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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.

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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 ≥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 CEREES (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)<50cp/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>50cp/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) ≥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≥50cp/mL or one VL≥50cp/mL followed by treatment modification. The date of virological failure was when the second VL was ≥50cp/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/mm^3 (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 ≥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 ≥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 ≥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 (≥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.

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

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.

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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