Original article

Very high pre-therapy viral load is a predictor of virological rebound in HIV-1-infected patients starting a modern first-line regimen

Daniele Armenia[1,2†], Domenico Di Carlo[3†], Alessandro Cozzi-Lepri[4], Andrea Calcagno[5], Vanni Borghi[6], Caterina Gori[7], Ada Bertoli[1], William Gennari[8], Rita Bellagamba[9], Antonella Castagna[10], Alessandra Latini[11], Carmela Pinnetti[9], Stefania Cicalini[9], Annalisa Saracino[12], Giuseppe Lapadula[13], Stefano Rusconi[14], Francesco Castelli[15], Simona Di Giambenedetto[16], Massimo Andreoni[17], Giovanni Di Perri[5], Andrea Antinori[9], Cristina Mussini[6], Francesca Ceccherini-Silberstein[1], Antonella D'Arminio Monforte[18], Carlo F Perno[7,19], Maria M Santoro[1], ICONA Foundation Study Group*

[1]Department of Experimental Medicine, University of Rome 'Tor Vergata', Rome, Italy
[2]Saint Camillus International University of Health Sciences, Rome, Italy
[3]Pediatric Clinical Research Center 'Romeo and Erica Invernizzi', University of Milan, Milan, Italy
[4]Institute for Global Health London, University College London, London, UK
[5]Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy
[6]Clinic of Infectious Diseases, University Hospital, University of Modena and Reggio Emilia, Modena, Italy
[7]Virology Unit, National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy
[8]Microbiology and Virology Unit, University Hospital Polyclinic, Modena, Italy
[9]Clinical Division of HIV/AIDS, National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy
[10]Infectious Diseases Department, IRCCS San Raffaele Scientific Institute & Vita-Salute University, Milan, Italy
[11]Unit of Dermatology and Sexually Transmitted Diseases, San Gallicano Dermatological Institute IRCCS, Rome, Italy
[12]Division of Infectious Diseases, University of Bari, Policlinic Hospital, Bari, Italy
[13]Division of Infectious Diseases, 'San Gerardo' Hospital, Monza, Italy
[14]Infectious Diseases Unit, DIBIC Luigi Sacco, University of Milan, Milan, Italy
[15]University Department of Infectious and Tropical Diseases, University of Brescia and Spedali Civili General Hospital, Brescia, Italy
[16]Infectious Diseases Unit, Catholic University of Sacred Heart, Rome, Italy
[17]Clinical Infectious Diseases, University Hospital 'Tor Vergata', Rome, Italy
[18]Department of Health Sciences, Clinic of Infectious Diseases, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy
[19]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

[†]Authors equally contributed to this work
*Corresponding author e-mail: santormaria@gmail.com

Background: Pre-cART (combined antiretroviral therapy) plasma viral load >500,000 copies/ml has been associated with a lower probability of achieving virological suppression, while few data about its role on maintenance of virological suppression are available. In this study we aimed to clarify whether high levels of pre-cART viraemia are associated with virological rebound (VR) after virological suppression.

Methods: HIV-infected individuals who achieved virological suppression after first-line cART were included. VR was defined as the first of two consecutive viraemia >50 copies/ml (VR50) or, in an alternative analysis, >200 copies/ml (VR200). The impact of pre-cART viraemia on the risk of VR was evaluated by survival analyses.

Results: Among 5,766 patients included, 59.2%, 31.4%, 5.2% and 4.2% had pre-cART viraemia ≤100,000, 100,001–500,000, 500,001–1,000,000 and >1,000,000 copies/ml, respectively. Patients with pre-cART viraemia levels >1,000,000 copies/ml had the highest probability of VR (>1,000,000; 500,000–1,000,000; 100,000–500,000; <100,000 copies/ml; VR50: 28.4%; 24.3%; 17.6%; 13.8%, *P*<0.0001; VR200: 14.4%; 11.1%; 7.2%; 7.6%; *P*=0.009).

By Cox multivariable analyses, patients with pre-cART viraemia >500,000 and >1,000,000 copies/ml showed a significantly higher risk of VR regardless of the VR end point used. No difference in the risk of VR was found between patients with pre-cART viraemia ranging 500,000–1,000,000 copies/ml and those with pre-cART viraemia >1,000,000 copies/ml, regardless of the VR end point used.

Conclusions: Pre-cART plasma viral load levels >500,000 copies/ml can identify fragile patients with poorer chance of maintaining virological control after an initial response. An effort in defining effective treatment strategies is mandatory for these patients that remain difficult to treat.

Introduction

Despite the overwhelming success of combined antiretroviral therapy (cART) [1–4], in some patients starting their first treatment, the effectiveness of cART is still not sufficient, with consequent residual viral replication and virological failure [5–8]. Studies underlined that high pre-cART plasma viral load significantly contributes to reduce the chances of HIV-1 suppression after first-line therapy [9–12]. Initial studies indicated that subjects with plasma viral load >100,000 copies/ml at cART initiation had a reduced chance of responding to treatment [13]. However, this threshold set at 100,000 copies/ml might be not optimal to identify patients with potentially less options to achieve and maintain virological suppression (VS), particularly at the time of new and more effective antiviral approaches. In fact, recent evidence highlighted that higher levels of pre-cART viral load (such as >500,000 copies/ml) are associated with a prolonged time and a lower probability of achieving VS both in randomized and observational studies [9–11,14]. An association between pre-cART viral load and the risk of virological rebound (VR) after the achievement of VS was also demonstrated [9,12], though data are still limited, mainly for patients with very high pre-cART viral load levels (>500,000 or >1,000,000 copies/ml).

Patients with very high pre-cART viral load induce uncertainty in clinicians' decision because treatment recommendations for this problematic category of patients are still missing [15,16]. In this context, treatments including integrase inhibitors (INIs) or even four-drug based strategies together with ritonavir/cobicistat boosted protease inhibitors (PIbs), seem to perform better than the classical 3-drug regimens based on PIs or non-nucleoside reverse transcriptase inhibitors (NNRTIs) [10,12].

In this analysis we evaluated the association between pre-cART viral load (with particular attention to values above 500,000 and 1,000,000 copies/ml) and the risk of VR (and potential subsequent selection of drug-resistance) in a large multi-centric cohort of HIV-1-infected patients who initially achieved VS.

Methods

Study population

HIV-1-infected patients were selected from: a large Italian anonymous database collecting data for HIV-1-infected patients followed at several clinical centres in Central Italy; the Icona Foundation Study, a cohort of HIV-infected patients, which superseded the original Italian Cohort of Antiretroviral-Naive Patients study [17]. Eligible individuals were those drug-naive who achieved VS (the first viraemia <50 copies/ml after cART start, regardless of therapy changes) after starting a first-line regimen. Participants had to satisfy the additional following criteria: age ≥18 years; first-line therapy based on at least three drugs among at least two antiretroviral classes; pre-cART viraemia >500 copies/ml; quantifiable viraemia at levels >500,000 copies/ml; at least one available viral load measurement after the date of achieving VS. Patients with documented acute infection were excluded. Analyses were performed by stratifying patients in the following pre-cART strata [9–11,18]: ≤100,000, 100,001–500,000, 500,001–1,000,000 and >1,000,000 copies/ml. Reference viraemia levels (dummies) were <100,000 and

500,000–1,000,000 copies/ml in order to evaluate the risks of VR in patients with pre-cART viraemia ranging 100,000–500,000 and >1,000,000 copies/ml.

Ethical approval

This study was conducted on data collected for clinical purposes. All data used in the study were previously anonymized, according to the requirements set by Italian Data Protection Code (leg. decree 196/2003) and by the General authorizations issued by the Data Protection Authority. Written informed consent for medical procedures/interventions performed for routine treatment purposes was collected for each patient included in the Icona Foundation Study or from other clinical centres involved in the study, in accordance with the ethics standards of the committee on human experimentation and the Helsinki Declaration (1983 revision).

Statistical analysis

All the analyses were performed using R open source environment for statistical computing (version 3.4.1, R Foundation for Statistical Computing, Vienna, Austria).

Survival analysis: virological rebound

Survival analysis was used to estimate the cumulative probability and predictors of experiencing VR. The date of VR was defined according the two following definitions: the date of the first of two consecutive viral load measurements >50 copies/ml (VR50); the date of the first of two consecutive viral load measurements >200 copies/ml (VR200). Survival analyses were performed by ignoring therapy changes and patients' follow-up was censored at the date of their last available viraemia measurement or at the time of the first therapy interruption (intention to treat approach [ITT]). Kaplan–Meier curves were performed to estimate the probability of VR according to pre-cART viraemia strata. Cox regression analysis was performed to evaluate the association of pre-cART viral load on risk VR after controlling for other potential confounding factors, such transgender, age, HIV-1 subtype, mode of HIV-1 transmission, year of cART initiation (per 1 increase of calendar year), pre-cART viral load (categorized as indicated above), pre-cART $CD4^+$ T-cell count (per 100 cells/mm^3 increase), type of initial regimen started, type of NRTI-backbone used, time (months) to achieving VS (categorised as: <6 months; 6–12 months; >12 months), and level of transmitted drug resistance detected at pre-cART genotypic resistance test (GRT). Cox regression models were performed under the assumption of proportionality of the hazards. To avoid any potential bias due to missing data, for variables containing missing values (HIV-1 subtype; risk factor; transmitted drug resistance, calculated according to the list of Bennet *et al.* [19]) a multiple imputation statistical approach was performed by using MICE (Multivariate Imputation via Chained Equations) package of R. Moreover, a model excluding variables with missing values was also built for confirmation.

Evaluation of resistance detected after virological rebound

Among patients with an available plasma GRT performed within 6 months after VR, the prevalence of primary resistance mutations (PRMs; according to the list panelled on Stanford HIV Drug Resistance Database) was evaluated and compared according to the levels of viraemia at GRT by using χ^2 test for trend.

Sensitivity analyses

To confirm the robustness of the results, the following additional Cox regression models were performed: excluding patients who started a rilpivirine-containing regimen because the usage of this NNRTI is recommended only in patients with pre-cART viraemia <100,000 copies/ml [15,16]; by censoring patients at the end of their first-line regimen (on treatment approach [OT]).

Results

Patients' characteristics

Overall, 5,766 patients were included in the study. Patients' characteristics are summarized in Table 1. Patients started their first-line regimen on average in 2011 (median [first quartile–third quartile: Q_1–Q_3] 2011 [2008–2013]). Stratifying patients according to viraemia ranks, 59.2%, 31.4%, 5.2% and 4.2% had their pre-cART viraemia value in the ranges of ≤100,000, 100,001–500,000, 500,001–1,000,000 and >1,000,000 copies/ml, respectively.

The proportion of patients who achieved VS before 6 months, over 6–12 months and after 12 months of the date of treatment initiation was 67.7%, 24.1% and 9.2%, respectively. Regarding first-line treatments, the most commonly used PIb was lopinavir (911, 34.1%), followed by atazanavir (861, 32.2%) and darunavir (780, 29.2%). Efavirenz (1,771, 71.7%) was the most commonly administered NNRTI, followed by rilpivirine (506, 20.5%) and nevirapine (189, 7.7%). Finally, raltegravir was the most commonly used INI (262, 49.6%), followed by elvitegravir (165, 31.3%) and dolutegravir (101, 19.1%). Patients with higher pre-cART viraemia levels were more likely to have started a triple therapy containing a PIb, an INI or a therapy containing at least four drugs, while people with lower pre-cART viraemia levels were mainly treated with an NNRTI-based triple therapy (P<0.001; Table 1).

Transmitted drug resistance was observed in the 9.6% (PI [0.8%]; NRTI [3.3%]; NNRTI [5.9%]) of patients with an available GRT at cART baseline (n=3,038).

Table 1. Characteristics of 5,766 drug-naive HIV-1-infected patients achieving virological suppression after the first-line therapy stratified for pre-cART plasma viral load

Characteristics	Overall (*n*=5,766)	Pre-cART plasma viral load, copies/ml ≤100,000 (*n*=3,407)	100,001–500,000 (*n*=1,814)	500,001–1,000,000 (*n*=302)	>1,000,000 (*n*=243)	*P*-value[a]
Male, *n* (%)	4,499 (78)	**2,591 (76.0)**	**1,492 (82.2)**	**234 (77.5)**	**182 (74.9)**	**0.039**
Median age, years (Q_1–Q_3)	39 (33–46)	**38 (32–46)**	**40 (33–47)**	**42 (34–49)**	**42 (34–50)**	**<0.0001↑**
Median pre-cART CD4⁺ T-cell count, cells/mm³ (Q_1–Q_3)	280 (153–390)	**313 (217–422)**	**238 (106–357)**	**135 (44–290)**	**99 (37–216)**	**<0.0001↓**
Time (months) of achieving VS						
<6, *n* (%)	3,846 (66.7)	**2,590 (76)**	**1,054 (58.1)**	**123 (40.7)**	**79 (32.5)**	**<0.0001**
6–12, *n* (%)	1,389 (24.1)	**593 (17.4)**	**573 (31.6)**	**128 (42.4)**	**95 (39.1)**	**<0.0001**
>12, *n* (%)	531 (9.2)	**224 (6.6)**	**187 (10.3)**	**51 (16.9)**	**69 (28.4)**	**<0.0001**
Risk factor						
Homosexual, *n* (%)	2,398 (41.6)	**1,369 (40.2)**	**783 (43.2)**	**145 (48)**	**101 (41.6)**	**0.021**
Heterosexual, *n* (%)	2,398 (41.6)	1,446 (42.4)	738 (40.7)	116 (38.4)	98 (40.3)	0.124
Drug abuser, *n* (%)	512 (8.9)	310 (9.1)	159 (8.8)	22 (7.3)	21 (8.6)	0.424
Other, *n* (%)	269 (4.7)	160 (4.7)	82 (4.5)	13 (4.3)	14 (5.8)	0.799
Unknown, *n* (%)	189 (3.3)	122 (3.6)	52 (2.9)	6 (2.0)	9 (3.7)	0.248
Transmitted drug resistance, *n* (%)[b,c]	292 (9.6)	173 (9.8)	83 (8.5)	24 (15.1)	12 (9.2)	0.702
Subtype						
B, *n* (%)	2,553 (44.3)	1,504 (44.1)	840 (46.3)	122 (40.4)	87 (35.8)	0.099
CRF02_AG, *n* (%)	217 (3.8)	**118 (3.5)**	**70 (3.9)**	**11 (3.6)**	**18 (7.4)**	**0.014**
F, *n* (%)	168 (2.9)	**77 (2.3)**	**64 (3.5)**	**13 (4.3)**	**14 (5.8)**	**<0.0001**
C, *n* (%)	142 (2.5)	92 (2.7)	31 (1.7)	9 (3.0)	10 (4.1)	0.870
Other, *n* (%)	358 (6.2)	**202 (5.9)**	**107 (5.9)**	**26 (8.6)**	**23 (9.5)**	**0.022**
Unknown, *n* (%)	2,328 (40.4)	1,414 (41.5)	702 (38.7)	121 (40.1)	91 (37.4)	0.065
First-line therapy						
2 NRTIs + 1 PIb, *n* (%)	2,675 (46.4)	**1,403 (41.2)**	**952 (52.5)**	**175 (57.9)**	**145 (59.7)**	**<0.0001**
2 NRTIs + 1 NNRTI, *n* (%)	2,469 (42.8)	**1,668 (49.0)**	**673 (37.1)**	**84 (27.8)**	**44 (18.1)**	**<0.0001**
2 NRTIs + 1 INI, *n* (%)	401 (7.0)	**257 (7.5)**	**114 (6.3)**	**19 (6.3)**	**11 (4.5)**	**0.024**
PIb + INI + ≥1 NRTI, *n* (%)	127 (2.2)	**33 (1.0)**	**37 (2.0)**	**20 (6.6)**	**37 (15.2)**	**<0.0001**
Other, *n* (%)	94 (1.6)	46 (1.4)	38 (2.1)	4 (1.3)	6 (2.5)	0.090
NRTI-backbone[d]						
TDF/TAF+FTC, *n* (%)	4,282 (74.4)	**2,516 (73.9)**	**1,317 (72.8)**	**250 (83.1)**	**199 (82.6)**	**0.001**
ABC+3TC, *n* (%)	451 (7.8)	**306 (9)**	**113 (6.2)**	**18 (6)**	**14 (5.8)**	**0.001**
AZT+3TC, *n* (%)	613 (10.6)	349 (10.3)	219 (12.1)	24 (8.0)	21 (8.7)	0.832
Other, *n* (%)	410 (7.2)	233 (6.8)	161 (8.9)	9 (3.0)	7 (2.9)	0.124
More than three drugs, *n* (%)	219 (3.8)	**82 (2.4)**	**75 (4.1)**	**22 (7.3)**	**40 (16.5)**	**<0.0001**
Median year of cART initiation, (Q_1–Q_3)	2011 (2008–2013)	**2011 (2008–2013)**	**2010 (2007–2012)**	**2011 (2009–2013)**	**2012 (2010–2013)**	**<0.0001↑**
Median plasma viral load follow-up length, years (Q_1–Q_3)	3 (1–6)	**3 (1–6)**	**3 (2–6)**	**3 (2–5)**	**3 (1–4)**	**0.010↑**
Median number of plasma viral load measurements per year (Q_1–Q_3)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	0.509

Bold font indicates factors significantly associated with pre-therapy plasma viral load ranges ($P<0.05$). [a]*P*-value was calculated by χ^2 test for trend for qualitative variables and by Jonckheere-Terpstra test (↑, alternative one-side hypothesis: increasing. ↓, alternative one-side hypothesis: decreasing) for quantitative variables. [b]Only for patients with available genotypic resistance test at baseline: *n*=3,038 (≤100,000, *n*=1,772. 100,001–500,000, *n*=976. 500,001–1,000,000, *n*=159. >1,000,000, *n*=131). [c]As the presence of at least one mutation from WHO surveillance transmitted resistance list (Bennet *et al.* [19]). [d]Only for patients under a nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) containing regimen: *n*=5,756 (≤100,000, *n*=3,404. 100,001–500,000, *n*=1,810. 500,001–1,000,000, *n*=301. >1,000,000, *n*=241). ABC, abacavir; AZT, zidovudine; cART, combined antiretroviral therapy; FTC, emtricitabine; INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PIbs, ritonavir/cobicistat protease inhibitors; Q_1, first quartile; Q_3, third quartile; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; VS, virological suppression; WHO, World Health Organization; 3TC, lamivudine.

Survival analyses: virological rebound

Virological rebound: 50 copies/ml threshold (VR50)

Overall, by 4 years from the date of achieving VS, the risk of experiencing VR50 was 16.2% (95% CI: 15%, 17.3%). Median (IQR) viral load at VR50 was 208 (87–5,834) copies/ml. Patients who experienced VR50 were under virological suppression since a median (IQR) time of 12.4 (4.3–30.8) months.

Stratifying patients by pre-cART viraemia ranges, those with pre-cART viraemia levels >1,000,000 copies/ml had the highest probability of VR50 and a dose–response relationship of decreasing risks with lower viraemia level was observed (>1,000,000: 28.4%; 500,001–1,000,000: 24.3%; 100,001–500,000: 17.6%; ≤100,000: 13.8%; $P<0.0001$; Figure 1A).

Cox multivariable regression model confirmed the impact of pre-cART viraemia onVR50. In fact, the adjusted hazard ratio (aHR) of experiencing VR50 was significantly higher in patients having pre-cART viraemia levels >1,000,000 copies/ml and 500,001–1,000,000 copies/ml, compared with those having pre-cART viraemia ≤100,000 copies/ml ($P<0.0001$; Table 2).

To evaluate potential differences in terms of VR risk between pre-cART viraemia strata 500,000–1,000,000 and >1,000,000 copies/ml, we repeated the same Cox regression models by considering as reference category for pre-cART viraemia the stratum 500,000–1,000,000 copies/ml (Table 2). We found no evidence that the risk of VR50 in patients with pre-cART viraemia >1,000,000 was different to that of those with pre-cART viraemia ranging 500,000–1,000,000 copies/ml (aHR [95% CI]: 1.03 [0.72, 1.49]; $P=0.860$). Whereas, compared to those belonging to the 500,000–1,000,000 copies/ml stratum, patients with pre-cART viraemia <100,000 and 100,000–500,000 copies/ml had a significant lower aHR of VR (Additional file 1).

Other factors associated with a higher risk of VR50, independently of pre-cART viraemia, were to be drug abuser versus to be homosexual and carrying a non-B subtype HIV-1 versus the more common B subtype (Table 2). In contrast, factors associated with a lower aHR of experiencing VR50 were a more recent year of first-line cART initiation, a first-line regimen based on two NRTIs plus one NNRTI (compared with PI-based regimens), and higher levels of pre-cART $CD4^+$ T-cell count.

Virological rebound: 200 copies/ml threshold (VR200)

Overall, by 4 years from the date of achieving VS, the risk of experiencing VR200 was 7.9% (95% CI: 7.0%, 8.7%). Median (IQR) viral load at VR200 was 6,938 (742–53,649) copies/ml. Patients who experienced VR200 were under virological suppression since a median (IQR) time of 16.7 (7.3–39.0) months.

Again, patients with pre-cART viraemia levels >1,000,000 and 500,001–1,000,000 copies/ml had the highest probability of VR200 (>1,000,000 copies/ml: 14.4%; 500,001–1,000,000 copies/ml: 11.1%; 100,001–500,000 copies/ml: 7.2%; ≤100,000 copies/ml: 7.6%; $P=0.009$; Figure 1B). Cox multivariable regression model showed results similar to those observed when using the >50 copies/ml threshold endpoint (Table 2).

Also, for the end point VR200, we found no evidence that the risk of VR in patients with pre-cART viraemia >1,000,000 was different to that of those with pre-cART viraemia ranging 500,000–1,000,000 copies/ml (aHR [95% CI]: 1.02 [0.60, 1.75]; $P=0.942$; Additional file 1).

Sensitivity analyses

Considering that rilpivirine has been approved only for treating patients with pre-cART viraemia <100,000 copies/ml [15,16], to avoid potential bias related with pre-cART viraemia due to this recommendation, we performed a Cox regression model after excluding patients who started ART with rilpivirine. Results of the model were superimposable with those obtained from the full set and the VR50 end point (Additional file 2).

We repeated Cox regression analyses by censoring patients' follow-up at the date of their last viraemia measurement available during the first-line treatment (OT approach); results were similar to those obtained from ITT approach (Additional file 3).

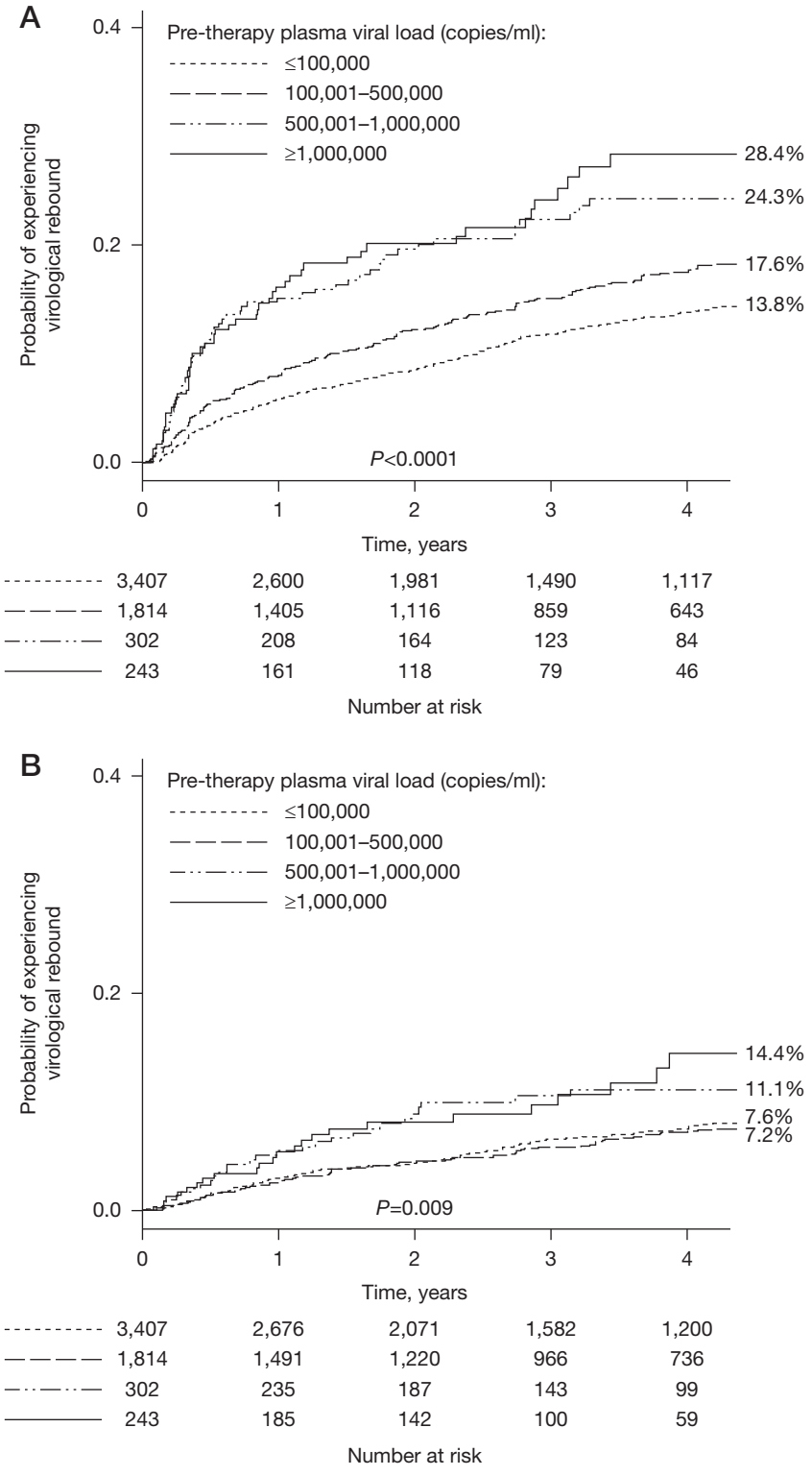
Finally, we repeated Cox regression analyses by excluding variables with missing values (HIV-1 subtype, risk factor and transmitted drug resistance). The final model showed results superimposable to the those observed in the model built by using MICE approach (data not shown).

Overview of resistance detected after VR

Among 709 patients experiencing VR, 170 (24.0%) had an available GRT after VR; 71 (41.8%) of them had at least one PRM. In particular, 9 (5.3%), 48 (28.2%) and 49 (28.8%) patients had at least one PI, NRTI and NNRTI PRM, respectively. The proportion of patients with resistance to any drug class was significantly higher with higher viraemia levels at the time of GRT after VR (ranging from 27.0% at GRT viraemia of 51–200 copies/ml to 36.4% at viraemia >100,000 copies/ml, with a peak of 61.5% at viraemia of 1,000–10,000 copies/ml; $P=0.007$; Figure 2).

We also evaluated the emergence of resistance after VR according to pre-cART viraemia levels. By cross tabulating the detection of PRMs with pre-cART viraemia levels, a considerable proportion of patients with resistance after VR was also found in those with very high

Figure 1. Kaplan–Meier curve estimates of cumulative probability of virological rebound according to pre-therapy plasma viral load ranges by 4 years



Kaplan–Meier cumulative probability estimates of virological rebound [**(A)** the first of two consecutive plasma viral load measurements >50 copies/ml; **(B)** the first of two consecutive plasma viral load measurements >200 copies/ml] at 4 years after achieving virological suppression was performed by stratifying patients according to pre-therapy viral load ranges (copies/ml). Analyses were performed regardless therapy changes and patients were censored at the last viraemia measurement available or at the time of the first therapy interruption. *P*-values were calculated by using log-rank test for trend. A *P*-value <0.05 was considered statistically significant.

Table 2. Factors associated with virological rebound in HIV-1-infected patients achieving virological suppression after the first-line therapy.

	Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral loads >50 copies/ml)				Hazard ratio of experiencing virological rebound (first of two consecutive plasma viral loads >200 copies/ml)			
Variables	Crude HR (95% CI)	Crude *P*-value	Adjusted[a] HR (95% CI)	Adjusted[a] *P*-value	Crude HR (95% CI)	Crude *P*-value	Adjusted[a] HR (95% CI)	Adjusted[a] *P*-value
Gender (female versus male[b])	**1.30 (1.12, 1.52)**	**0.001**	1.08 (0.92, 1.27)	0.346	**1.65 (1.34, 2.04)**	**<0.0001**	**1.27 (1.02, 1.58)**	**0.034**
Age (per 5 years increase)	1.02 (0.98, 1.05)	0.338	1.00 (0.97, 1.04)	0.831	0.96 (0.92, 1.01)	0.126	0.96 (0.91, 1.02)	0.170
HIV-1 subtype[c] (non-B versus B[b])	**1.36 (1.17, 1.59)**	**<0.0001**	**1.46 (1.24, 1.72)**	**<0.0001**	**1.45 (1.17, 1.80)**	**0.001**	**1.60 (1.27, 2.01)**	**<0.0001**
Mode of HIV-1 transmission[c]								
Homosexual[b]	1		1		1		1	
Heterosexual	1.09 (0.94, 1.27)	0.270	1.13 (0.97, 1.32)	0.116	1.21 (0.96, 1.51)	0.100	1.23 (0.98, 1.54)	0.072
Drug abuser	**1.79 (1.45, 2.21)**	**<0.0001**	**1.49 (1.20, 1.85)**	**<0.0001**	**2.60 (1.97, 3.41)**	**<0.0001**	**2.08 (1.57, 2.75)**	**<0.0001**
Other	1.05 (0.74, 1.51)	0.775	0.98 (0.68, 1.41)	0.918	0.94 (0.53, 1.65)	0.822	0.87 (0.49, 1.55)	0.645
Year of cART initiation (per 1 year increase)	**0.89 (0.87, 0.91)**	**<0.0001**	**0.89 (0.87, 0.92)**	**<0.0001**	**0.86 (0.83, 0.88)**	**<0.0001**	**0.88 (0.84, 0.92)**	**<0.0001**
Pre-cART viral load								
≤100,000[b] copies/ml	1		1		1		1	
100,001–500,000 copies/ml	**1.29 (1.11, 1.50)**	**0.001**	1.16 (0.99, 1.36)	0.068	0.92 (0.74, 1.15)	0.476	0.90 (0.72, 1.13)	0.376
500,001–1,000,000 copies/ml	**2.04 (1.57, 2.65)**	**<0.0001**	**1.92 (1.46, 2.54)**	**<0.0001**	**1.59 (1.08, 2.32)**	**0.018**	**1.85 (1.24, 2.76)**	**0.003**
>1,000,000 copies/ml	**2.32 (1.75, 3.08)**	**<0.0001**	**1.99 (1.46, 2.71)**	**<0.0001**	**1.84 (1.23, 2.77)**	**0.003**	**1.89 (1.21, 2.95)**	**0.005**
Pre-cART CD4$^+$ T-cell count (per 100 cells/mm^3 increase)[c]	**0.85 (0.81, 0.88)**	**<0.0001**	**0.94 (0.90, 0.99)**	**0.010**	**0.89 (0.83, 0.94)**	**<0.0001**	0.97 (0.91, 1.03)	0.366
Type of initial regimen started								
2 NRTIs + 1 PIb[b]	1		1		1		1	
2 NRTIs + 1 NNRTI	**0.67 (0.58, 0.78)**	**<0.0001**	**0.68 (0.59, 0.80)**	**<0.0001**	0.85 (0.70, 1.04)	0.120	**0.79 (0.64, 0.98)**	**0.030**
2 NRTI + INI	**0.46 (0.29, 0.74)**	**0.001**	0.87 (0.54, 1.42)	0.586	**0.26 (0.10, 0.70)**	**0.008**	0.52 (0.19, 1.41)	0.198
PIb + INI + ≥1NRTI	0.65 (0.35, 1.23)	0.186	0.69 (0.34, 1.40)	0.301	0.63 (0.24, 1.71)	0.366	0.70 (0.22, 2.23)	0.546
Other	1.22 (0.79, 1.87)	0.371	0.90 (0.57, 1.41)	0.634	1.31 (0.73, 2.35)	0.359	0.82 (0.45, 1.52)	0.534
Type of NRTI-backbone used								
TDF + FTC[b]	1		1		1		1	
ABC + 3TC	0.97 (0.72, 1.31)	0.841	0.88 (0.65, 1.19)	0.414	1.19 (0.78, 1.81)	0.425	1.08 (0.70, 1.65)	0.739
AZT + 3TC	**2.14 (1.79, 2.56)**	**<0.0001**	1.08 (0.85, 1.37)	0.529	**2.65 (2.08, 3.39)**	**<0.0001**	1.25 (0.89, 1.74)	0.200
Other	**1.95 (1.58, 2.40)**	**<0.0001**	1.01 (0.77, 1.34)	0.928	**2.87 (2.20, 3.76)**	**<0.0001**	1.32 (0.91, 1.91)	0.142
Time to achieving VS								
<6[b] months	1		1		1		1	
6–12 months	1.13 (0.95, 1.33)	0.163	0.98 (0.83, 1.17)	0.857	0.88 (0.69, 1.14)	0.336	0.85 (0.65, 1.09)	0.204
>12 months	**1.69 (1.38, 2.08)**	**<0.0001**	1.02 (0.81, 1.27)	0.880	**1.63 (1.22, 2.17)**	**0.001**	0.93 (0.68, 1.26)	0.631
TDR detected at pre-cART GRT[c,d]	0.84 (0.66, 1.05)	0.127	0.85 (0.67, 1.07)	0.159	0.80 (0.57, 1.11)	0.184	0.86 (0.61, 1.20)	0.379

Bold font indicates factors that were significantly associated ($P<0.05$) with virological rebound. [a]Adjusted for: gender, age, HIV-1 subtype, mode of HIV-1 transmission, year of combined antiretroviral therapy (cART) initiation, pre-cART viral load, pre-cART CD4$^+$ T-cell count, type of initial regimen started, type of nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)-backbone used, time to achieving virological suppression (VS) and level of transmitted drug resistance (TDR) detected at pre-cART genotypic resistance test (GRT). [b]Reference group (dummy). [c]A multiple imputation approach was performed to fill missing values. [d]As the presence of at least one mutation from World Health Organization (WHO) surveillance TDR list (Bennet *et al.* [19]). ABC, abacavir; AZT, zidovudine; FTC, emtricitabine; HR, hazard ratio; INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PIb, ritonavir-cobicistat boosted protease inhibitor; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine.

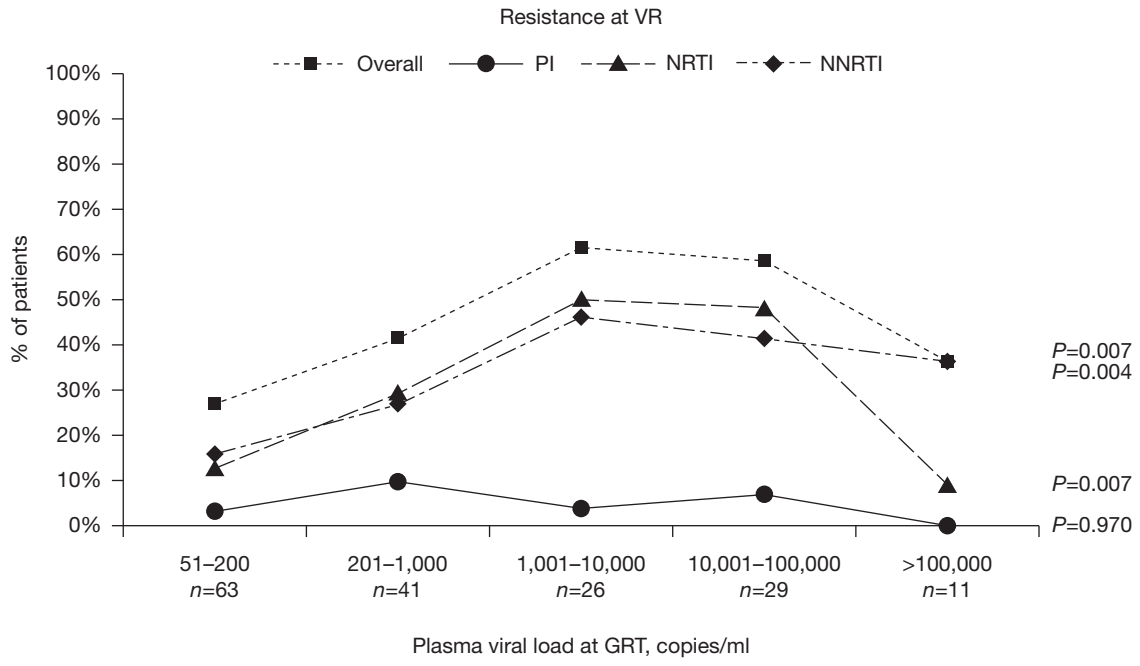
pre-cART viraemia (pre-cART viraemia [copies/ml]: % of patients with ≥1 PRM after VR: <100,000: 39.6%; 100,001–500,000: 48.6%; 500,001–1,000,000: 27.3%; >1,000,000: 44.4%; P=0.854).

A total of 67 out of 170 patients had the GRT available after VR under the first-line treatment without any therapy switch. Among them, the proportion of patients with at least one PRM after VR was lower among subjects receiving a PI-based cART (12/37, 32.4%) compared with those receiving a NNRTI-based cART (17/30, 56.7%; P=0.046).

Regarding INI resistance, integrase GRT after VR was available for 31 patients. Among these, three patients (9.7%) developed the INI PRM N155H.

We also performed an analysis on a sub-group of patients who had both a GRT before cART start and a GRT after VR (114/170, 67.1%), to evaluate the extent of new PRMs selected. Among the 40 (35.1%) patients

Figure 2. Resistance prevalence detected after VR according to pre-therapy viral load ranges



Line plots represent the proportion of patients with resistance detected after virological rebound (VR) considering resistance to any drug (square with dotted line), protease inhibitor (PI) resistance (circle with solid line), nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) resistance (triangle with dotted line) and non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance (diamond with dotted line). Resistance was stratified according to pre-therapy viral load ranges. *P*-values were calculated by using χ^2 test for trend. A *P*-value <0.05 was considered statistically significant. GRT, genotypic resistance test.

with resistance after VR, almost all (38, 95%) developed new PRMs.

Discussion

In the present manuscript, by analysing a large cohort of HIV-1-infected patients followed in several clinical centres in Italy who initially achieved VS, we observed a strong association between pre-cART viral load and the risk of VR after VS (by both ITT and OT approaches, also after adjusting for several confounding factors), confirming the negative role of very high viraemia levels (>500,000 copies/ml) on the maintenance of virological control [9]. While in previous analysis we compared patients with a viral load below or above 500,000 copies/ml [9], here, thanks to the much larger sample size, we could explore the issue more in depth by using finer viraemia categories, including values >1,000,000 copies/ml. We observed a significantly raised risk of VR in patients who started with pre-cART viraemia levels of 500,001–1,000,000 copies/ml as compared with those who started with a value <100,000 copies/ml. No further increase in risk was significantly present for patients starting with viraemia values >1,000,000 copies/ml. Thus, our results indicate that very high pre-cART viraemia (>500,000 copies/ml) seems to be more strongly associated with a negative response to first-line therapy, compared with the widely used levels of 100,000 copies/ml.

Raffi *et al.* [12] in a recent publication also showed that pre-cART viral load is negatively associated with the chance of maintaining virological control after first-line therapy initiation. However, the authors found a difference in risk of VR when comparing pre-cART viraemia levels > or <100,000 copies/ml but no further differences in the strata of 100,000–500,000 and >500,000 copies/ml [12]. This discrepancy might be explained by the fact that only patients with treatments including two NRTIs plus one PI or one INI or efavirenz were included in this French study. Indeed, selection might underestimate the number of patients with very high pre-cART viral load that might receive alternative treatments [10]. For this reason, in our analysis, we decided to include all patients treated with at least three drugs among at least two antiretroviral classes (regardless the recommended first-line regimens) to better represent real settings.

Of note, we found that NNRTI-based therapies (regardless of rilpivirine use) were associated with a

significant lower risk of VR than PIb-based therapies. However, we found that the usage of NNRTIs is associated with a higher rate of resistance selection after VR compared to PIs, confirming that NNRTIs, despite their high potency, have a lower genetic barrier to develop resistance compared with PIs.

Regarding virological response under INIs, also this drug class performed better than PIs, even though only at univariable analyses (probably due to the very low number of rebounds documented under INI-based treatments; Table 2). These results regarding the drug class comparison are consistent with data already available [10,12,20,21].

In our study we also explored the prevalence of resistance after VR according to viraemia levels at GRT. We detected a considerable rate of resistance also at low-level viraemia (27% at viraemia 51–200 copies/ml), in line with other previous studies [22–25], confirming that the presence of resistance at these low viraemia ranges is not a rare event. In this context, considering that GRT is reliable even at low-level viraemia [22–25], resistance in patients experiencing rebound, especially with high pre-cART plasma viral load, should be promptly tested after rebound regardless viraemia magnitude to avoid virological failure and/or loss of treatment options related to resistance development. Indeed, resistance detected at low-level viraemia has been already associated with an increased risk of virological failure [26].

Our analysis has a number of limitations. First, important potential non-measured confounders such as adherence levels and information about acute/recent seroconversion were not evaluated because they were poorly recorded in our database. Concerning adherence, even though patients might have a good initial compliance because the majority of them achieved undetectability under their first drug regimen, we found that drug abuser patients had an increased risk of experiencing VR. These results might reflect an indirect association between low adherence and VR, as recently observed in study conducted on Swiss Cohort that confirmed the association of drug abuse with poorer adherence and consequently with virological failure [27]. By contrast, we found that patients receiving an NNRTI-based regimen had a lower of risk of experiencing VR compared with those treated with a PI-based regimen. This finding may reflect clinician preference for prescribing PI-based cART to patients perceived to be at risk for poor adherence [28]. Another point is that we cannot extrapolate robust results from patients starting INI-based treatment. Due to the extraordinary INIs efficacy, we observed very few rebound events. Further studies including a larger number of patients treated with INIs are required to provide more robust results regarding this drug class.

In conclusion, pre-cART viraemia >500,000 copies/ml is a condition that can identify patients with lower chances of maintaining virological control after initial undetectability. An effort in defining effective treatment strategies is mandatory for these patients that remain difficult to treat.

Acknowledgements

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BIOLOGICAL BANK INMI: F Carletti, S Carrara, A Di Caro, S Graziano, F Petroni, G Prota, S Truffa.
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MA Ursitti (Reggio Emilia); M Andreoni, A Antinori, R Cauda, A Cristaudo, V Vullo, R Acinapura, D Moschese, M Capozzi, A Mondi, A Cingolani, M Rivano Capparuccia, G Iaiani, A Latini, R Gagliardini, MM Plazzi, G De Girolamo, A Vergori (Roma); M Cecchetto, F Viviani (Rovigo); G Madeddu, A De Vito(Sassari); B Rossetti, F Montagnani (Siena); A Franco, R Fontana Del Vecchio (Siracusa); D Francisci, C Di Giuli (Terni); P Caramello, G Di Perri, S Bonora, GC Orofino, M Sciandra (Torino); M Bassetti, A Londero (Udine); V Manfrin, G Battagin (Vicenza); G Starnini, A Ialungo (Viterbo).

ICONA Foundation is supported by unrestricted grants from Gilead Sciences, Janssen, Merck Sharp and Dohme and ViiV Healthcare.

This work was also financially supported by the Italian Ministry of Education, University and Research (MIUR) [Bandiera InterOmics grant number PB05 1° and PRIN 2012 grant number 2012L783TW] and an unrestricted grant from AVIRALIA foundation.

Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: A table showing factors associated with virological rebound in HIV-1-infected patients achieving virological suppression after the first-line therapy starting can be found at https://www.intmedpress.com/uploads/documents/4455_Armenia_Addfile1.pdf

Additional file 2: A table showing factors associated with virological rebound in HIV-1-infected patients achieving virological suppression after the first-line therapy (by excluding 502 patients under a rilpivirine-containing regimen) can be found at https://www.intmedpress.com/uploads/documents/4455_Armenia_Addfile2.pdf

Additional file 3: A table showing factors associated with virological rebound under the first-line therapy in HIV-1-infected patients achieving virological suppression (on treatment approach) can be found at https://www.intmedpress.com/uploads/documents/4455_Armenia_Addfile3.pdf

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Accepted 27 February 2019; published online 12 April 2019