



HAL
open science

Decrease in CD4 T-Cell Count and Risk of Severe Morbid Conditions in People With Human Immunodeficiency Virus Infection With Controlled Viral Load After Initiating Combination Antiretroviral Therapy Between 2006 and 2018

Maria Choufany, Laurence Weiss, Alain Makinson, H el ene Roul, Jean-Michel Livrozet, Val erie Pourcher, Giovanna Melica, Christophe Rioux, Jean-Paul Viard, Esaie Marshall, et al.

► To cite this version:

Maria Choufany, Laurence Weiss, Alain Makinson, H el ene Roul, Jean-Michel Livrozet, et al.. Decrease in CD4 T-Cell Count and Risk of Severe Morbid Conditions in People With Human Immunodeficiency Virus Infection With Controlled Viral Load After Initiating Combination Antiretroviral Therapy Between 2006 and 2018. *Clinical Infectious Diseases*, 2023, 76 (8), pp.1364-1371. 10.1093/cid/ciac939 . hal-04420551

HAL Id: hal-04420551

<https://hal.sorbonne-universite.fr/hal-04420551v1>

Submitted on 26 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche fran ais ou  trangers, des laboratoires publics ou priv es.

Decrease in CD4 count and risk of severe morbidity in PWH with controlled viral load after initiating cART between 2006 and 2018

Maria Choufany¹, Laurence Weiss², Alain Makinson³, H  l  ne Roul¹, Jean-Michel Livroz  t⁴, Val  rie Pourcher^{1,5}, Giovanna Melica⁶, Christophe Rioux C⁷, Jean-Paul Viard², Esaie Marshall¹, Sophie Grabar^{1,8}, Dominique Costagliola¹.

¹ Sorbonne Universit  , INSERM, Institut Pierre Louis d'  pid  miologie et de Sant   Publique, F75013, Paris, France

² Clinical Immunology Department, AP-HP, H  tel Dieu Hospital, and Inserm U1016 - CNRS UMR8104, Institut Cochin, Universit   Paris Cit  , Paris France

³ University of Montpellier, Inserm U1175, Montpellier, France

⁴ Department of Infectious and Tropical Diseases, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France

⁵ Department of Infectious and Tropical Diseases, Piti  -Salp  tr  ri  re Hospital, AP-HP, Paris, France

⁶ Infectious Diseases Department, Henri-Mondor/Albert-Chenevier Hospital Group, Cr  teil, France

⁷ Infectious and Tropical Diseases Department, Bichat-Claude Bernard University Hospital, AP-HP; Paris, France

⁸ Public Health Department, Saint-Antoine Hospital, AP-HP, F75012 Paris, France

Keywords: HIV, PWH, CD4 decline, severe morbidity, Cohort study

Running title (37 characters): CD4 decline in viremia controlled PWH

Key points (40 words): In viremia controlled PWH, CD4 decline was a rare event, with global lymphopenia. Older age and lower viral load at treatment initiation were associated with CD4 decline. It was associated with the risk of severe morbidity during the first six months.

Part of this work was presented at the Conference on Retroviruses and Opportunistic Infections. Virtual. February 12-16, 2022, Abstract 770.

Corresponding author: Dominique Costagliola, Institut Pierre Louis d'  pid  miologie et de Sant   Publique, Sorbonne Universit  , INSERM 56 Bd V Auriol, CS81393, 75646 PARIS Cedex 13, France Tel: 33(0)615190379 e-mail: dominique.costagliola@iplesp.upmc.fr

Alternate orresponding author: Sophie Grabar, Institut Pierre Louis d'  pid  miologie et de Sant   Publique, Sorbonne Universit  , INSERM 56 Bd V Auriol, CS81393, 75646 PARIS Cedex 13, France Tel: 33(0)607864528 e-mail: sophie.grabar@iplesp.upmc.fr

Abstract (250 words)

Background: A previous study showed CD4 decline in people with HIV infection (PWH) with viral suppression to be associated with an increased risk of severe morbidity. We aimed to assess the risk of CD4 decline, determine associated factors, and evaluate its association with the risk of severe morbidity (cardiovascular disease, cancer, death).

Methods: From the ANRS-CO4 FHDH cohort, we selected PWH older than 18 years and followed for at least two years after viral suppression following cART initiation between 2006 and 2018. CD4 decline was defined as two consecutive relative differences $\geq 15\%$. Among participants with CD4 decline, we modeled CD4, CD8, and total lymphocyte counts before and after CD4 decline using spline regression. The remaining objectives were assessed using Poisson regression, with the association between CD4 decline and the risk of severe morbidity evaluated during or after six months of decline.

Results: Among 15,714 participants (75,417 person-years (PY)), 181 presented CD4 decline (IR: 2.4/1000 PY (95%CI: 2.1-2.8)). CD8 and total lymphocyte counts also showed a similar decline. Older current age and lower viral load at treatment initiation were associated with the risk of CD4 decline. The risk of severe morbidity was 11-fold higher during the first six months for participants who presented CD4 decline versus those who did not (IR ratio: 10.8(5.1-22.8)), with no significant difference after six months.

Conclusion: In PWH with viral suppression, CD4 decline was rare and related to global lymphopenia. CD4 decline was associated with a higher risk of severe morbidity during the first six months.

Introduction

CD4 levels have been shown to be one of the strongest prognostic factors of AIDS-defining diseases, and are used to monitor immune response among people with HIV infection (PWH) after combined antiretroviral treatment (cART) initiation [1,2]. PWH have an increased risk of developing cancer [3–7], cardiovascular diseases [8–10], and death [11,12]. With the advent of new antiretroviral drugs, better tolerated and more efficient, CD4 counts increase [13,14] and the risk of most severe age-related morbidities decreases for PWH with a controlled viral load [15]. However, some PWH who remain virologically controlled shows a decline in CD4 counts. Helleberg et al. studied this phenomenon in the Danish HIV Cohort Study between 1995 and 2010, with the hypothesis that CD4 decline might be a marker of cancer or cardiovascular disease [16]. They found an association between CD4 decline and an increased risk of severe morbidities. Although their study suggests that a decline in CD4 counts could be associated with lymphopenia, they were unable to model CD8 or total lymphocyte counts over time. In addition, this CD4 decline may be less frequent in recent years, with an increasing number of participants who restore their CD4 counts after the initiation of treatments, and an increasing number of PWH treated at higher CD4 counts.

Using data from a large hospital cohort of PWH living in France, ANRS-CO4-FHDH, we aimed to estimate the risk of developing CD4 decline in a recent period and determine its associated factors, to model CD4, CD8, and total lymphocyte counts before and after the decline, and to assess the association between the risk of developing severe morbidity and CD4 decline.

Methods

Data source

We selected the population from the ANRS-CO4 French-Hospital-Database-on-HIV (FHDH). This is an ongoing nationwide hospital-based open cohort that includes data on PWH followed in hospitals. In 2020, 184 hospitals were participating in data collection in all French regions. Participants are eligible if they are ≥ 18 years of age, have documented human immunodeficiency virus (HIV)-1 or HIV-2 infection, and have provided written informed consent to participate [17].

The cohort was initially approved by the French data protection agency (CNIL) on November 27, 1991 (Official Journal, January 17, 1992). The research authorization was updated to comply with new regulations, including General Data Protection Regulations. The ANRS-CO4-FHDH cohort was approved by the CERES (Comité d'Expertise pour les Recherches, les Études et les Évaluations dans le domaine de la Santé) on July 20, 2018, and as a hospital data warehouse by the French data protection authority (Commission Nationale de l'Informatique et des Libertés) on February 19, 2021. Authorization to conduct research projects on the data warehouse of the cohort was received from CNIL on March 30, 2021.

Study population

Participants with HIV-1 who initiated antiretroviral treatment between 2006 and 2018 with at least two years of follow-up were selected. Antiretroviral treatment was defined as boosted protease inhibitor (PI) monotherapy, dual therapy of either two nucleoside reverse transcriptase inhibitors (NRTIs), or two antiretroviral drugs including at least one boosted protease inhibitor or one integrase inhibitor (INSTI), triple therapy combining two NRTIs with one boosted PI, one non-nucleoside reverse transcriptase inhibitor (NNRTI), or one INSTI. The NRTIs were either tenofovir, tenofovir alafenamide, or abacavir and either emtricitabine or lamivudine. The boosted PIs were darunavir/ritonavir or atazanavir/ritonavir. The NNRTI were nevirapine, efavirenz, etravirine, rilpivirine, or doravirine, and the INSTIs were either raltegravir, dolutegravir, elvitegravir or bictegravir.

Additional participant inclusion criteria were two HIV viral loads (VL) < 50 cp/mL after treatment initiation and a period of viral suppression ≥ 9 months, during which a minimum of

five measurements of CD4 counts were recorded with an interval of at least two months between consecutive measurements. The fifth measurement was considered as the index date to avoid immortal time bias. If during the 9 months period, there was one viral load $>50\text{cp/mL}$, the participant was excluded. We excluded participants who had cancer or cardio-vascular diagnoses before the index date, and those who had an HCV infection or were treated with Interferon or Peg-Interferon before the CD4 decline because these treatments can cause a drop in CD4 counts.

Outcomes

CD4 decline was defined according to the method described in [16]. We used the moving average (MA) of the mean over three consecutive CD4 counts to reduce the effect of random outliers. CD4 decline was defined as two consecutive relative differences (RD) $\geq 15\%$ as detailed in Supplementary Materials A. When a decline occurred, the date of the beginning of the CD4 decline was defined as the first CD4 count of the first MA and the date of CD4 decline was defined as the third CD4 count in the second consecutive MA. Severe morbidities of interest were cardiovascular diseases and cancer, coded according to the *International Classification of Diseases, 10th Revision (ICD-10)*: I20-I25 (ischemic heart disease) or I60-I69 (cerebrovascular disease) for cardiovascular diseases and C00-C97 for cancer.

Statistical analysis

The time was computed from the index date until the occurrence of an outcome (CD4 decline, cardiovascular event, cancer, or death), the date of the last follow-up, one year after the last viral load (VL) measurement or CD4 count, or virological failure, whatever occurred first. Virological failure was defined as two consecutive VLs $\geq 50\text{cp/mL}$ or one VL $\geq 50\text{cp/mL}$ followed by treatment modification. The date of virological failure was when the second VL was $\geq 50\text{cp/mL}$ or a switch of treatment occurred.

We estimated the risk of CD4 decline and its associated factors by computing the incidence rate (IR) and incidence-rate-ratio (IRR) of CD4 decline using univariable and multivariable Poisson regression. To study the evolution of CD4, CD8, and total lymphocyte counts before and after the beginning of the CD4 decline, we analyzed the counts as a time-continuous

variable using restricted cubic splines, with knots at 0.5, 1, 2, 3, 4, and 5 years before and after the beginning of CD4 decline [18].

We evaluated the relative risk of a cardiovascular event, cancer, death, or either of these events by estimating the IR and IRR by univariable and multivariable Poisson regression for participants with no CD4 decline, in the first six months after the decline, and after the first six months of the decline. The multivariate Poisson regression models were adjusted for the following factors: (1) age in years, (2) a composite variable combining sex, HIV exposure risk, and geographical origin, consisting of five categories: men who have sex with men, regardless of geographical origin (MSM), other men from Sub-Saharan Africa, other men, women from Sub-Saharan Africa, and other women, (3) CD4 count, (4) viral load, and (5) CD4/CD8 ratio at treatment initiation, (6) time to viral suppression in months, and (7) AIDS at the index date. In the model analyzing factors associated with the risk of CD4 decline, age was modelled as current age, while in the models analyzing factors associated with the risk of severe morbidities, age was modelled as age at treatment initiation. Analyses were performed using SAS version 9.4.

Results

Population

The study flowchart is presented in Supplementary Figure 1. In total, 15,714 participants were enrolled with a follow-up of 75,417 person-years (PY), corresponding to a median follow-up time of 4.3 (interquartile range(IQR)=2.3-6.8) years. The demographic and clinical characteristics are summarized in Table 1. Men accounted for most of the participants (71.5%), of whom 66.3% were MSM. At treatment initiation, the median age was 38 years (IQR=31-47), the median CD4 count was 345/mm³ (IQR=229-480), and the median viral load was 43,183 copies/mL (IQR=11,980-125,893).

CD4 decline, concomitant evolution of CD8 and total lymphocyte counts

In total, 181 participants presented a CD4 decline, with a median follow-up time of 5.4 (IQR=3.0-8.9) years and an incidence rate (IR) of 2.4/1000 PY (95% confidence interval (CI): 2.1-2.8). Aside from the CD4 decline, these participants also experienced a decline in CD8 and total lymphocyte counts (Table 3). The median interval between the date of the beginning of the CD4 decline and the date of the CD4 decline was 14.8 months. The CD4, CD8, and total lymphocyte counts showed a similar evolution before and after the date of the first CD4 decline: an increase in the counts before the decline, followed by an increase (Figure 1).

In multivariable analysis (Figure 2), factors associated with CD4 decline were higher current age and a lower viral load at treatment initiation. The IRR was estimated as 4,0 (95%CI:1.6-9.8) for participants ≥ 50 years of age versus participants < 30 years of age and as 0.5 (95%CI:0.3-0.8) for participants with a viral load $\geq 100,000$ copies/mL versus participants with viral load $< 5,000$ copies/mL.

Morbidity and mortality

Among the 181 participants with a CD4 decline, two were diagnosed with a cardiovascular event (myocardial infarction, cerebral atherosclerosis) and 10 with cancer (4 Hodgkin's disease, 1 non-Hodgkin's lymphoma, and 1 prostate, 1 lung, 1 pancreatic, 1 bone marrow,

and 1 anal cancer) and three died (1 from infective endocarditis, 1 from a lung cancer diagnosed before the CD4 decline, and 1 from prostate cancer) (Table 2). The adjusted IR for a cardiovascular event was 1.4/1000 PY for those who did not have a decline and 5.7/1000 PY in the first six months and 0.8/1000 PY beyond the first six months for those who had a CD4 decline, representing higher risk of developing a cardiovascular event in the first six months after the CD4 decline than for those who did not have a CD4 decline, with a non-significant adjusted rate-ratio of 4.1 (95%CI:0.6-29.2). The adjusted IR for cancer was 2.1/1000 PY for those who did not have a CD4 decline and 32.5/1000 PY in the first six months and 4.5/1000 PY beyond the first six months for those who had a CD4 decline. Thus, participants who had a CD4 decline had an increased risk of developing cancer in the first six months after the CD4 decline than those who did not have a CD4 decline (IRR:15.3 (95%CI:6.3-36.9)). The adjusted IR of death was 0.9/1000 PY for those who did not have a decline and 16.9/1000 PY in the first six months and 1.1/1000 PY beyond the first six months for those who had a CD4 decline. Mortality rates were higher during the first six months after the CD4 decline than for those who did not have a CD4 decline (IRR:19.2 (95%CI:4.7-78.6)). For cardiovascular events, cancer, and death combined, the risk increased 11-fold in the first six months after CD4 decline relative to the risk for those who did not have a CD4 decline (IRR:10.8 (95%CI:5.1-22.8)).

Sensitivity analysis

We performed a sensitivity analysis considering the occurrence of CD4 decline with two consecutive relative differences $\geq 10\%$ instead of 15%. In this analysis, 673 participants had a CD4 decline (IR:9.2/1000 PY (95%CI:8.6-9.8)), of whom 7 were diagnosed with cardiovascular events, 18 with cancer, and 10 died after the CD4 decline. In the first six months after CD4 decline, the association between CD4 decline and the risk of severe morbidities or death was IRR:1.3 (95%CI:0.2-9.6) for cardiovascular events, IRR:6.7 (95%CI:3.1-14.1) for cancer, and IRR:9.2 (95%CI:2.9-29) for death. Results of the sensitivity analyses are presented in Supplementary Table 1.

Discussion

Using data from a large hospital cohort, the ANRS-CO4-FHDH, we show that a CD4 decline was a rare event related to global lymphopenia in PWH with viral suppression after initiating cART between 2006 and 2018 (IR:2.4/1000 PY (95%CI:2.1-2.8)). The risk of CD4 decline was associated with higher current age and lower viral load at cART initiation. The risk of severe morbidities was 11-fold higher during the first six months for participants who had a CD4 decline versus those with no decline but not significantly different after the first six months.

The strength of our study was its large size, with 15,714 participants followed for a median of four years, and the availability of the total lymphocyte, CD4, and CD8 counts in the ANRS CO4 FHDH database, allowing modeling of the trajectories of CD4, CD8, and total lymphocyte counts before and after CD4 decline. On the other hand, there are no national cancer or cardiovascular registries in France, and the ANRS CO4 FHDH is not linked with the death registry. This may have led to underreporting of morbidity and mortality, given that data are collected from infectious and internal medicine wards. Because only a relatively small number (n=181) of participants experienced a CD4 decline, only small numbers of severe morbidities or death after CD4 decline could be analyzed, resulting in large confidence intervals when estimating the risk of these events and the inability to study each cancer separately. For this study, the definition of CD4 decline was based on the work from the Danish cohort [16]. When a less strict definition of decline, one that considered relative differences $\geq 10\%$, was used, the occurrence of CD4 decline remained rare, and the association with the risk of severe morbidities or mortality remained significant.

Compared to the results obtained in the Danish cohort, CD4 decline was rare, with a lower incidence observed in the French cohort (IR:2.4/1000 PY (95%CI:2.1-2.8) vs. 4.2/1000 PY (95%CI:3.2-5.4) for the Danish cohort). This may be due to the fact that we considered a more recent study period (2006-2018 vs. 1995-2010 in the Danish cohort). In a preliminary analysis conducted within the ANRS-CO4-FHDH cohort over the 2006-2014 period, the incidence of CD4 decline (IR:3.7/1000 PY (95%CI:2.7-4.7)) was closer to that observed in the Danish cohort and higher than that in 2006-2018. The smaller incidence of CD4 decline in our study could be explained by the fact that participants were treated for a longer period of

time, likely initiated treatment more rapidly after diagnosis with more effective combinations leading more rapidly to suppression, as highlighted by a median time from HIV diagnosis to viral suppression of 29 months in the Danish study and only 12 months in the current study.

Furthermore, we observed that CD4 decline occurred simultaneously with a decline in CD8 and total lymphocyte counts. CD4, CD8, and total lymphocytes showed similar trajectories before and after CD4 decline and thus reflect global lymphopenia. This was only suggested in the study of Helleberg et al. because they lacked longitudinal data for CD8, and total lymphocyte counts [16]. Together with innate immunity, T lymphocytes are an important component of cancer immunosurveillance. In the general population, lymphopenia has been associated with increased cancer incidence [19]. However, lymphoma or cancer can also result in low blood lymphocyte counts. In addition, lymphopenia has been shown to be a prognostic factor for overall and progression-free survival for several solid cancers [20]. Lymphopenia has also been associated with an increased risk of all-cause and cause-specific mortality such as non-hematologic cancers, hematologic cancers, cardiovascular diseases, respiratory diseases, and infectious diseases. [21]

We showed that 50 years of age or older individuals have a higher risk of CD4 decline. This is not surprising, as the efficiency of the immune system declines with age [22]. Altered lymphopoiesis, which may result partly from systemic immune activation, and which can only be partially reversed with prolonged cART, is more prevalent with age [23]. In people with high viral loads ($\geq 100,000$ copies/mL) at treatment initiation, the risk of CD4 decline was lower. It is possible that some of their CD4 cells are trapped in lymphoid organs. Once cART is initiated, a rapid increase in circulating CD4 cells is observed due to redistribution from the lymphoid organs to the circulation. However, it is unclear how this could be related to a CD4 decline occurring many months after treatment initiation.

The IRR of severe morbidities was significantly elevated in the first six months after CD4 decline and dropped after six months. This result is similar to that observed in the Danish cohort study (Supplementary Table 2). Modeling CD4 before and after the decline showed an increase in CD4 counts after the decline. This could explain the rarer events six months later. Of note, the number of events in the first six months or after six months of a CD4

decline was small in both studies, leading to unstable estimates of the IRR, with large confidence intervals. This precludes any precise discussion on the size of the effect. Nevertheless, the IRR of cancer beyond the first six months after CD4 decline was estimated to be 2 in both studies and close to significance. Although we cannot draw any firm conclusions, this may warrant additional studies.

In our study, 4 of 10 participants who developed cancer after CD4 decline had Hodgkin lymphoma. Previous studies [24–26] have shown that the risk of Hodgkin's disease increases as the most recent CD4 count decreases and that the incidence of Hodgkin lymphoma is not reduced after cART initiation. Therefore, these results support the hypothesis already made by Helleberg et al. that CD4 decline may be a consequence of cancer, not the cause. For the other events (non-Hodgkin's lymphoma, prostate, lung, pancreas, bone marrow, and anal cancers, and cardiovascular events), it is still uncertain as to whether CD4 decline leads to a risk of severe morbidities or mortality or whether the latter leads to CD4 decline.

In line with our results, occurrence of a drop in CD4-T-cell count, associated with a drop in CD8-T-cell count or in lymphocyte count, in the absence of viral rebound, should serve as a trigger in PWH on CART and leads to additional investigations given the increased risk of severe morbidity. Additional investigations (e.g., computed tomography) should consider the context, such as smoking and cancer family history.

In conclusion, we found that CD4 decline occurred rarely among virally suppressed PWH followed between 2006 and 2020 in France and that it is related to global lymphopenia. We show an association between CD4 decline and higher current age and lower viral load at treatment initiation. CD4 decline was associated with an increased risk of cardiovascular disease, cancer, and death, notably during the first six months after the decline. However, it is difficult to conclude whether the observed severe morbidities and mortality are a cause or a consequence of CD4 decline.

Funding

The ANRS CO4 FHDH is supported by the ANRS-MIE (Agence Nationale de Recherche sur le Sida et les hépatites virales-Maladies Infectieuses Emergentes), INSERM (Institut National de la Santé et de la Recherche Médicale) and the French Ministry of Health.

Acknowledgments

The authors are grateful to all ANRS CO4-FHDH participants and research assistants, without whom this work would not have been possible.

Members of ANRS CO4-FHDH are listed at <https://anrs-co4.fhdh.fr/>

For the purpose of Open Access, a CC-BY 4.0 public copyright license has been applied by the authors to the present document and will be applied to all subsequent versions up to the Author Accepted Manuscript arising from this submission (<https://creativecommons.org/licenses/by/4.0/>).

Conflict of Interest

LW reports grants from Gilead and Pfizer, and personal fees from MSD, outside the submitted work. AM reports grant from MSD, and lecture fees from ViiV, Janssen, MSD, and Gilead, and support for attending meetings from Gilead, MSD, Janssen, ViiV. VP reports consulting and lecture fees from Gilead, ViiV, MSD, outside the submitted work. GM reports lecture fees from Pfizer, Astellas, Gilead, outside the submitted work, and support for attending meetings from Astellas and Gilead. DC reports an HIV grant from Janssen (2019-2020), and personal fees from Gilead (2020) and Pfizer (2022) for lectures outside the submitted work. MC, HR, JML, CR, JPV, EM, and SG report no conflict of interest.

Authors Contribution

MC, SG, DC, LW, and HR designed the study. LW, AM, JML, VP, GM, CR, and JPV included PWH. MC analyzed the data. MC, SG, DC, EM, and LW drafted the manuscript. MC, SG, DC, and EM had full access to the data, verified the data, and had final responsibility for the decision to submit the study for publication. All authors were involved in the interpretation of the data and critical revision of the manuscript and approved the final version.

References

- [1] Hoffman J, van Griensven J, Colebunders R, McKellar M. Role of the CD4 count in HIV management. *HIV Therapy*, 2010; 4(1) :27-39.
- [2] Ford N, Meintjes G, Pozniak A, Bygrave H, Hill A, Peter T, et al. The future role of CD4 cell count for monitoring antiretroviral therapy. *Lancet Infect Dis*, 2015; 15(2):241-7.
- [3] Hleyhel M, Belot A, Bouvier AM, Tattevin P, Pacanowski J, Genet P, et al. Risk of AIDS-defining cancers among HIV-1-infected patients in France between 1992 and 2009: Results from the FHDH-ANRS CO4 cohort. *Clin Infect Dis*, 2013; 57(11):1638-47. *Clin Infect Dis* 2013; 57(11):1638-47.
- [4] Hleyhel M. Risk of non-AIDS-defining cancers among HIV-1-infected individuals in France between 1997 and 2009: Results from a French cohort. *AIDS*, 2014; 28(14):2109-18.
- [5] Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet*, 2007; 370(9581):59-67.
- [6] Hernández-Ramírez RU, Shiels MS, Dubrow R, Engels EA. Cancer risk in HIV-infected people in the USA from 1996 to 2012: a population-based, registry-linkage study. *Lancet HIV*, 2017; 4(11):e495-e504.
- [7] Hessol NA, Whittemore H, Vittinghoff E, Hsu LC, Ma D, Scheer S, et al. Incidence of first and second primary cancers diagnosed among people with HIV, 1985–2013: a population-based, registry linkage study. *Lancet HIV*, 2018; 5(11):e647-e655.
- [8] Baldé A, Lang S, Wagner A, Ferrières J, Montaye M, Tattevin P, et al. Trends in the risk of myocardial infarction among HIV-1-infected individuals relative to the general population in France: Impact of gender and immune status. *PLoS One*, 2019; 14(1):e0210253.
- [9] Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab*, 2007; 92(7):2506-12.
- [10] Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, et al. Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS*, 2010; 24(8):1228-30.
- [11] The Antiretroviral Therapy Cohort Collaboration. Mortality of HIV-infected patients starting potent antiretroviral therapy: Comparison with the general population in nine industrialized countries. *Int J Epidemiol*, 2009;38(6):1624-33.
- [12] Croxford S, Kitching A, Desai S, Kall M, Edelstein M, Skingsley A, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. *The Lancet Public Health*, 2017; 2(1):e35-e46.
- [13] Roul H, Mary-Krause M, Ghosn J, Delaugerre C, Pialoux G, Cuzin L, et al. CD4R cell count recovery after combined antiretroviral therapy in the modern combined antiretroviral therapy era. *AIDS*, 2018; 32(17):2605-2614.
- [14] Kaufmann GR, Perrin L, Pantaleo G, Opravil M, Furrer H, Telenti A, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: The Swiss HIV cohort study. *Arch Intern Med*, 2003; 163(18):2187-95.

- [15] Rasmussen LD, May MT, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, et al. Time trends for risk of severe age-related diseases in individuals with and without HIV infection in Denmark: A nationwide population-based cohort study. *Lancet HIV*, 2015 Jul;2(7):e288-98.
- [16] Helleberg M, Kronborg G, Larsen CS, Pedersen G, Pedersen C, Obel N, et al. CD4 decline is associated with increased risk of cardiovascular disease, cancer, and death in virally suppressed patients with HIV. *Clin Infect Dis*, 2013 Jul; 57(2):314-21.
- [17] Mary-Krause M, Grabar S, Lièvre L, Abgrall S, Billaud E, Boué F, et al. Cohort profile: French hospital database on HIV (FHDH-ANRS CO4). *Int J Epidemiol*, 2014; 43(5):1425-36.
- [18] Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*, 1989; 8(5):551-61.
- [19] Ménétrier-Caux C, Ray-Coquard I, Blay JY, Caux C. Lymphopenia in Cancer Patients and its Effects on Response to Immunotherapy: An opportunity for combination with Cytokines? *J Immunother Cancer*, 2019; 7(1):85.
- [20] Ray-Coquard I, Cropet C, van Glabbeke M, Sebban C, Le Cesne A, Judson I, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res*, 2009; 69(13):5383-91.
- [21] Warny M, Helby J, Nordestgaard BG, Birgens H, Bojesen SE. Incidental lymphopenia and mortality: a prospective cohort study. *CMAJ*, 2020;192(2):E25-E33.
- [22] Grabar S, Weiss L, Costagliola D. HIV infection in older patients in the HAART era. *J Antimicrob Chemother*, 2006; 57(1):4-7.
- [23] Sauce D, Larsen M, Fastenackels S, Pauchard M, Ait-Mohand H, Schneider L, et al. HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood*, 2011; 117(19):5142-51.
- [24] Bohlius J, Schmidlin K, Boué F, Fätkenheuer G, May M, Caro-Murillo AM, et al. HIV-1-related Hodgkin lymphoma in the era of combination antiretroviral therapy: Incidence and evolution of CD4+ T-cell lymphocytes. *Blood*, 2011; 117(23):6100-8.
- [25] Gupta RK, Marks M, Edwards SG, Smith K, Fletcher K, Lee SM, et al. A declining CD4 count and diagnosis of HIV-associated Hodgkin lymphoma: Do prior clinical symptoms and laboratory abnormalities aid diagnosis? *PLoS One*, 2014; 9(2):e87442.
- [26] Shepherd L, Ryom L, Law M, Hatleberg CI, de Wit S, D'Arminio Monforte A, et al. Differences in virological and immunological risk factors for non-Hodgkin and Hodgkin lymphoma. *J Natl Cancer Inst*, 2018; 110(6):598-607.

Table 1. Participant characteristics according to CD4 decline

| | Total | | CD4 Decline | | No CD4 Decline | |
|---|--------------------------------|------|-------------------------------|------|--------------------------------|------|
| | N = 15 714 | % | N = 181 | % | N = 15 533 | % |
| Demographic characteristics | | | | | | |
| Age at cART initiation (years) | 38.2 [30.9-46.6] | | 41.7 [31.4-50.6] | | 38.2 [30.9-46.6] | |
| < 30 | 3484 | 22.2 | 37 | 20.4 | 3447 | 22.2 |
| [30-50[| 9491 | 60.4 | 96 | 53.1 | 9395 | 60.5 |
| ≥ 50 | 2739 | 17.4 | 48 | 26.5 | 2691 | 17.3 |
| Transmission x Origin x Sex | | | | | | |
| Men who have sex with men | 7450 | 47.4 | 79 | 43.6 | 7371 | 47.5 |
| Other men from SSA | 1330 | 8.5 | 17 | 9.4 | 1313 | 8.5 |
| Other women | 2021 | 12.9 | 19 | 10.5 | 2423 | 15.6 |
| Women from SSA | 2457 | 15.6 | 34 | 18.8 | 2002 | 12.8 |
| Other men | 2456 | 15.6 | 32 | 17.7 | 2424 | 15.6 |
| Clinical characteristics | | | | | | |
| CD4 at cART initiation (/mm3) | 345 [229-480] | | 324 [207-432] | | 345 [229-481] | |
| < 100 | 1604 | 10.2 | 19 | 10.5 | 1585 | 10.2 |
| [100-200[| 1629 | 10.4 | 26 | 14.4 | 1603 | 10.3 |
| [200-350[| 4797 | 30.5 | 58 | 32.0 | 4739 | 30.5 |
| [350-500[| 4130 | 26.3 | 50 | 27.6 | 4080 | 26.3 |
| ≥ 500 | 3554 | 22.6 | 28 | 15.5 | 3526 | 22.7 |
| Viral Load at cART initiation (copies/mL) | 43 183 [11 980-125 893] | | 42 015 [10 700-86 184] | | 43 234 [12 000-126 101] | |
| < 5 000 | 2277 | 14.5 | 35 | 19.4 | 2242 | 14.4 |
|]5 000 - 30 000] | 4335 | 27.6 | 46 | 25.4 | 4289 | 27.6 |
|]30 000 - 100 000] | 4361 | 27.7 | 61 | 33.7 | 4300 | 27.7 |
| ≥ 100 000 | 4741 | 30.2 | 39 | 21.5 | 4702 | 30.3 |
| CD4/CD8 at cART initiation | 0.4 [0.2-0.6] | | 0.3 [0.2-0.5] | | 0.4 [0.2-0.6] | |
| [0-0.5[| 1568 | 10.0 | 122 | 67.4 | 9149 | 58.9 |
| [0.5-0.8[| 9271 | 59.0 | 31 | 17.1 | 3145 | 20.2 |
| [0.8-1[| 3176 | 20.2 | 3 | 1.7 | 842 | 5.4 |
| ≥ 1 | 845 | 5.4 | 4 | 2.2 | 850 | 5.5 |
| Missing | 854 | 5.4 | 21 | 11.6 | 1547 | 10.0 |
| Time to viral load control | 3.5 [2.1-5.4] | | 3.3 [1.9-5.0] | | 3.5 [2.1-5.4] | |
| [0-2[months | 3791 | 24.1 | 48 | 26.5 | 3743 | 24.1 |
| [2-4.5[| 6413 | 40.8 | 77 | 42.5 | 6336 | 40.8 |
| [4.5-7.5[| 4492 | 28.6 | 46 | 25.5 | 4446 | 28.6 |
| ≥ 7.5 | 1018 | 6.5 | 10 | 5.5 | 1008 | 6.5 |
| AIDS at index date | | | | | | |
| No | 14 563 | 92.7 | 160 | 88.4 | 14 403 | 92.7 |
| Yes | 1151 | 7.3 | 21 | 11.6 | 1130 | 7.3 |

For quantitative data, the median and interquartile range (median [IQR]) were calculated

Table 2. Risk of severe morbidity and death before and after CD4 Decline

| CD4 Decline | No. events | PY | IR/1000 PY (95%CI) | | IRR (95%CI) | |
|--|------------|--------|---------------------|---------------------|-----------------|-----------------|
| | | | Non-adjusted | Adjusted | Non-adjusted | Adjusted |
| Cardiovascular event | | | | | | |
| No decline | 173 | 78 559 | 2.20 (1.90-2.56) | 1.41 (1.00-1.97) | 1 | 1 |
| < 6 months after decline | 1 | 83 | 12.10 (1.71-85.91) | 5.73 (0.78-41.92) | 5.5 (0.8-39.2) | 4.1 (0.6-29.2) |
| ≥ 6 months after decline | 1 | 603 | 1.67 (0.23-11.78) | 0.81 (0.11-5.92) | 0.8 (0.1-5.4) | 0.6 (0.1-4.1) |
| Cancer | | | | | | |
| No decline | 291 | 78 355 | 3.71 (3.31-4.42) | 2.14 (1.65-2.77) | 1 | 1 |
| < 6 months after decline | 5 | 71 | 70.40 (29.30-169.1) | 32.48 (13.01-81.07) | 19.0 (7.8-45.9) | 15.3 (6.3-36.9) |
| ≥ 6 months after decline | 5 | 558 | 8.95 (3.72-21.51) | 4.5 (1.81-11.15) | 2.4 (1.0-5.8) | 2.1 (0.9-5.1) |
| Death | | | | | | |
| No decline | 92 | 77 811 | 1.18 (0.96-1.45) | 0.88 (0.58-1.33) | 1 | 1 |
| < 6 months after decline | 2 | 70 | 28.54 (7.14-114.1) | 16.90 (4.00-71.50) | 24.1 (5.9-98.0) | 19.2 (4.7-78.6) |
| ≥ 6 months after decline | 1 | 548 | 1.83 (0.26-12.96) | 1.09 (0.15-8.02) | 1.5 (0.2-11.1) | 1.2 (0.2-9.0) |
| Cardiovascular or cancer or death | | | | | | |
| No decline | 541 | 73 327 | 7.38 (6.78-8.02) | 4.78 (3.98-5.57) | 1 | 1 |
| < 6 months after decline | 7 | 69 | 101.1 (48.22-212.2) | 51.55 (24.03-110.6) | 13.7 (6.5-28.9) | 10.8 (5.1-22.8) |
| ≥ 6 months after decline | 7 | 515 | 13.60 (6.48-28.52) | 7.66 (3.59-16.36) | 1.8 (0.9-3.9) | 1.6 (0.8-3.4) |

Abbreviations: IR, incidence rate; IRR, incidence rate ratio; PY, person years; CI, confidence interval

ICD Codes for cardiovascular events: I20 to I25 (ischemic heart disease) and I60 to I69 (cerebrovascular disease) and cancer: C00 to C97

Note: One of the participants died of prostate cancer after the CD4 decline. This was counted as a cancer and a death.

Table 3. CD4, CD8, and total lymphocytes counts and CD4/CD8 ratio before and at the date of CD4 decline among the 122 participants with CD4 decline

| | | Missing Values | Median | Interquartile Range |
|----------------------------|---------------------|----------------|--------------|---------------------|
| CD4 / mm^3 | Before CD4 decline | 0 | 728 | 540-960 |
| | Date of CD4 decline | 0 | 328 | 171-506 |
| CD8 / mm^3 | Before CD4 decline | 13 | 788 | 610-1151 |
| | Date of CD4 decline | 15 | 389 | 275-691 |
| CD4/CD8 | Before CD4 decline | 13 | 0.81 | 0.60-1.16 |
| | Date of CD4 decline | 15 | 0.79 | 0.52-1.04 |
| Total lymphocytes / mm^3 | Before CD4 decline | 39 | 2 168 | 1660-2822 |
| | Date of CD4 decline | 34 | 1 041 | 725-1570 |

Figure 1: CD4, CD8, Lymphocytes counts (a) before and (b) after CD4 decline modelled with cubic splines

Figure 2. Factors associated with CD4 decline

Abbreviations: No. events, number of events; IRR, incidence rate ratio; PY, person years; CI, confidence interval; VL, viral load