Fatal and nonfatal AIDS and non-AIDS events in HIV-1-positive individuals with high CD4 cell counts according to viral load strata

Joanne Reekie[a], Jose M. Gatell[b], Israel Yust[c], Elzbieta Bakowska[d], Aza Rakhmanova[e], Marcelo Losso[f], Maksym Krasnov[g], Patrick Francioli[h], Justyna D. Kowalska[i], Amanda Mocroft[a], for EuroSIDA in EuroCoord

Background: This study compared the incidence of fatal and nonfatal AIDS and non-AIDS events in HIV-positive individuals with a CD4 cell count more than 350 cells/μl among viral load strata: low (<500 copies/ml), intermediate (500–9999.9 copies/ml) and high (≥10000 copies/ml).

Methods: Individuals contributed person-years at risk if their most recent CD4 cell count was more than 350 cells/μl. Follow-up was censored if their CD4 cell count dropped below 350 cells/μl. Poisson regression analysis investigated the relationship between viraemia and the incidence of AIDS and non-AIDS events.

Results: Three hundred and fifty-four AIDS events occurred during 51 732 person-years of follow-up (PYFU), crude incidence rate of AIDS across the three strata was 0.53, 0.90 and 2.12 per 100 PYFU, respectively. After adjustment, a higher rate of AIDS was observed in individuals with moderate [incidence rate ratio (IRR) 1.44, 1.02–2.05, $P = 0.03$] and high viraemia had a higher rate (IRR 3.91, 2.89–5.89, $P < 0.0001$) compared with low viraemia. Five hundred and seventy-two non-AIDS events occurred during 43 784 PYFU, the crude incidence rates were 1.28, 1.52, and 1.38 per 100 PYFU, respectively. After adjustment, particularly for age, region of Europe and starting combination antiretroviral therapy, there was a 61% (IRR 1.61, 1.21–2.14, $P = 0.001$) and 66% (IRR 1.66, 1.17–2.32, $P = 0.004$) higher rate of non-AIDS in individuals with intermediate and high viraemia compared with low viraemia.

Conclusion: In individuals with a CD4 cell count more than 350 cells/μl, an increased incidence of AIDS and a slightly increased incidence of non-AIDS was found in those with uncontrolled viral replication. The association with AIDS was clear and consistent. However, the association with non-AIDS was only apparent after adjustment and no differences were observed between intermediate and high viraemia.

AIDS 2011, **25**:2259–2268

Keywords: AIDS, CD4 cell count, non-AIDS events, viral load

[a]University College London Medical School, London, UK, [b]Hospital Clinic, Barcelona, Spain, [c]Ichilov Hospital, Tel Aviv, Israel, [d]Centrum Diagnostyki i Terapii AIDS, Warsaw, Poland, [e]Medical Academy Botkin Hospital, St Petersburg, Russia, [f]Hospital JM Ramos Mejia, Buenos Aires, Argentina, [g]Kharkov State Medical University, Kharkov, Ukraine, [h]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, and [i]Copenhagen HIV Programme, University of Copenhagen, Copenhagen, Denmark.

Correspondence to Joanne Reekie, HIV Epidemiology and Biostatistics Group, Research Department of Infection and Population Health, University College London Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.

Tel: +44 20 7830 2239; fax: +44 20 7794 1224; e-mail: j.reekie@ucl.ac.uk

Received: 9 May 2011; revised: 31 August 2011; accepted: 7 September 2011.

DOI:10.1097/QAD.0b013e32834cdb4b

Introduction

There is evidence that clinical progression to AIDS is associated with a low CD4 cell count [1–3]. Additionally, some non-AIDS-defining illnesses have also been found at an increased rate in HIV-positive individuals who are immunocompromised [4,5]. However, the occurrence of both AIDS-defining and non-AIDS-defining illnesses in HIV-positive individuals who are not immunocompromised has not been fully investigated. A recent EuroSIDA study found that there was a continuum of decreasing risk of developing new AIDS events as the CD4 cell count increased which was not observed in non-AIDS events in which no decrease in risk was found in CD4 cell counts more than 350 cells/μl [6]. The Strategies for Management of Anti-Retroviral Therapy (SMART) study [7] compared a CD4 cell count-guided treatment strategy in which antiretroviral therapy (ART) was only started when the patients CD4 cell count fell below 250 cells/μl and was stopped if the CD4 cell count increased to more than 350 cells/μl, with continuous ART designed to achieve maximum and continuous suppression of HIV replication. A higher rate of opportunistic infections, cardiovascular, hepatic and renal disease and death was observed in the CD4 cell count-guided arm, raising questions about the contribution of uncontrolled viral replication on the risk of these clinical events. Deeks *et al.* [8] also reported that in HIV-positive individuals on long-term protease inhibitor-based combination ART (cART) who were experiencing virological failure (>500 copies/ml), their CD4 cell counts remained higher than their pretherapy levels and they had a lower CD4 cell decline than that in a historical control group of untreated patients. In addition, a recent study by Ferry *et al.* [5] reported that uncontrolled HIV replication may be an independent risk factor associated with the occurrence of non-AIDS events. It is clinically relevant and important to understand the risk of AIDS and non-AIDS events during these periods of virological replication. The aim of this study was, therefore, to compare the incidence of fatal and nonfatal AIDS and non-AIDS events occurring in HIV-positive individuals with high CD4 cell counts (>350 cells/μl) in different viral load strata.

Methods

Patients

The EuroSIDA study is a prospective, observational pan European study of more than 16 000 HIV-positive individuals across Europe, Israel and Argentina. Details of the study have been described previously [9]. In brief, HIV-positive individuals were enrolled in eight different cohorts from May 1994. To be eligible for inclusion into EuroSIDA, individuals must be aged 16 or older and have a prebooked outpatient appointment at the centre. On standardized recruitment and follow-up forms, data are collected on demographics, for example, age, sex, ethnic origin and clinical information, such as the date and type of development of any AIDS-defining illnesses, opportunistic infection or death and a Coding of Death in HIV (CoDe) case report form is additionally completed for each fatal case [10]. Additionally, from 1 January 2001 onwards, data have also been collected on any serious non-AIDS related clinical events, such as cardiovascular disease (CVD), liver-related and non-AIDS-defining malignancies (NADMs). Furthermore, details on all laboratory measurements since last follow-up are recorded, such as CD4 cell counts, viral load and hepatitis virology/serology. Therapeutic information is collected on the date of starting or stopping any antiretroviral drug, diabetes or lipid-lowering medication. Data are collected and updated at 6-month intervals. These updates include data on all visits and laboratory measurements in the previous 6 months. An extensive quality assurance program has been established that includes data checking at the coordinating office, as well as regular monitoring visits with source verification of all new major events. Ethical approval for each participating centre is sought according to local regulation.

Statistical analysis

For a given month, an individual contributed person-years at risk if their most recent CD4 cell count (measured within the previous 6 months and after 1 January 1997) was more than 350 cells/μl, in addition to a viral load measured within the 6 months before. Follow-up was censored if there was no CD4 cell count or viral load measured in the previous 6 months, or their CD4 cell count dropped below 350 cells/μl, and after their last recorded visit or death. Therefore, person-years contributed by an individual were not necessarily consecutive, depending on the availability of viral load measurements, CD4 cell counts and whether they were greater than 350 cells/μl. Person-years were split into three different viral load strata (<500, 500–9999.9 and ≥10000 copies/ml).

All fatal and nonfatal AIDS and non-AIDS events, occurring during the follow-up time, were recorded and the incidence rates calculated as the number of events per 100 person-years of follow-up (PYFU) calculated. Recurrences of the same diagnosis were excluded, for example, NADM followed by another NADM, only the first NADM would be classed as an event. Non-AIDS events were further split into individual components (e.g. NADM, CVD) in which there were a sufficient number of events ($n > 30$). As non-AIDS events were not collected until January 2001, analyses for this endpoint were left censored at 1 January 2001.

Poisson regression analysis was used to determine predictors of different clinical events, adjusted for repeated

events per individual. Factors investigated included demographics such as sex, ethic origin, HIV exposure group and region of Europe. In addition, age, year of follow-up, hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) antibody status, anaemia, diabetes, hypertension, smoking status, starting cART (yes/no), prior AIDS, CD4 cell count and viral load were included as time-updated covariates. Hypertension was defined as a DBP of at least 90 mmHg or a SBP of at least 140 mmHg or receiving antihypertensive medication. An individual was defined as having diabetes if they had a diagnosis of insulin-dependent diabetes or were receiving diabetic medication or insulin. Anaemia was defined as a haemoglobin level of 12 mg/dl or less or 14 mg/dl or less for females and males, respectively [11]. All deaths were classified as either AIDS-related or non-AIDS-related following the Euro-SIDA three-step hierarchical process for coding cause of death [12]. cART was defined as receiving at least three antiretrovirals after 1 January 1996. All analyses were performed using SAS 9.1 (SAS Institute, Cary, North Carolina, USA).

Results

Eleven thousand, four hundred and ninety-two HIV-positive individuals contributed 51 732 PYFU to the fatal and nonfatal AIDS events analysis (Table 1). The majority of follow-up (82%) were in individuals with a viral load less than 500 copies/ml, 11% of the follow-up was in individuals with a viral load between 500 and 9999.9 copies/ml and 7% of the follow-up was in individuals with a viral load of at least 10 000 copies/ml. Ninety, 61 and 36% of the follow-up in each stratum, respectively, was in individuals who were currently receiving cART. For the non-AIDS events analysis, 10 869 HIV-positive individuals contributed 43 784 PYFU, slightly less than for the AIDS events analysis, as follow-up was only included from 2001 onwards; however, from Table 1, the distribution of the follow-up was similar to that of the AIDS events.

Table 2 shows the most commonly occurring AIDS and non-AIDS events reported during the follow-up period. Three hundred and fifty-four AIDS events occurred during follow-up, the crude incidence rate was 0.68 per 100 PYFU [95% confidence interval (CI) 0.61–0.76], the most common reported AIDS event was oesophageal candidiasis. Five hundred and seventy-two non-AIDS events occurred under follow-up (incidence rate 1.31 per 100 PYFU, 95% CI 1.20–1.41), the most common of which were cardiovascular events and NADMs.

A higher crude rate of AIDS events was observed in individuals with a higher viral load; this same relationship was not observed for non-AIDS events. Individuals with a viral load of less than 500 copies/ml had an incidence rate of 0.53 AIDS events per 100 PYFU (95% CI 0.46–0.59), compared with 0.90 per 100 PYFU (95% CI 0.66–1.15) in individuals with a viral load between 500 and 9999.9 copies/ml and 2.12 per 100 PYFU (95% CI 1.66–2.58) in individuals with a viral load of at least 10 000 copies/ml. A similar incidence rate of non-AIDS events was observed in individuals with a viral load of less than 500 copies/ml (incidence rate 1.28 per 100 PYFU, 95% CI 1.17–1.39), between 500 and 9999.9 copies/ml (incidence rate 1.52, 95% CI 1.12–1.92) and at least 10 000 copies/ml (incidence rate 1.38 per 100 PYFU, 95% CI 0.95–1.81).

Figure 1 shows that after adjustment for the fixed variables of sex, HIV exposure group, region of Europe, time-updated variables, HBsAg and HCV antibody status, smoking status, hypertension, year of follow-up, CD4 cell count and starting cART, there remained a higher rate of AIDS events in individuals with a viral load between 500 and 9999.9 copies/ml compared with a viral load of less than 500 copies/ml [incidence rate ratio (IRR) 1.44, 95% CI 1.02–2.05, $P = 0.03$]. Individuals with a viral load of at least 10 000 copies/ml had almost a four times significantly higher rate of AIDS events than those with a viral load of less than 500 copies/ml (IRR 3.91, 95% CI 2.89–5.89, $P < 0.0001$). For non-AIDS events, after adjustment for fixed variables HIV exposure group, region of Europe, time updated variables peak viral load, CD4 cell count, age, HBsAg and HCV antibody status, diabetes, hypertension, smoking status, prior AIDS and started cART, there was a 61% (IRR 1.61, 95% CI 1.21–2.14, $P = 0.001$) and 66% (IRR 1.66, 95% CI 1.17–2.34, $P = 0.004$) higher incidence of non-AIDS events in individuals with a viral load between 500 and 9999.9 copies/ml and at least 10 000 copies/ml, respectively, compared with a viral load of less than 500 copies/ml. This increase was seen particularly after adjustment for age, region of Europe and whether or not the individual had started cART. Adjusting for current use of cART, rather than cART experience, reduced the association, but there was still a significantly higher incidence of non-AIDS events in individuals with a viral load between 500 and 9999.9 copies/ml (IRR 1.56, 95% CI 1.16–2.08, $P = 0.002$) and at least 10 000 copies/ml (IRR 1.58, 95% CI 1.10–2.30, $P = 0.01$) compared with a viral load of less than 500 copies/ml. The association with AIDS events and higher viral load was consistent after adjusting for current use of cART (data not shown).

The effect of viral load on the incidence of NADM, CVD, liver-related and pancreatitis events was investigated separately, as more than 30 events were observed for each of these specific non-AIDS events (Fig. 2). A marginally higher incidence of CVD was observed in individuals with a viral load between 500 and 9999.9 copies/ml (IRR 1.65, 95% CI 1.03–2.66, $P = 0.03$); however, this was not observed in those with

Table 1. Distribution of the person years of follow-up included in each viral load strata.

		Fatal and nonfatal AIDS events analysis		
		Viral load strata (copies/ml)		
		<500 cells/μl	500–9999.9 cells/μl	≥10000 cells/μl
Total PYFU (% of total)		42 267 (82)	5641 (11)	3824 (7)
Sex (PYFU, %)	Male	32 462 (77)	4027 (71)	2867 (75)
Ethnic origin (PYFU, %)	White	36 508 (86)	4974 (88)	3422 (89)
HIV exposure group (PYFU, %)	Homosexual	20 267 (48)	2429 (43)	1793 (47)
	IDU	7018 (17)	1173 (21)	739 (19)
	Heterosexual	11 936 (28)	1713 (30)	1087 (28)
Region of Europe (PYFU, %)	South	12 079 (29)	2287 (41)	1309 (34)
	West central	12 115 (29)	1502 (27)	952 (25)
	North	12 533 (30)	1067 (19)	893 (23)
	East central	3983 (9)	430 (8)	332 (9)
	East	926 (2)	251 (4)	276 (7)
	Argentina	629 (1)	103 (2)	61 (2)
On cART (PYFU, %)		38 201 (90)	3420 (61)	1359 (36)
CD4 cell count (PYFU, %)	<500 cells/μl	13 934 (33)	2736 (49)	2134 (56)
	500–749 cells/μl	17 476 (41)	2162 (38)	1298 (34)
	≥750 cells/μl	10 858 (26)	743 (13)	392 (10)
Age (PYFU, %)	<30 years	6567 (16)	1283 (23)	1003 (26)
	30–39 years	18 942 (45)	2696 (48)	1722 (45)
	≥40 years	16 759 (39)	1662 (29)	1099 (29)
		Fatal and nonfatal non-AIDS events analysis		
		Viral load strata (copies/ml)		
		<500 cells/μl	500–9999.9 cells/μl	≥10000 cells/μl
Total PYFU (% of total)		37263 (85)	3690 (8)	2830 (6)
Sex (PYFU, %)	Male	28 626 (77)	2605 (71)	2092 (74)
Ethnic origin (PYFU, %)	White	32 203 (86)	3280 (89)	2548 (90)
HIV exposure group (PYFU, %)	Homosexual	17 784 (48)	1553 (42)	1284 (45)
	IDU	6169 (17)	753 (20)	518 (18)
	Heterosexual	10 597 (28)	1184 (32)	873 (31)
Region of Europe (PYFU, %)	South	10 422 (28)	1447 (39)	927 (33)
	West central	10 448 (28)	889 (24)	689 (24)
	North	10 905 (29)	610 (17)	563 (20)
	East central	3926 (11)	387(10)	314 (11)
	East	932 (3)	253 (7)	277 (10)
	Argentina	629 (2)	104 (3)	61 (2)
On cART (PYFU, %)		34 168 (92)	2292 (62)	865 (31)
CD4 cell count (PYFU, %)	<500 cells/μl	11 702 (31)	1630 (44)	1468 (52)
	500–749 cells/μl	15 455 (41)	1475 (40)	1024 (36)
	≥750 cells/μl	10 106 (27)	585 (16)	339 (12)
Age (PYFU, %)	<30 years	5669 (15)	877 (24)	788 (28)
	30–39 years	16 469 (44)	1708 (46)	1217 (43)
	≥40 years	15 125 (41)	1105 (30)	826 (29)

For fatal and nonfatal AIDS events, patients were included from first CD4 cell count of more than 350 cells/μl measured after 1 January 1997, while enrolled in EuroSIDA. For fatal and nonfatal non-AIDS events, patients were included from first CD4 cell count of more than 350 cells/μl measured after 1 January 2001, while enrolled in EuroSIDA. cART, combination antiretroviral therapy; IDU, injection drug use; PYFU, person-years of follow-up.

Table 2. Specific AIDS and non-AIDS events observed during follow-up.

AIDS events	*N* (% of AIDS events) 354 (100)	Non-AIDS events	*N* (% of non-AIDS events) 572 (100)
Oesophageal candidiasis	54 (15)	Cardiovascular events	208 (36)
Pulmonary tuberculosis	49 (14)	Non-AIDS-defining malignancies	204 (36)
Non-Hodgkin's lymphoma	44 (12)	Liver-related events	36 (6)
Herpes simplex virus ulcers (>1 month duration)	32 (9)	Pancreatitis	36 (6)
Extra pulmonary tuberculosis	27 (8)	End-stage renal disease	16 (3)
Kaposi's sarcoma	27 (8)	Deaths due to non-AIDS-related causes	72 (13)
Bacterial pneumonia (recurrent)	21 (6)		
Cervical carcinoma	15 (4)		
Deaths due to AIDS-related causes	14 (4)		

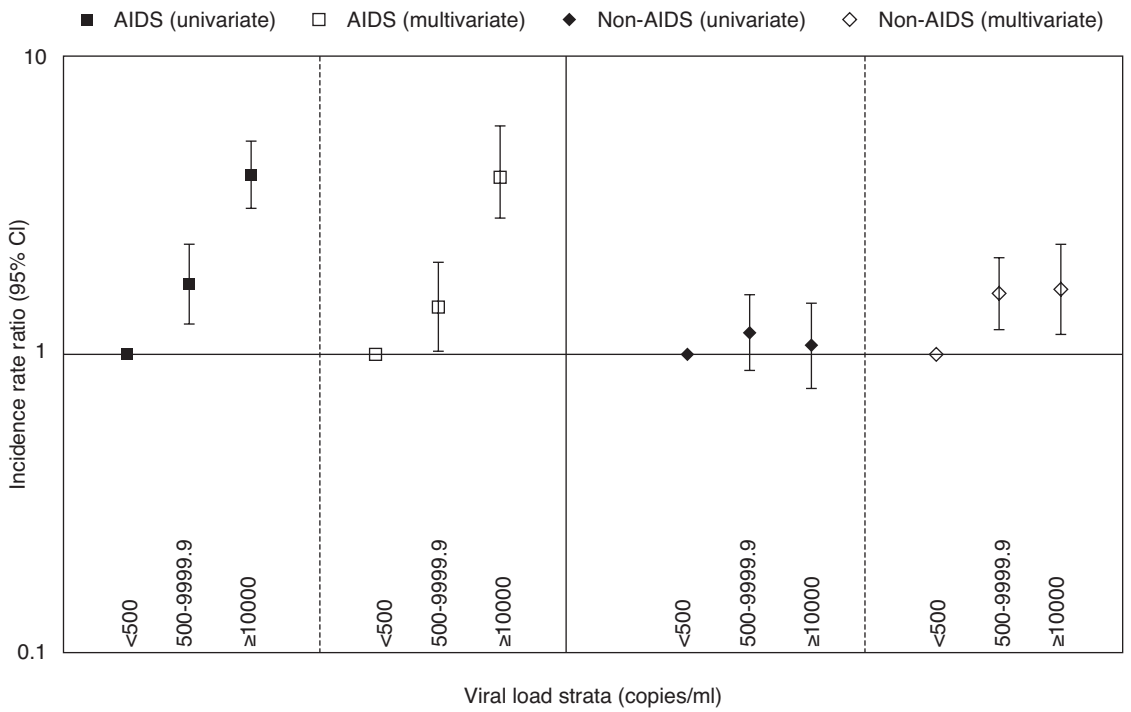

Fig. 1. Unadjusted and adjusted incidence rate ratios for AIDS and non-AIDS events by viral load strata. In AIDS univariate model, only viral load strata was included in the model. AIDS multivariate model was also adjusted for sex, HIV exposure group, region of Europe, hepatitis B surface antigen (HBsAg)* and hepatitis C virus (HCV) antibody status*, smoking status*, hypertension*, year of follow-up*, CD4 cell count* and starting combination antiretroviral therapy (cART)*. In non-AIDS univariate model, only viral load strata was included in the model. Non-AIDS multivariate model was also adjusted according to HIV exposure group, region of Europe, peak viral load*, CD4 cell count*, age*, HBsAg* and HCV antibody status*, diabetes*, hypertension*, smoking status*, prior AIDS* and starting cART*. The symbol '*' denotes time-updated variables.

a viral load of at least 10 000 copies/ml (IRR 1.00, 95% CI 0.50–2.02, $P = 0.99$) compared with individuals with a viral load of less than 500 copies/ml. For NADM, liver-related and pancreatitis events, no significant differences in incidence were observed across the viral load strata.

Figure 3 shows the effect of viral load on the incidence of each endpoint across different CD4 cell count strata. Compared with individuals with a CD4 cell count between 350 and 499 cells/μl, a nonsignificant lower rate of AIDS events was observed in individuals with a CD4 cell count between 500 and 749 cells/μl (IRR 0.88, 95% CI 0.70–1.12, $P = 0.31$) and at least 750 cells/μl (IRR 0.76, 95% CI 0.54–1.05, $P = 0.09$) after adjusting for other factors including viral load. For non-AIDS events, no association was found between current CD4 cell count and risk of a non-AIDS events, there was no significant difference in incidence rate between individuals with a current CD4 cell count between 350 and 499 cells/μl and those with a CD4 cell count between 500 and 749 cells/μl (IRR 1.01, 95% CI 0.84–1.22, $P = 0.92$) or at least 750 cells/μl (IRR 0.98, 95% CI 0.79–1.23, $P = 0.89$). For both of the endpoints, the effect of viral load was independent of CD4 cell count, with a test for interaction P value of 0.10 for AIDS events and 0.69 for non-AIDS events.

Discussion

In HIV-positive individuals with a CD4 cell count of more than 350 cells/μl, we observed an association with uncontrolled viral replication, and a higher incidence of fatal and nonfatal AIDS events and a slightly increased incidence of fatal and nonfatal non-AIDS events. The higher incidence of AIDS events was observed in individuals in both crude and adjusted analysis. However, the increased incidence of non-AIDS events was only apparent after adjustment, particularly for age, region of Europe and whether or not the individual had started cART. In addition, no differences were observed between intermediate and high viral replication and the incidence of non-AIDS events.

Early studies investigating the prognostic effect of viral load on clinical progression to AIDS demonstrated the association with higher viral replication and an increased risk of the development of AIDS events [13,14];

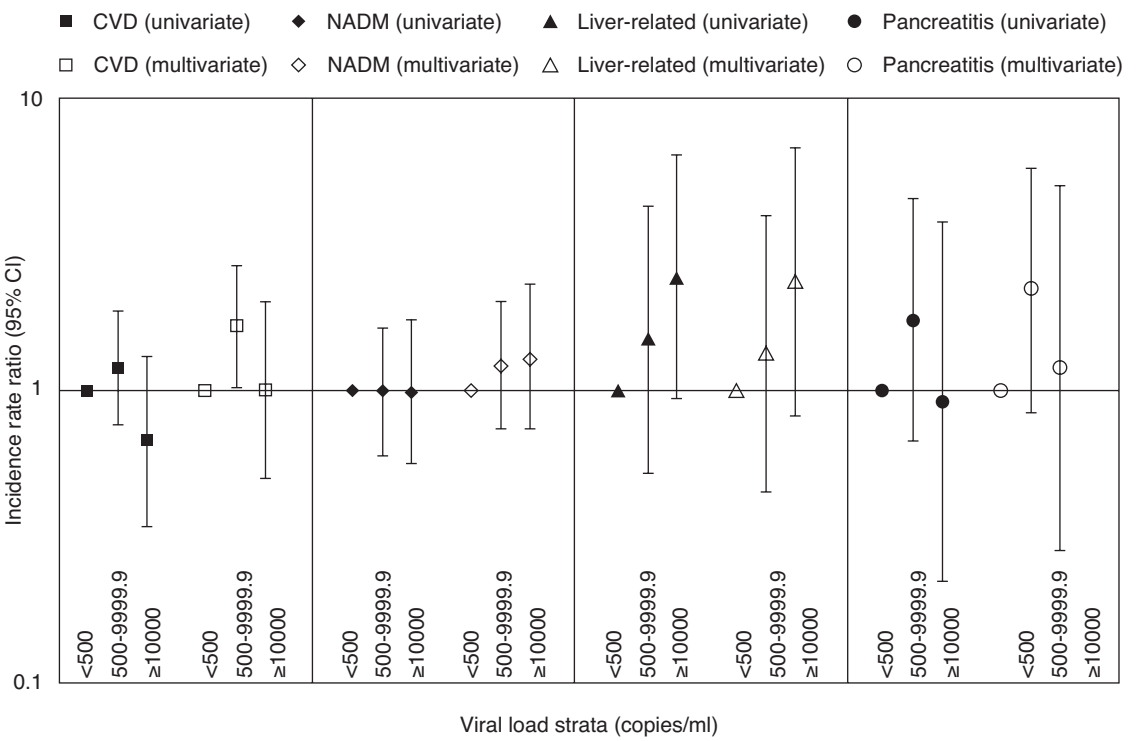

Fig. 2. Unadjusted and adjusted incidence rate ratios for specific non-AIDS events by viral load strata. In cardiovascular disease (CVD) univariate model, only viral load strata was included in the model. CVD multivariate model also adjusted for sex, ethnic origin, region of Europe, age*, hepatitis B surface antigen (HBsAg)*, diabetes*, smoking status*, hypertension*, year of follow-up*, peak viral load, CD4 cell count* and starting combination antiretroviral therapy (cART)*. In non-AIDS-defining malignancy (NADM) univariate model, only viral load strata was included in the model. NADM multivariate model also adjusted for sex, ethnic origin, HIV exposure group, region of Europe, age*, HBsAg*, diabetes*, prior AIDS* peak viral load* and CD4 cell count*. In the liver-related univariate model, only viral load strata were included in the model. Liver-related multivariate model also adjusted for HIV exposure group, HBsAg* and hepatitis C virus (HCV) antibody status*, smoking status*, CD4 cell count* and starting cART*. In pancreatitis univariate model, only viral load strata were included in the model. Pancreatitis multivariate model also adjusted of sex, age*, HBsAG* and HCV antibody status* and prior AIDS. The symbol '*' denotes time-updated variables.

this finding was consistent even in HIV-positive individuals with high CD4 cell counts [15,16] and this study supports these findings in patients with CD4 cell counts of more than 350 cells/μl. The association we found between viral replication and the incidence of AIDS events appears to be almost linear and was independent of current CD4 cell count.

The association with non-AIDS events and viral replication is harder to interpret. Previous findings have reported a decline in the rate of non-AIDS related deaths when compared with the pre-cART era [17,18], suggesting that HIV may play a role in non-AIDS related diseases as well as those classed as AIDS-defining. In addition, in studies looking at treatment interruption strategies, a higher incidence of some non-AIDS events, such as infection, cardiovascular, renal and hepatic complications was seen in individuals who interrupted therapy compared with those on continuous ART and it was hypothesized that this was due to uncontrolled HIV replication [19,20]. However, these studies did not focus specifically on individuals with high CD4 cell counts, although they did account for current CD4 cell count in the analysis.

This analysis selected only individuals with a high CD4 cell count (>350 cells/μl) and the results were found to be independent of current CD4 cell count. An increased incidence of non-AIDS events was found with intermediate or high viral replication, but it was only apparent after adjustment. Furthermore, unlike the AIDS events, no difference in incidence was observed between moderate and high viral replication. A study by Ferry *et al.* [5] looked specifically at the factors associated with the first non-AIDS event and found an increased rate of non-AIDS events in individuals with uncontrolled viral replication, after adjustment for current CD4 cell count. They hypothesized that uncontrolled HIV replication may induce a state of immune suppression independent of CD4 cell count. In a study that looked at individuals with high CD4 cell counts, Lodwick *et al.* [21] found no association in risk of all-cause death and viral replication in treatment-naive HIV-positive individuals.

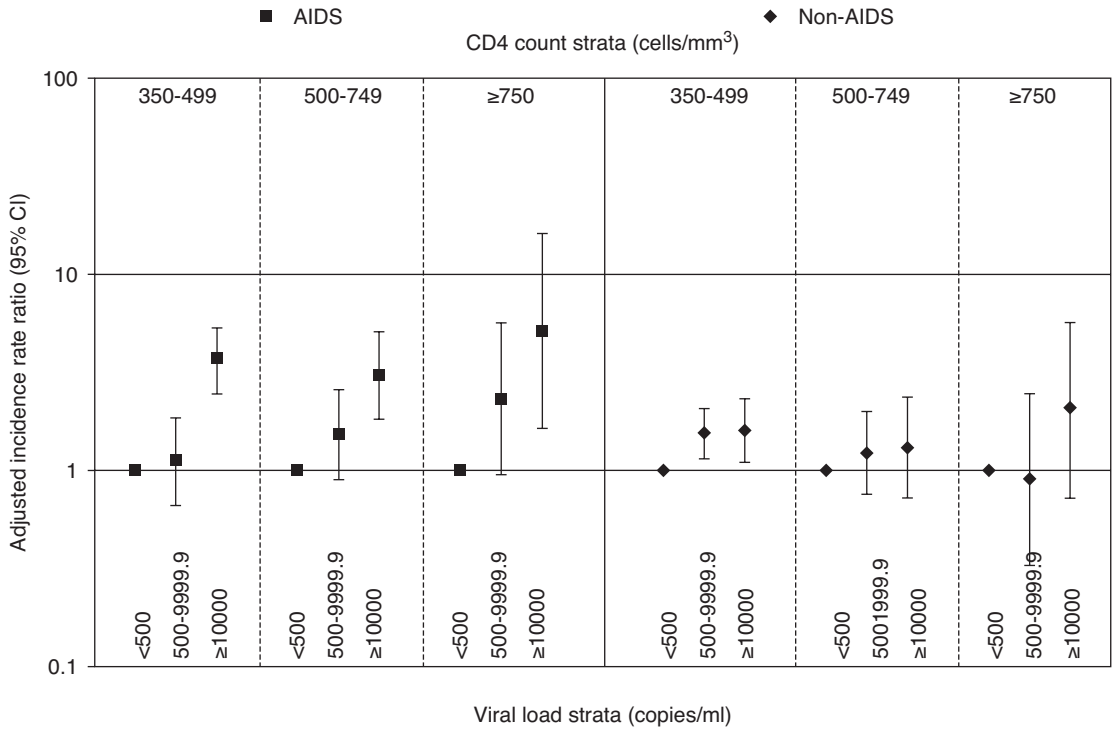

Fig. 3. Adjusted incidence rate ratios for AIDS and non-AIDS events by viral load strata stratified by current CD4 cell count. The AIDS models also adjusted for sex, HIV exposure group, region of Europe, hepatitis B surface antigen (HBsAg)* and hepatitis C virus (HCV) antibody status*, smoking status*, hypertension*, year of follow-up* and starting combination antiretroviral therapy (cART)*. The non-AIDS models also adjusted for HIV exposure group, region of Europe, peak viral load*, age*, HBsAg* and HCV antibody status*, diabetes*, hypertension*, smoking status*, prior AIDS* and starting cART*. The symbol '*' denotes time-updated variables. CI, confidence interval

Looking at specific non-AIDS events in our study, no association was found after adjustment between viral replication and the incidence of NADM, CVD, liver-related or pancreatitis events. However, these results should be interpreted with caution due to the small number of events in each group. Other studies, including Ferry *et al.* [5], have found no association with high viral replication and the development of NADM or related death after accounting for CD4 cell count [4,22,23]. However, this is a very heterogeneous group, with many malignancies developing due to different causes. Studies looking at specific NADM found no association between Hodgkin's lymphoma, liver cancer or lung cancer. However, the risk of anal cancer has been found to be higher in individuals with a viral load of more than 100 000 copies/ml [23]. A study by the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) collaboration looking at causes of non-AIDS-related death and immunodeficiency, found a higher risk of CVD related death with high levels of HIV-RNA after accounting for CD4 cell count and whether or not the individual was on cART [22]. The SMART study [19] similarly observed an increased risk of cardiovascular events in individuals with a viral load above 400 copies/ml, after adjustment for most recent CD4 cell count. In our study, a slightly increased incidence of CVD was observed in patients with moderate viral replication; however, this was not seen in those with high viral replication. The CASCADE study [22] also found a moderately significantly increased risk of liver-related death in individuals with a viral load of more than 500 copies/ml and not on cART, but not in those on cART. In our analysis, we observed a nonsignificant trend toward an increased risk. Again this analysis lacked power, as only a small number of liver-related events were observed and, therefore, wide CIs were seen. In the analysis by Ferry *et al.* [5], there was a strong association with viral load and non-AIDS bacterial infection which we were unable to look at as a specific endpoint. Deaths due to non-AIDS-related bacterial infection were included in this analysis, but more specific events were not. A larger study with longer follow-up would be needed to analyse the association between each specific non-AIDS events and viral replication further.

Previously in EuroSIDA, we reported that there was a continuum of decreasing risk of AIDS events with increased CD4 cell count even in patients with high CD4 cell counts, but that this same relationship was not seen for non-AIDS events with no further decrease in risk

in patients with CD4 cell counts of more than 350 cells/μl [6]. This earlier analysis included all patients enrolled in EuroSIDA after 1 January 2001, whereas in this current analysis only individuals who were not immunocompromised were selected and the focus was specifically on the relationship of viraemia and the development of AIDS and non-AIDS events and whether this relationship differed at different CD4 cell counts. Our results were consistent with the previous analysis in that we found a lower rate of AIDS events in patients with higher CD4 cell counts, although in this analysis the difference was nonsignificant, and again the relationship was not seen for non-AIDS events. In addition, we also found no evidence that the relationship with viraemia and risk of experiencing an AIDS or non-AIDS event differed at different CD4 cell counts.

There are some limitations to this analysis. These data are from a large observational cohort study and although a number of associated variables were adjusted for in this analysis, there may be other unmeasured or unknown confounders that could not be accounted for. In this study, individuals with a high level of HIV replication are either treatment-naive, have stopped using cART or are on a virologically failing cART regimen. As these individuals were not randomly allocated to starting cART and the situation they are currently in, there may be some confounding between HIV viral load and the reasons why individuals are in these situations. It could be that those individuals with a high viral load are a subpopulation of difficult to manage, or poorly managed individuals who may be noncompliant to treatment, and have exhausted all their treatment options or have a lifestyle that leads to an excess risk of morbidity or mortality. As such, rather than a high viral load being detrimental to the individual, it may be that this is a surrogate marker for a difficult to manage subpopulation. Additionally, there are many clinical situations in which an individual may experience an increase in viral load, such as during a non-AIDS infection or while experiencing drug–drug interaction resulting in nontherapeutic levels of antiretroviral drugs. Some of these situations are unavoidable and it is important to understand any increase in risk these individuals may experience in order for them to receive the appropriate level of care.

Although results such as ours from observational studies help to provide clues which are relevant for making clinical decisions, such as whether to start ART, by far the most optimal means of addressing questions about clinical strategy is through randomized trials. The randomized Strategic Timing of AntiRetroviral Therapy (START) trial is currently in the process of recruiting HIV-positive individuals with a CD4 cell count of more than 500 cells/μl, with the aim to determine whether immediate ART initiation leads to a reduced rate of serious AIDS and serious non-AIDS diseases compared with deferral of ART until the CD4 cell count has reached 350 cells/μl [24]. As well as answering the important strategy question, it should also help increase our knowledge and understanding of the role of uncontrolled HIV-replication in both AIDS and non-AIDS events,

In conclusion, in HIV-positive individuals with a CD4 cell count of more than 350 cells/μl, an increased incidence of AIDS and a slightly increased incidence of non-AIDS were found in those with uncontrolled viral replication. The association with AIDS was clear and consistent. However, the association with non-AIDS was only apparent after adjustment and no differences were observed between intermediate and high viremia.

Acknowledgements

The EuroSIDA Study Group, the multicentre study group on EuroSIDA (national coordinators in parenthesis) are as follows.

The EuroSIDA study group

The multi-centre study group on EuroSIDA (national coordinators in parenthesis): *Argentina*: (M.L.), C. Elias, Hospital JM Ramos Mejia, Buenos Aires. *Austria*: (N. Vetter), PulmologischesZentrum der Stadt Wien, Vienna; R. Zangerle, Medical University Innsbruck, Innsbruck. *Belarus*: (I. Karpov), A. Vassilenko, Belarus State Medical University, Minsk, V.M. Mitsura, Gomel State Medical University, Gomel; O. Suetnov, Regional AIDS Centre, Svetlogorsk. *Belgium*: (N. Clumeck), S. De Wit, M. Delforge, Saint-Pierre Hospital, Brussels; R. Colebunders, Institute of Tropical Medicine, Antwerp; L. Vandekerckhove, University Ziekenhuis Gent, Gent. *Bosnia-Herzegovina*: (V. Hadziosmanovic), KlinickiCentarUniverziteta Sarajevo, Sarajevo. *Bulgaria*: (K. Kostov), Infectious Diseases Hospital, Sofia. *Croatia*: (J. Begovac), University Hospital of Infectious Diseases, Zagreb. *Czech Republic*: (L. Machala), D. Jilich, Faculty Hospital Bulovka, Prague; D. Sedlacek, Charles University Hospital, Plzen. *Denmark*: (J. Nielsen), G. Kronborg, T. Benfield, M. Larsen, Hvidovre Hospital, Copenhagen; J. Gerstoft, T. Katzenstein, A.-B.E. Hansen, P. Skinhøj, Rigshospitalet, Copenhagen; C. Pedersen, Odense University Hospital, Odense; L. Ostergaard, Skejby Hospital, Aarhus. *Estonia*: (K. Zilmer), West-Tallinn Central Hospital, Tallinn; J. Smidt, NakkusosakondSiseklinik, Kohtla-Järve. *Finland*: (M. Ristola), Helsinki University Central Hospital, Helsinki. *France*: (C. Katlama), Hôpital de la Pitié-Salpétière, Paris; J.-P. Viard, Hôpital Necker-EnfantsMalades, Paris; P.-M. Girard, Hospital Saint-Antoine, Paris; J.M. Livrozet, HôpitalEdouard Herriot, Lyon; P. Vanhems, University Claude Bernard, Lyon; C. Pradier, Hôpital de l'Archet, Nice; F. Dabis, D. Neau, Unité INSERM, Bordeaux. *Germany*: (J. Rockstroh), UniversitätsKlinik Bonn; R. Schmidt, MedizinischeHochschule Hannover; J. van

Lunzen, O. Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, Hamburg; H.J. Stellbrink, IPM Study Center, Hamburg; S. Staszewski, JW Goethe University Hospital, Frankfurt; J. Bogner, MedizinischePoliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne. *Greece*: (J. Kosmidis), P. Gargalianos, G. Xylomenos, J. Perdios, Athens General Hospital; G. Panos, A. Filandras, E. Karabatsaki, 1st IKA Hospital; H. Sambatakou, IppokrationGenereal Hospital, Athens. *Hungary*: (D. Banhegyi), SzentLásló Hospital, Budapest. *Ireland*: (F. Mulcahy), St. James's Hospital, Dublin. *Israel*: (I.Y.), D. Turner, M. Burke, Ichilov Hospital, Tel Aviv; S. Pollack, G. Hassoun, Rambam Medical Center, Haifa; S. Maayan, Hadassah University Hospital, Jerusalem. *Italy*: (S. Vella), IstitutoSuperiore di Sanità, Rome; R. Esposito, I. Mazeu, C. Mussini, Università Modena, Modena; C. Arici, OspedaleRiuniti, Bergamo; R. Pristera, OspedaleGeneraleRegionale, Bolzano; F. Mazzotta, A. Gabbuti, Ospedale S Maria Annunziata, Firenze; V. Vullo, M. Lichtner, University di Roma la Sapienza, Rome; A. Chirianni, E. Montesarchio, M. Gargiulo, PresidioOspedaliero AD Cotugno, Monaldi Hospital, Napoli; G. Antonucci, A. Testa, P. Narciso, C. Vlassi, M. Zaccarelli, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A. Lazzarin, A. Castagna, N. Gianotti, Ospedale San Raffaele, Milan; M. Galli, A. Ridolfo, Osp. L. Sacco, Milan; A. d'Arminio Monforte, Istituto Di Clinica Malattie Infettive e Tropicale, Milan. *Latvia*: (B. Rozentale), I. Zeltina, Infectology Centre of Latvia, Riga. *Lithuania*: (S. Chaplinskas), Lithuanian AIDS Centre, Vilnius. *Luxembourg*: (R. Hemmer), T. Staub, Centre Hospitalier, Luxembourg. *Netherlands*: (P. Reiss), Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam. *Norway*: (V. Ormaasen), A. Maeland, J. Bruun, Ullevål Hospital, Oslo. *Poland*: (B. Knysz) J. Gasiorowski, Medical University, Wroclaw; A. Horban, E.B., Centrum DiagnostykiiTerapii AIDS, Warsaw; A. Grzeszczuk, R. Flisiak, Medical University, Bialystok; A. Boron-Kaczmarska, M. Pynka, M. Parczewski, Medical Univesity, Szczecin; M. Beniowski, E. Mularska, OsrodekDiagnostykiiTerapii AIDS, Chorzow; H. Trocha, Medical University, Gdansk; E. Jablonowska, E. Malolepsza, K. Wojcik, WojewodzkiSzpitalSpecjalistyczny, Lodz. *Portugal*: (F. Antunes), M. Doroana, L. Caldeira, Hospital Santa Maria, Lisbon; K. Mansinho, Hospital de Egas Moniz, Lisbon; F. Maltez, Hospital Curry Cabral, Lisbon. *Romania*: (D. Duiculescu), Spitalul de BoliInfectioasesi Tropicale: Dr. Victor Babes, Bucarest. *Russia*: (A.R.), Medical Academy Botkin Hospital, St Petersburg; N. Zakharova, St Petersburg AIDS Centre, St Peterburg; S. Buzunova, Novgorod Centre for AIDS, Novgorod. *Serbia*: (D. Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade. *Slovakia*: (M. Mokráš), D. Staneková, Dérer Hospital, Bratislava. *Slovenia*: (J. Tomazic), University Clinical Centre Ljubljana, Ljubljana. *Spain*: (J. González-Lahoz), V. Soriano, P. Labarga, J. Medrano, Hospital Carlos III, Madrid; S. Moreno, J.M. Rodriguez, Hospital Ramon y Cajal, Madrid; B. Clotet, A. Jou, R. Paredes, C. Tural, J. Puig, I. Bravo, Hospital Germans TriasiPujol, Badalona; J.M.G., J.M. Miró, Hospital Clinic i Provincial, Barcelona; P. Domingo, M. Gutierrez, G. Mateo, M.A. Sambeat, Hospital Sant Pau, Barcelona. *Sweden*: (A. Karlsson), Venhaelsan-Sodersjukhuset, Stockholm; L. Flamholc, Malmö University Hospital, Malmö. *Switzerland*: (B. Ledergerber), R. Weber, University Hospital, Zürich; P.F., M. Cavassini, Centre Hospitalier Universitaire Vaudois, Lausanne; B. Hirschel, E. Boffi, Hospital Cantonal Universitaire de Geneve, Geneve; H. Furrer, Inselspital Bern, Bern; M. Battegay, L. Elzi, University Hospital Basel. *Ukraine*: (E. Kravchenko), N. Chentsova, Kiev Centre for AIDS, Kiev; V. Frolov, G. Kutsyna, Luhansk State Medical University; Luhansk; S. Servitskiy, Odessa Region AIDS Center, Odessa; M.K., Kharkov State Medical University, Kharkov. *United Kingdom*: (S. Barton), St. Stephen's Clinic, Chelsea and Westminster Hospital, London; A.M. Johnson, D. Mercey, Royal Free and University College London Medical School, London (University College Campus); A. Phillips, M.A. Johnson, A.M., Royal Free and University College Medical School, London (Royal Free Campus); M. Murphy, Medical College of Saint Bartholomew's Hospital, London; J. Weber, G. Scullard, Imperial College School of Medicine at St. Mary's, London; M. Fisher, Royal Sussex County Hospital, Brighton; C. Leen, Western General Hospital, Edinburgh.

Steering Committee: J.M.G., B. Gazzard, A. Horban, B. Ledergerber, M.L., J. Lundgren, A. d'Arminio-Monforte, C. Pedersen, A. Phillips, A.R., P. Reiss, M. Ristola, J. Rockstroh (Chair), S. De Wit (Vice-Chair).

Coordinating Centre Staff: J. Lundgren, O. Kirk, A.M., A. Cozzi-Lepri, D. Grint, M. Ellefson, D. Podlekareva, J. Kjær, L. Peters, J. R., J.K., J. Tverland, A.H. Fischer, J. Nielsen.

Primary support for EuroSIDA is provided by the European Commission BIOMED 1 (CT94–1637), BIOMED 2 (CT97–2713), the 5th Framework (QLK2–2000–00773), the 6th Framework (LSHP-CT-2006–018632) and the 7th Framework (FP7/2007–2013, EuroCoord number 260694) programmes. Current support also includes unrestricted grants by Gilead, Pfizer and Merck and Co. The participation of centres from Switzerland was supported by the Swiss National Science Foundation (grant 108787).

Conflicts of interest

There are no conflicts of interest.

This study was presented at the 10th International Congress on Drug Therapy in HIV Infection; 7–11 November 2010; Glasgow, UK.

References

1. Darbyshire J, Schoenfeld DA, Weller IV. **Clinical trials in HIV infection: current problems and future directions.** *AIDS* 1991; **5 (Suppl 2)**:S167–S173.
2. Miller V, Phillips AN, Clotet B, Mocroft A, Ledergerber B, Kirk O, *et al*. **Association of virus load, CD4 cell count, and treatment with clinical progression in human immunodeficiency virus-infected patients with very low CD4 cell counts.** *J Infect Dis* 2002; **186**:189–197.
3. Mocroft A, Katlama C, Johnson AM, Pradier C, Antunes F, Mulcahy F, *et al*. **AIDS across Europe, 1994–98: the EuroSIDA study.** *Lancet* 2000; **356**:291–296.
4. Monforte A, Abrams D, Pradier C, Weber R, Reiss P, Bonnet F, *et al*. **HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malignancies.** *AIDS* 2008; **22**:2143–2153.
5. Ferry T, Raffi F, Collin-Filleul F, Dupon M, Dellamonica P, Waldner A, *et al*. **Uncontrolled viral replication as a risk factor for non-AIDS severe clinical events in HIV-infected patients on long-term antiretroviral therapy: APROCO/COPILOTE (ANRS CO8) cohort study.** *J Acquir Immune Defic Syndr* 2009; **51**:407–415.
6. Mocroft A, Reiss P, Gasiorowski J, Ledergerber B, Kowalska J, Chiesi A, *et al*. **Serious fatal and nonfatal non-AIDS-defining illnesses in Europe.** *J Acquir Immune Defic Syndr* 2010; **55**:262–270.
7. Lundgren JD, Babiker A, El-Sadr W, Emery S, Grund B, Neaton JD, *et al*. **Inferior clinical outcome of the CD4+ cell count-guided antiretroviral treatment interruption strategy in the SMART study: role of CD4+ cell counts and HIV RNA levels during follow-up.** *J Infect Dis* 2008; **197**:1145–1155.
8. Deeks SG, Barbour JD, Martin JN, Swanson MS, Grant RM. **Sustained CD4(+) T cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection.** *J Infect Dis* 2000; **181**: 946–953.
9. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, *et al*. **Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group.** *Lancet* 1998; **352**:1725–1730.
10. Kowalska JD, Friis-Moller N, Kirk O, Bannister W, Mocroft A, Sabin C, *et al*. **The Coding Causes of Death in HIV (CoDe) Project: initial results and evaluation of methodology.** *Epidemiology* 2011; **22**:516–523.
11. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, *et al*. **Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group.** *AIDS* 1999; **13**:943–950.
12. Kowalska JD, Mocroft A, Ledergerber B, Florence E, Ristola M, Begovac J, *et al*. **Cause of death in HIV infection as AIDS or non-AIDS related: results from the EuroSIDA Study.** *HIV Clin Trials* 2011; **12**:109–117.
13. Loveday C, Hill A. **Prediction of progression to AIDS with serum HIV-1 RNA and CD4 count.** *Lancet* 1995; **345**:790–791.
14. Sabin CA, Devereux H, Phillips AN, Janossy G, Loveday C, Lee CA. **Immune markers and viral load after HIV-1 seroconversion as predictors of disease progression in a cohort of haemophilic men.** *AIDS* 1998; **12**:1347–1352.
15. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, *et al*. **Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection.** *Ann Intern Med* 1997; **126**:946–954.
16. Phillips A. **Short-term risk of AIDS according to current CD4 cell count and viral load in antiretroviral drug-naive individuals and those treated in the monotherapy era.** *AIDS* 2004; **18**:51–58.
17. Mocroft A, Brettle R, Kirk O, Blaxhult A, Parkin JM, Antunes F, *et al*. **Changes in the cause of death among HIV positive subjects across Europe: results from the EuroSIDA study.** *AIDS* 2002; **16**:1663–1671.
18. Grabar S, Lanoy E, Allavena C, Mary-Krause M, Bentata M, Fischer P, *et al*. **Causes of the first AIDS-defining illness and subsequent survival before and after the advent of combined antiretroviral therapy.** *HIV Med* 2008; **9**:246–256.
19. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, *et al*. **CD4+ count-guided interruption of antiretroviral treatment.** *N Engl J Med* 2006; **355**:2283–2296.
20. Danel C, Moh R, Minga A, Anzian A, Ba-Gomis O, Kanga C, *et al*. **CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial.** *Lancet* 2006; **367**:1981–1989.
21. Lodwick RK, Sabin CA, Porter K, Ledergerber B, van Sighem A, Cozzi-Lepri A, *et al*. **Death rates in HIV-positive antiretroviral-naive patients with CD4 count greater than 350 cells per microL in Europe and North America: a pooled cohort observational study.** *Lancet* 2010; **376**:340–345.
22. Marin B, Thiebaut R, Bucher HC, Rondeau V, Costagliola D, Dorrucci M, *et al*. **Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy.** *AIDS* 2009; **23**:1743–1753.
23. Guiguet M, Boue F, Cadranel J, Lang JM, Rosenthal E, Costagliola D. **Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study.** *Lancet Oncol* 2009; **10**:1152–1159.
24. *START protcol synopsis*. http://insight.ccbr.umn.edu/official_documents/START/protocol_documents/START_ProtocolSynopsis.pdf. [Accessed 19 November 2009]