD4+ lymphocyte count made after month 6 and is referred as the "response-defining lymphocytes count". Those with no CD4+ lymphocyte count between month 6 and month 12 were excluded. The definition of low (or paradoxical) CD4+ lymphocyte count response depended on the month that the blood samples were obtained. If the response-defining CD4+ lymphocyte count was taken in month 7 then an increase from baseline below  $50/\text{mm}^3$  was called "low". For month 8 the cut-off was  $55/\text{mm}^3$ , month 9:  $60/\text{mm}^3$ , month 10:  $65/\text{mm}^3$ , month 11:  $70/\text{mm}^3$  and month 12:  $75/\text{mm}^3$ . Those values were considered as the lowest normal CD4+ lymphocyte response after initiation of HAART [9,11]. The group of patients with a low CD4+ lymphocyte 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+ lymphocyte count response.

Statistical methods:

The univariate analysis included demographic, therapeutic, clinical and biologic variables. The Chi-squared test was used for the analysis of categorical variables. Continuous variables were analysed with the Student's t-test or the Wilcoxon-Mann-Whitney test when the assumption of normality did not hold. Difference in outcome was expressed as an odds ratio and its 95% confidence interval. A two-sided p-value below 0.05 was considered significant. A multiple regression analysis using the logistic regression model was used to assess associations between the significant variables from the univariate analyses. All statistical analyses were performed using SAS statistical software, version 6.12.

## Results

Of the 8556 patients, a total of 780 patients satisfied the eligibility criteria for this analysis. The population was predominantly male, aged around 40 years (table 2). Most of the patients acquired the infection through sexual contact. The patients were mainly distributed across western Europe as East European patients have only recently been included in the study. A large proportion of patients (61%) had received at least one nucleoside analogue before starting HAART. The patients were severely immunosuppressed at baseline and had a high pVL, most of them started a protease inhibitor-containing regimen. The median month of the response-defining CD4+ lymphocyte count was month 8 (interquartile range IQR 7; 10) and this was the same for the patients with low CD4+ lymphocyte count and for those with a good response. A low CD4+ lymphocyte count response was observed in 225 persons (29%). For those defined as having a low CD4+ lymphocyte count response the median difference from baseline of the response-defining CD4+ lymphocyte count was +22/mm<sup>3</sup> (IQR -8; 41). The equivalent value for those with a good response was +154/mm<sup>3</sup> (IQR 103; 245). Table 3 shows the univariable and multivariable associations with the odds of a low CD4+ lymphocyte count response. The only factors found to be independently related to the odds of low CD4+ lymphocyte count response were older age, lower CD4+ lymphocyte count at baseline, higher increase from the nadir to baseline CD4+lymphocyte counts

and lower pVL at baseline. A previous episode of Kaposi's sarcoma was also slightly significant. 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 were not significantly associated with a low CD4+ lymphocyte count increase in a logistic regression model. The patients were studied for a period of four years, during which the availability of antiretroviral products has increased and the treatment guidelines were modified several times. Apparently 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. The type of HAART prescribed influenced the CD4+ lymphocyte 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 CD4+ lymphocyte increase despite a good virological control are not fully described and the physiopathology remains unknown. Several hypotheses have been raised:

The low CD4+ lymphocyte increase results from irreversible damages to the immune system of patients in an advanced stage of the disease [12]. The impaired immunological recovery following HAART may be related to a reduced thymic function such as the thymic involution observed with age [13].

A low level of viral replication under treatment would be responsible for a continuous damage on the immune system and thereby preventing a reconstitution of the CD4+ lymphocyte pool [14]. It remains unclear whether patients with an undetectable pVL 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 & zalcitabine) an immune toxicity has been documented in a murine model and on blood from healthy donors [15,16]. Few studies were performed on blood from HIV seropositive persons [17] and only one study has tested the potential toxicity of PIs [18]. 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 the time and the definition used for immune restoration). In the EuroSIDA cohort nearly 30% of the patients with a good virological response had a poor immunological outcome which represents 9.6% of the patients who started HAART (n=2347). This is comparable to previous studies with the same follow-up period [7,19]. Our results might have been biased by the fact that at least one CD4+ lymphocyte count had to be available between months 6 and 12 to allow inclusion in the study. It is however unlikely that patients who were lost to follow up or died early had an undetectable pVL which would have allowed them to be included in the study.

The following risk factors have been associated with a poor immune reconstitution during HAART in other studies: older age [13,20,21,22], HIV transmission group [19], hepatitis C virus (HCV) co-infection [23], pre-treatment with NRTI [19], type of protease-inhibitor prescribed [19], low CD4+ lymphocyte count and a high pVL at baseline [19].

In our population of patients with low CD4+ lymphocyte count at baseline and a good virological control on HAART, we found that older age, lower baseline and nadir CD4+ lymphocyte count, higher increase from nadir to baseline CD4+ lymphocytes and lower baseline pVL were independently associated with a poor immunological reconstitution during the first year after initiation of HAART. The association between baseline CD4+ lymphocyte 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 [24].

The lack of CD4+ lymphocyte count increase could be also to some extent due to NRTI pre-exposure. In some patients, HIV 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+ lymphocyte response may already have occurred as shown by the high odds ratio associated with the CD4+ lymphocyte increase from nadir to baseline. For those reasons some CD4+ lymphocyte 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+ lymphocyte response. A previous history of Kaposi's sarcoma was slightly associated with a poorer CD4+ lymphocyte response and this may have been caused by the specific treatment received (chemotherapy, corticosteroids or irradiation). Our study was maybe not powered enough to detect a relation between HCV co-infection and a low CD4+ lymphocyte response since a lot of observations regarding the HCV serological status were missing. 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) [23]. Despite their potential cytotoxic properties, nucleoside analogues were not associated with a poorer immunological outcome. Patients who received more potent antiretroviral combinations composed of at least one product of each class experienced a better CD4+ lymphocyte response. Those antiretroviral combinations might enhance the suppression of HIV replication and allow a better immune reconstitution [25].

In two studies, half of the patients with a poor immunological response at 6 months presented a significant increase in CD4+ lymphocyte count at 12 months [19] and at 30 months [26], 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 [19] compared to full responders, even when the virus was fully suppressed.

In conclusion, a poor immune reconstitution despite a good virological control seems frequent during the first year after initiation of HAART and patients 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. 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 adjuvant therapy currently under investigation such as interleukin-2 [27] and therapeutic vaccination [28]. Older patients should also maybe start HAART earlier. Secondly, a residual viral replication prevents immunological reconstitution. In this case monitoring tools finer than pVL, like proviral DNA measurement [29], should be developed for routine follow-up. The patients should in this case be considered as treatment failure and be switched to a more potent antiretroviral treatment [25].

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