

## Low-level viraemia, measured as viraemia copy-years, as a prognostic factor for medium–long-term all-cause mortality: a MASTER cohort study

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**Objectives:** We investigated the association between persistent low-level viraemia, measured as viraemia copy-years (VCY), and all-cause mortality.

**Methods:** We included 3271 HIV-infected patients who initiated their first combined ART (cART) during 1998–2012 enrolled in the multicentre Italian MASTER cohort. VCY was defined as the area under the curve of plasma viral load (pVL) and expressed in  $\log_{10}$  copies·years/mL. VCY was evaluated from cART initiation until the end of follow-up [VCY-overall (VCY-o)], and stratified into before [VCY-early (VCY-e)] and after [VCY-late (VCY-l)] the eighth month from starting cART, and as the ratio of VCY-l to follow-up duration (VCY-l/FUD).

**Results:** The risk of death increased of about 40% for higher than the median levels of VCY-o and VCY-e. Compared with subjects with permanently suppressed pVL after the eighth month from starting cART, mortality increased by 70% for those with VCY-l  $\geq 3 \log_{10}$  copies·years/mL, and by about 20-fold for those with VCY-l/FUD  $\geq 2.3 \log_{10}$  copies/mL. Patients who maintained low levels of VCY-l ( $< 3 \log_{10}$  copies·years/mL) or VCY-l/FUD ( $< 2.3 \log_{10}$  copies/mL) had a risk of death similar to patients with permanently suppressed pVL. CD4 cell count at baseline was predictive of high risk of death only in subjects with VCY-l  $\geq 3 \log_{10}$  copies·years/mL.

**Conclusions:** The risk of death did not increase in HIV-infected patients with low levels of VCY-l compared with patients with permanent virological suppression.

### Introduction

The quantification of HIV-1 RNA in plasma is used to verify the efficacy of combined ART (cART). Most guidelines indicate that treatment should aim to achieve a viral load of  $\leq 50$  copies/mL, but there is no consensus about the definition of virological failure. The most recent guidelines of the European AIDS Clinical Society define treatment failure as a confirmed HIV RNA value  $> 50$  copies/mL  $\geq 6$  months after starting therapy (initiation or modification).<sup>1</sup> However, other guidelines propose different HIV RNA serum levels for defining failure; British guidelines, for example, consider virological failure when plasma viral load (pVL) is  $> 200$  copies/mL after 6 months of therapy without ever

achieving pVL  $\leq 50$  copies/mL or evidence of confirmed virological rebound to  $> 200$  copies/mL after achieving a pVL below the limit of detection (ordinarily  $< 40$ – $50$  copies/mL).<sup>2</sup> The range of 50–200 copies/mL (low replication) is a 'grey zone' in which there is no consensus regarding patient management.

Several groups have focused on whether viraemia  $< 200$  or even  $< 50$  copies/mL may be predictive of subsequent treatment failure or associated with the development of drug resistance.<sup>3–9</sup> However, very little is known regarding the consequences of the persistence of low-level replication on the clinical evolution of HIV infection with respect to the emergence of AIDS- and non-AIDS-related diseases or overall survival. Data from 18 cohorts in Europe and North America, contributing to the ART Cohort Collaboration, showed

that low-level viraemia (defined as  $<500$  copies/mL) was not associated with clinical outcomes considering AIDS-defining events or deaths. Moreover, modification of the ART regimen during viraemia  $<200$  copies/mL did not influence either the clinical or the virological outcome.<sup>10</sup>

To evaluate the impact of HIV replication on mortality and morbidity, however, most studies measured plasma viraemia at baseline, during follow-up or at the last visit. The impact of cumulative exposure to HIV viraemia is a growing focus of concern. Only few studies have focused on the clinical impact (rate of CD4 cell gain, AIDS events or mortality) of the overall HIV viral load burden represented as the lifelong cumulative HIV viraemia during cART, measured as viraemia copy-years (VCY).<sup>11–13</sup> However, these studies investigated the impact of cumulative HIV viraemia using a median-dichotomized variable and no information about the relationship between low-level viraemia, measured as VCY, and risk of death is available at present.

We aimed to investigate the association between persistent low-level viraemia, measured as VCY, and all-cause mortality in a cohort of HIV-infected individuals. We further examined the relationship between the CD4+ T cell count at the start of cART and mortality through all causes, according to VCY after 8 months of treatment.

## Patients and methods

### Setting and data sources

The Italian MASTER cohort is a hospital-based, multicentre, dynamic cohort established in the mid-1990s with retrospective patient enrolment since 1986. Enrolment in MASTER is independent of HIV disease stage, degree of immunosuppression or use of ART.<sup>14</sup> Collected data include demographic features, date of HIV diagnosis, route of transmission, AIDS-defining events, CD4+ T cell count, pVL and cART.

Data are updated annually. Vital status and date of death were ascertained through clinical charts or record linkage with local health authority mortality registers in about one-third of patients. The Italian healthcare system provides free tax-supported HIV treatment including cART to all HIV-infected individuals who are resident in Italy.

### Ethics

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Informed consent was obtained according to the standards of the local ethics committees.

### Study populations

In this study, we included all antiretroviral-naïve HIV-infected patients enrolled in the MASTER cohort who started their first cART between January 1998 and December 2012 with follow-up  $>8$  months and at least three pVL measurements: baseline, eighth month from baseline and one afterwards. Date of starting cART was considered as baseline.

### Description and categorization of exposure variables, outcomes and covariates

The primary outcome of this study was all-cause mortality. Deaths occurring during the first 8 months after starting cART were not considered, because they were probably deaths related to severe illness at HIV diagnosis (i.e. late presenters).

We retrieved data on gender, age, country of origin, HIV exposure risk factors, year of cART initiation, viral hepatitis C and B at baseline and all pVL measurements from the MASTER electronic database.

VCY was defined as the area under the individual curve of pVL, with the assumption of linearity (trapezoidal integration) between two successive pVL measures, and expressed in  $\log_{10}$  copies·years/mL.<sup>15</sup>

VCY was evaluated as overall VCY (VCY-o), i.e. from the start of ART until the end of follow-up, and stratified into the first 8 months (VCY-early, VCY-e) and after the eighth month from starting cART (VCY-late, VCY-l). We used the eighth month as cut-off, because patients are expected to reach an undetectable viral load after 6–8 months of therapy in accordance with a previous study.<sup>11</sup> If no pVL was available at the eighth month from baseline, we estimated pVL using linear interpolation between the closest measures available before and after that time. All pVL values equal to or below the limit of detection ( $<50$  copies/mL) were set to zero to avoid an artificial increase of VCY with increasing duration of follow-up even without detectable pVL, as previously described.<sup>11</sup> At the other end of the measurement scale, because of the assay sensitivity limit, we set  $pVL >1000000$  copies/mL as 1000000 copies/mL.<sup>13</sup> Finally, we also evaluated VCY as the ratio between VCY-l and the corresponding follow-up duration (FUD) (VCY-l/FUD) according to the method described by Chirouze *et al.*<sup>11</sup>

### Statistical analysis

For each patient included in the study, person-years at risk have been calculated from the date of starting cART up to the end of follow-up (31 December 2012), last follow-up visit or death, whichever occurred first.

### VCY and risk of death

The distributions of VCY-o, VCY-e, VCY-l and VCY-l/FUD were examined and their approximation to normality was checked using the graphic method quantile–quantile plot. Associations of VCY-o, VCY-e, VCY-l and VCY-l/FUD were tested by univariate and multivariate analyses using Cox proportional hazards models. In time-independent models, we included all the covariates as measured at enrolment. Furthermore, we made a sensitivity analysis to evaluate the association between VCY-o and all-cause mortality in time-dependent models dividing the study period into 1 year intervals and including gender, age at enrolment and intravenous drug use as fixed covariates, whereas VCY-o (continuous or dichotomized at the median) and CD4 cell count were included as time-dependent covariates. VCYs were considered as both continuous and categorical variables. VCY-o and VCY-e were dichotomized at the median (6.16 and 6.15  $\log_{10}$  copies·years/mL, respectively), whereas VCY-l was divided into three categories: VCY-l suppressed (pVL values equal to or below the limit of detection of 50 copies/mL after the eighth month after initiating cART and maintained during all the follow-up); low-level VCY-l (VCY-l  $<3 \log_{10}$  copies·years/mL but not suppressed); and VCY-l  $\geq 3 \log_{10}$  copies·years/mL. The cut-off of  $3 \log_{10}$  copies·years/mL for VCY-l was calculated as  $\log_{10}(200 \text{ copies/mL} \times 4.95)$ , 4.95 years being the mean duration of follow-up. Likewise, VCY-l/FUD was also divided into three categories: VCY-l/FUD suppressed (pVL values equal to or below the limit of detection of 50 copies/mL after the eighth month after initiating cART and maintained during all the follow-up); low-level VCY-l/FUD (VCY-l/FUD  $<2.3 \log_{10}$  copies/mL but not suppressed); and VCY-l/FUD  $\geq 2.3 \log_{10}$  copies/mL per year of follow-up. The cut-off of  $2.3 \log_{10}$  copies/mL for VCY-l/FUD was calculated as  $\log_{10}(200 \text{ copies/mL})$  per year of follow-up.

To evaluate whether the associations of VCY with risk of death were not linear, we also fitted multivariate Cox models with a cubic spline for VCY.<sup>16</sup>

The probabilities of survival were estimated at 10 years according to Kaplan–Meier methods with Greenwood standard error (SE).

### pVL as predictor of long-term mortality in subjects with low-level VCY-l/FUD

We also assessed the relationship between the percentage of pVL  $>500$  copies/mL and risk of death in subjects with low levels of VCY-l/FUD using a multivariate Cox regression model including the following

**Table 1.** Patient characteristics; *n* = 3271

Variable	
Male, <i>n</i> (%)	2358 (72.1)
Age (years), mean (SD)	39.8 (9.9)
<35, <i>n</i> (%)	1114 (34.1)
35–44, <i>n</i> (%)	1289 (39.4)
≥45, <i>n</i> (%)	868 (26.5)
Intravenous drug use, <i>n</i> (%)	593 (18.1)
HCV or HBV coinfection, <i>n</i> (%)	1106 (33.8)
Log <sub>10</sub> viral load, median (IQR)	4.8 (4.2–5.3)
VCY-o (log <sub>10</sub> copies·years/mL), median (IQR)	6.16 (5.58–6.71)
VCY-e (log <sub>10</sub> copies·years/mL), median (IQR)	6.15 (5.57–6.71)
VCY-l suppressed, <i>n</i> (%)	1248 (38.2)
VCY-l (log <sub>10</sub> copies·years/mL), median (IQR)	1.47 (0–3.72)
VCY-l/FUD (log <sub>10</sub> copies/mL), median (IQR)	0.28 (0–0.75)
CD4 cell count (cells/mm <sup>3</sup> ), median (IQR)	245 (109–346)
<200, <i>n</i> (%)	1310 (40.0)
≥200, <i>n</i> (%)	1961 (60.0)
Deaths, <i>n</i> (%)	193 (5.9)

HCV, hepatitis C virus; HBV, hepatitis B virus; VCY-o, VCY-overall; VCY-e, VCY-early; VCY-l, VCY-late; VCY-l/FUD, VCY-l divided by the follow-up duration.

covariates: age, gender, intravenous drug use and CD4+ T cell count at baseline. A non-linear relationship between percentage of pVL > 500 copies/mL and risk of death was also investigated using a restricted cubic spline.

### CD4+ T cell count at baseline as predictor of mortality according to VCY

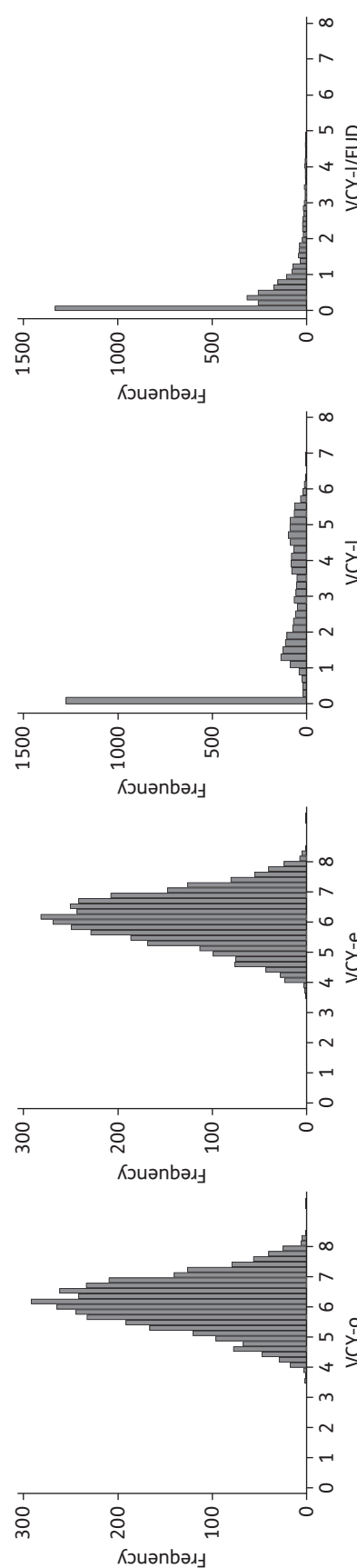
We assessed the association between CD4+ T cell count at baseline and overall mortality according to three categories of VCY-l using univariate and multivariate Cox proportional hazards models. CD4+ T cell count was considered both as continuous and dichotomized on 200 cells/mm<sup>3</sup>.

Cox proportional hazards models provided estimates of HRs, 95% CIs and *P* values using the Wald test. The proportional hazards assumption was investigated for each covariate and globally by analysing Schoenfeld residuals. We first produced graphical plots and then carried out formal statistical tests of their independence over the rank transformation of time. No departures from the proportional hazards assumption were found for all models.

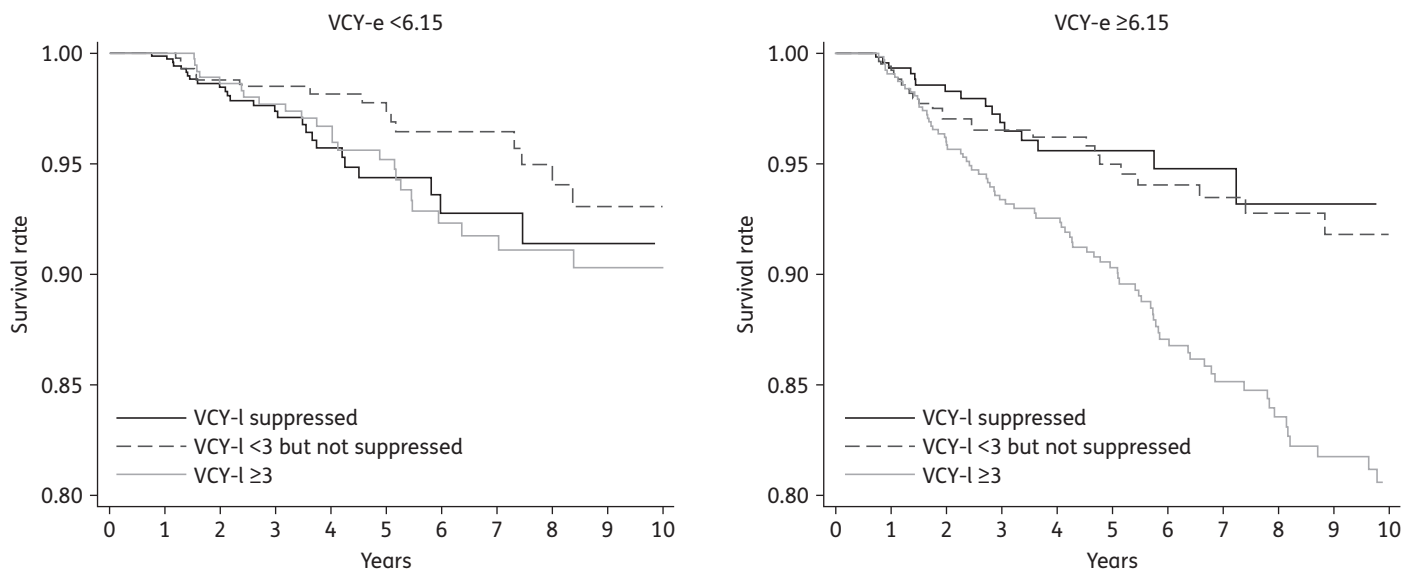
All the statistical tests were two-sided, assumed a level of significance of 0.05 and were performed using Stata version 12.1 (StataCorp, College Station, TX, USA).

## Results

A total of 3271 HIV-infected patients were enrolled (72.1% males and mean age 39.8 years). The cumulative probability of loss to follow-up at 3 years after enrolment was 10.0% (95% CI = 8.9%–11.2%). During a median follow-up of 4.1 years with a total of 16 197.4 person-years, 193 (5.9%) patients died. The



**Figure 1.** Distribution of VCY-o, VCY-e, VCY-l and VCY-l/FUD. VCY-o, VCY calculated from the start of ART until the end of follow-up (VCY-overall); VCY-e, VCY calculated in the first 8 months after cART initiation (VCY-early); VCY-l, VCY calculated after the eighth month from starting cART and during all follow-up (VCY-late); VCY-l/FUD, VCY-l divided by the follow-up duration.



**Figure 2.** Kaplan–Meier curves according to VCY-e and VCY-I. VCY-e, VCY calculated in the first 8 months after cART initiation (VCY-early); VCY-I, VCY calculated after the eighth month from starting cART and during all follow-up (VCY-late).

median number of HIV RNA tests per year was 4.5. The median CD4+ T cell count and median HIV RNA serum level were 245 cells/mm<sup>3</sup> and 4.8 log<sub>10</sub> copies/mL, respectively; 18.1% of patients were IVDUs and 33.8% were coinfecting with hepatitis C or hepatitis B (Table 1). VCY-o and VCY-e approximated normal distribution, whereas VCY-I and VCY-I/FUD had asymmetric, non-normal distribution (Figure 1).

### VCY and risk of death

Survival estimates at 10 years using Kaplan–Meier methodology were 91.5% and 86.1% for VCY-o < 6.16 and VCY-o ≥ 6.16, respectively; 91.4% and 86.2% for VCY-e < 6.15 and VCY-e ≥ 6.15, respectively; and 92.2%, 92.4% and 84.1% for VCY-I suppressed, low-level VCY-I and VCY-I ≥ 3, respectively (Figure S1, available as Supplementary data at JAC Online). We also assessed survival estimates by VCY-I according to VCY-e levels. In subjects with VCY-e < 6.15, survival estimates at 10 years were 91.4% (95% CI=86.1%–94.7%), 93.0% (95% CI=87.9%–96.3%) and 90.3% (95% CI=85.5%–93.5%) for VCY-I suppressed, low-level VCY-I and VCY-I ≥ 3, respectively (log-rank test, *P*=0.3) (Figure 2a). In subjects with VCY-e ≥ 6.15, survival estimates were 93.2% (95% CI=87.6%–96.3%), 91.8% (95% CI=87.3%–94.7%) and 80.6% (95% CI=75.9%–84.4%) for VCY-I suppressed, low-level VCY-I and VCY-I ≥ 3, respectively (log-rank test, *P*<0.001) (Figure 2b).

Table 2 shows the prognostic role of VCYs using multivariate Cox regression models, including gender, age, CD4+ T cell count at baseline and intravenous drug use. VCY-o and VCY-e levels higher than the median were associated with a higher risk of death (HR=1.40 and HR=1.39, respectively, *P*<0.05 for each). Similarly, subjects with VCY-I ≥ 3 log<sub>10</sub> copies·years/mL or VCY-I/FUD ≥ 2.3 log<sub>10</sub> copies/mL, compared with those suppressed, had higher risk of death (HR=1.68 and HR=19.5, respectively). We observed no difference in risk of death comparing VCY-I suppressed with low-level VCY-I (HR=0.86) and VCY-I/FUD suppressed with low-level VCY-I/FUD (HR=0.78). Also age,

**Table 2.** Association of VCYs with death for all causes using multivariate Cox regression models

Variable	HR	95% CI	<i>P</i>
VCY-o (log <sub>10</sub> copies·years/mL)			
<6.16	ref.		
≥6.16	1.40	1.03–1.90	0.033
continuous	1.48	1.23–1.79	<0.001
VCY-e (log <sub>10</sub> copies·years/mL)			
<6.15	ref.		
≥6.15	1.39	1.11–2.01	0.036
continuous	1.47	1.22–1.78	<0.001
VCY-I (log <sub>10</sub> copies·years/mL)			
suppressed	ref.		
low-level VCY-I	0.86	0.56–1.31	0.477
≥3	1.68	1.16–2.44	0.006
continuous	1.15	1.07–1.24	<0.001
VCY-I/FUD (log <sub>10</sub> copies/mL)			
suppressed	ref.		
low-level VCY-I/FUD	0.78	0.54–1.12	0.182
≥2.3	19.5	11.10–34.26	<0.001
continuous	3.54	2.98–4.20	<0.001

VCY-o, VCY-overall; VCY-e, VCY-early; VCY-I, VCY-late; VCY-I/FUD, VCY-I divided by the follow-up duration.

The models were adjusted for age, gender, CD4 cell count at start of cART and intravenous drug use at baseline.

intravenous drugs use as risk factors and low CD4+ T cell count at baseline were associated with higher risk of death (Table S1). These results were confirmed considering these variables as continuous. We also performed a sensitivity analysis to evaluate the association between VCY-o as a time-dependent covariate and

risk of death in Cox regression models. We found an HR of 2.12 (95% CI=1.46–3.06,  $P<0.001$ ) for VCY-o  $\geq 6.16$  versus  $<6.16$  and an HR of 1.50 (95% CI=1.19–1.89,  $P=0.001$ ) when considering VCY-o as a continuous covariate.

An increasing risk of death was shown with increasing VCY-o and VCY-e over the median, VCY-l  $>3 \log_{10}$  copies·years/mL and VCY-l/FUD for any value (linear dose–response relationship), using multivariate Cox regression models with restricted cubic-spline terms for VCYS (Figure 3).

**pVL as predictor of long-term mortality in subjects with low-level VCY-l/FUD**

We restricted the analyses to subjects with low-level VCY-l/FUD for assessing the relationship between percentage of pVL  $>500$  copies/mL and risk of death. Of 1704 subjects with low-level VCY-l/FUD, 44.8% never had pVL  $>500$  copies/mL, 23.9% had  $<15\%$  of their measurements  $>500$  copies/mL and 31.2% had  $\geq 15\%$  in the follow-up (data not shown).

Using a multivariate Cox regression model, patients with low-level VCY-l/FUD and  $>15\%$  of pVL measurements  $>500$  copies/mL in the follow-up, compared with subjects with no measurement of pVL  $>500$  copies/mL, had higher risk of death (HR=2.3, 95% CI=1.44–3.76,  $P=0.001$ ), whereas no increased risk of death was found in subjects with  $<15\%$  of pVL measurements  $>500$  copies/mL (HR=0.76, 95% CI=0.41–1.41,  $P=0.4$ ). This result was confirmed including percentage of pVL  $>500$  copies/mL as a continuous variable in a Cox regression model with a restricted cubic spline (Figure 4).

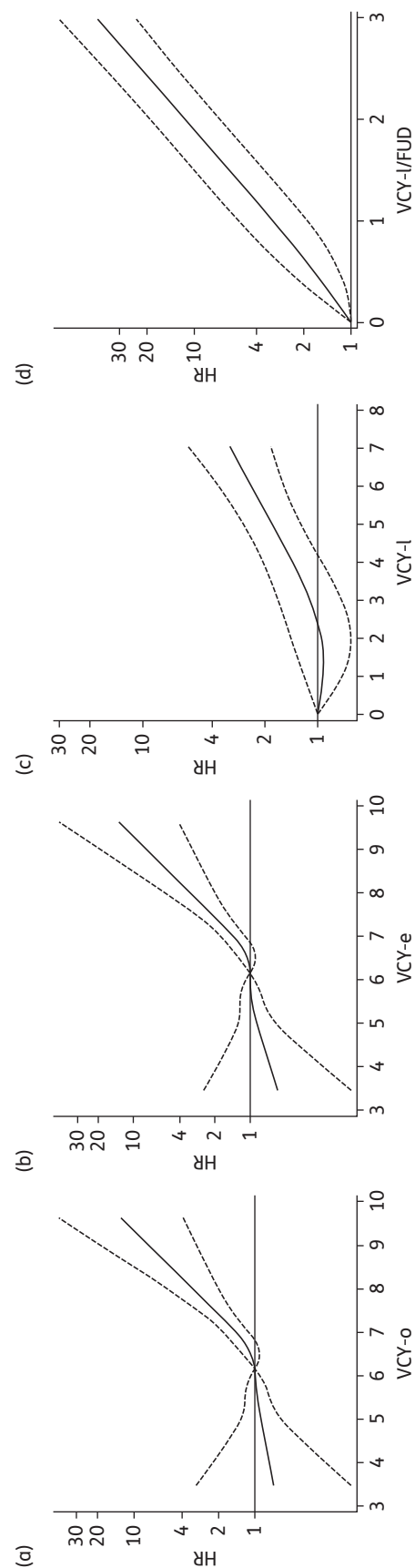
**CD4+ T cell count at baseline as predictor of mortality according to VCY**

Table S2 shows the patients’ characteristics at baseline according to VCY-l. Patients with VCY-l  $\geq 3$ , compared with those with VCY-l  $<3$ , were younger, had a higher proportion of IVDUs, hepatitis C or hepatitis B coinfection, and a lower proportion of men.

The prognostic role of the CD4+ T cell count was also assessed according to the three categories of VCY-l, using multivariate Cox regression models including age, sex and intravenous drug use as covariates (Table 3). CD4+ T cell count at baseline  $<200$  cells/mm<sup>3</sup>, compared with  $>200$  cells/mm<sup>3</sup>, was associated with a higher risk of death in all categories of VCY-l, but this association was statistically significant only in subjects with VCY-l  $>3$  (HR=1.84,  $P=0.002$ ). Similar results were found including in the models CD4+ T cell count at baseline as continuous or adjusting for VCY-e. Figure 5 shows non-linear relationships between CD4+ T cell counts and risk of death: for subjects with VCY-l  $\geq 3$  (Figure 5c), a decreasing risk of death with increasing CD4+ T cell count up to 400 cells/mm<sup>3</sup> was observed without significant variation above this level. No relationship was found for low-level VCY-l or VCY-l suppressed (Figure 5a and b).

**Discussion**

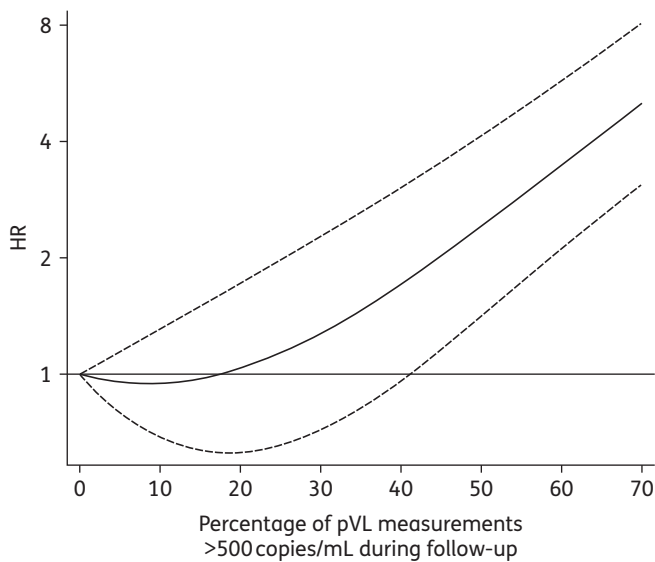
In people receiving cART, we observed a positive association between VCY-o, VCY-e, VCY-l and VCY-l/FUD with risk of death by all causes adjusting for the most important factors associated with negative outcomes in HIV-infected patients. The risk of death increased by about 40% for higher than the median levels of VCY-o and VCY-e. Compared with subjects with permanently



**Figure 3.** Risk of death (HR) according to distribution of VCY-o (a), VCY-e (b), VCY-l (c) and VCY-l/FUD (d). VCYS were modelled by cubic spline (continuous line) in Cox regression models adjusted for gender, age, intravenous drug use and CD4 cell count. The reference values are 6.16, 6.15 and 0  $\log_{10}$  copies·years/mL, respectively. The 95% confidence limits are shown as broken lines. Vertical axes have a logarithmic scale. VCY-o, VCY calculated from the start of ART until the end of follow-up (VCY-overall); VCY-e, VCY calculated in the first 8 months after cART initiation (VCY-early); VCY-l, VCY calculated after the eighth month from starting cART and during all follow-up (VCY-late); VCY-l/FUD, VCY-l divided by the follow-up duration.



suppressed pVL after the eighth month from starting cART, mortality increased by 70% for those with VCY-I  $\geq 3 \log_{10}$  copies-years/mL, and by about 20-fold for those with VCY-I  $\geq 2.3 \log_{10}$  copies/mL. Patients who maintained low-level VCY-I (calculated as pVL  $< 200$  copies/mL over a mean of 4.95 years of follow-up) or low-level VCY-I/FUD (calculated as pVL  $< 200$  copies/mL per year of follow-up) did not have a higher risk of death than patients with permanently suppressed VCY-I or VCY-I/FUD (HR=0.86 and HR=0.78, respectively). Also, CD4+ T cell count at baseline showed a negative predictive role for risk of death, but it was statistically significant only in subjects with VCY-I  $\geq 3 \log_{10}$  copies-years/mL.



**Figure 4.** Association between percentage of pVL  $> 500$  copies/mL and mortality risk (continuous line). Percentage of pVL was modelled as a restricted cubic spline term. Broken lines represent the 95% CI for the spline model.

Among patients with low-level VCY-I/FUD, 45% had never experienced pVL  $> 500$  copies/mL whereas  $> 30\%$  had  $> 15\%$  of pVL measurements  $> 500$  copies/mL during follow-up. Patients with  $> 15\%$  of pVL measurements  $> 500$  copies/mL, compared with those with no value, had a higher risk of death (HR=2.3,  $P=0.001$ ).

cART controls HIV plasma replication in most patients, leading to a reduction in morbidity and mortality. However, HIV replication remains an important clinical challenge because it is the major cause of chronic inflammation and persistent immune activation during cART<sup>17–20</sup> and both inflammation and immune activation have been related to AIDS and non-AIDS events and all-cause mortality.<sup>21–23</sup>

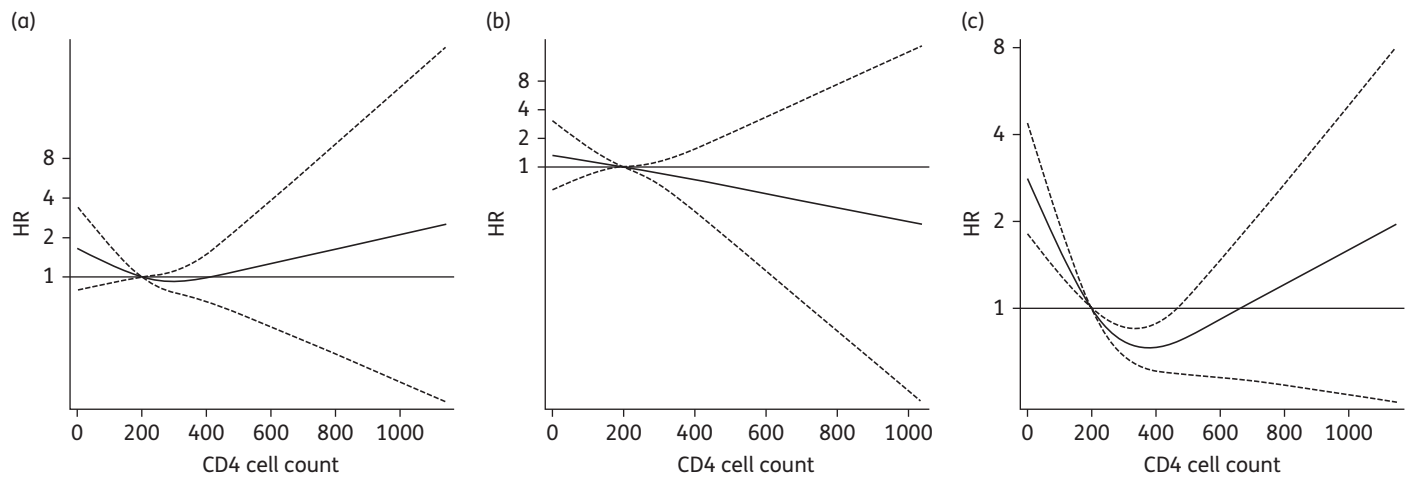
Very few studies have focused on cumulative HIV viral load, measured as VCY. Mugavero et al.<sup>12</sup> showed that VCY after the first 6 months of cART predicts all-cause mortality independently of CD4+ T cell count in HIV-infected patients initiating their first cART, suggesting that cumulative HIV replication may cause damage independently of its effect on immunodeficiency. Chirouze et al.<sup>11</sup> have recently confirmed this association in patients initiating their first antiretroviral PI-containing regimen, though not in a subgroup of cART-naïve patients, suggesting that VCY may be a useful marker of persistent viral replication for clinical care. Wright et al.<sup>13</sup> found that high VCY values were predictive of all-cause mortality when adjusting for recent CD4+ T cell count and recent pVL. In all these studies, VCY was analysed as a continuous variable or dichotomized at the median, computed for the whole period or after the first 6 months of therapy.

Their approach, however, did not investigate the shape of the dose–effect relationship between VCY and all-cause mortality; particularly, they did not evaluate whether low, but not null, values of VCY are related to a negative outcome. Instead, in our study we evaluated this relationship showing a linear increase of the risk of death for increasing levels of VCY-o, VCY-e, VCY-I and VCY-I/FUD. These findings may be useful for better comprehension of mechanisms related to death during HIV infection.

**Table 3.** Association of CD4 cell count at start of cART with death by all causes using a multivariate Cox regression model, according to VCY-I levels

Variable	VCY-I suppressed			Low-level VCY-I			VCY-I $\geq 3$		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Gender									
female	ref.			ref.			ref.		
male	1.02	0.47–2.22	0.967	2.10	0.82–5.39	0.121	1.16	0.73–1.82	0.529
Age (years)									
$< 35$	ref.			ref.			ref.		
35–44	1.82	0.72–4.64	0.206	1.57	0.61–4.06	0.350	1.30	0.81–2.09	0.284
$\geq 45$	2.80	1.10–7.12	0.030	3.68	1.45–9.35	0.006	3.03	1.83–5.02	$< 0.001$
Intravenous drug use									
no	ref.			ref.			ref.		
yes	2.20	1.06–4.54	0.034	3.16	1.54–6.47	0.002	3.02	2.05–4.45	$< 0.001$
CD4 cell count (cells/mm <sup>3</sup> )									
$\geq 200$	ref.			ref.			ref.		
$< 200$	1.57	0.85–2.89	0.146	1.37	0.74–2.54	0.323	1.84	1.25–2.71	0.002

VCY-I, VCY-late.



**Figure 5.** Risk of death (HR) according to CD4 cell count for VCY-I suppressed (a), VCY-I <3 log<sub>10</sub> copies-years/mL and not suppressed (b) and VCY-I ≥3 (c). CD4 cell counts were modelled by cubic spline (continuous line) in Cox regression models adjusted for gender, age and intravenous drug use. The CD4 reference value is 200 cells/mm<sup>3</sup>. The 95% confidence limits are shown as broken lines. Vertical axes have a logarithmic scale. VCY-I, VCY calculated after the eighth month from starting cART and during all follow-up (VCY-late).

Effective cART is expected to determine viral suppression 8–24 weeks after initiation.<sup>1</sup> Unfortunately, some patients experience treatment-related decrease of baseline HIV-1 levels that, however, does not achieve, or does not maintain, undetectable levels of viraemia over time.<sup>24</sup> Evidence concerning the effect of low, detectable levels (<200 copies/mL) of HIV viraemia on clinical outcomes is scant.<sup>10,18,23</sup> Our study is the first, to our knowledge, to evaluate the effect of low-level viraemia, measured as VCY-I, on all-cause mortality. Our findings show that patients who maintain low-level VCY-I have a risk of death similar to those with suppressed VCY during follow-up. These findings are in agreement with some previous studies carried out using pVL as a marker of HIV replication.<sup>10,18,23</sup> The reason for this lack of increased risk of death for subjects with low-level VCY is unclear. One hypothesis is that low replication could improve HIV-specific responses, positively influencing clinical outcome in HIV-infected patients.<sup>25,26</sup> On the other hand, it is possible that some episodes of low-level viraemia are the result of the different techniques used or biological variability and therefore do not reflect a ‘real’ increase in pVL. Indeed, various studies have demonstrated that repeated analysis of the same sample can show variations of up to 40%, in particular when HIV viraemia is low or depending on the assay used.<sup>27–29</sup>

Discrepancies are present in the literature about the virological impact of intermittent or persistent low-level HIV viraemia. Several authors have described that transient viraemic increases (‘blips’) are usually not associated with viroimmunological failure.<sup>30,31</sup> In contrast, other studies reported a correlation between persistent low-level viraemia and virological failure with detection of drug resistance.<sup>6,32,33</sup> A recent study shows that long episodes of pVL fluctuating at ~50 copies/mL under boosted PI-based regimens rarely result in drug resistance or virological failure.<sup>34</sup> The CASCADE study on HIV seroconverters showed a stronger benefit of cART in patients with higher VCY before initiating treatment as regards the risk of AIDS or death occurrence. However, the effect depended on the CD4+ T cell level: the benefit of cART was evident and similar for any VCY level at CD4+ <350 cells/mm<sup>3</sup>, whereas in patients with CD4+ ≥350 cells/mm<sup>3</sup> there was some evidence of

benefit only for high VCY.<sup>35</sup> This last finding is in agreement with our data showing that CD4+ T cell count at baseline was inversely related to mortality only in patients with higher VCY-I values. This is not surprising, as CD4+ T cell count at initiation of cART is a well-recognized predictive factor of disease progression or death, independent of age, sex and plasma HIV RNA.<sup>36</sup>

Our study has some limitations. First, the median duration of follow-up in our study was 4.1 years, which might be insufficient to assess the long-term impact of low-level viraemia, measured as VCY-I or VCY-I/FUD, on mortality. Second, VCY was calculated from cART initiation, therefore the impact of cumulative HIV exposure before this time was not evaluated. Probably, patients with lower CD4+ T cell count at baseline would have longer duration of HIV infection and therefore higher VCY before cART initiation.

In conclusion, our study shows that low levels of VCY-I are not associated with an increased risk of death for all causes compared with VCY-I permanently suppressed. However, among these patients, those with >15% of pVL measures >500 copies/mL had a double risk of death compared with those with none.

Our results support current treatment guidelines regarding the management of patients with low pVL, which suggest a regimen switch as soon as possible if pVL >500 copies/mL. Low CD4+ T cell count value at baseline seems to be predictive of risk of death only in the presence of high values of VCY-I, suggesting that these patients should be monitored carefully during follow-up. The clinical consequences of cumulative HIV viraemia on specific AIDS and non-AIDS events are still to be defined.

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## Transparency declarations

None to declare.

## Supplementary data

Figure S1 and Tables S1 and S2 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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