Original article

Impact of pre-therapy viral load on virological response to modern first-line HAART

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Background: We tested whether pre-HAART viraemia affects the achievement and maintenance of virological success in HIV-1-infected patients starting modern first-line therapies.

Methods: A total of 1,430 patients starting their first HAART (genotype-tailored) in 2008 (median; IQR: 2006–2009) were grouped according to levels of pre-HAART viraemia (≤30,000, 30,001–100,000, 100,001–300,000, 300,001–500,000 and >500,000 copies/ml). The impact of pre-therapy viraemia on the time to virological success (viraemia ≤50 copies/ml) and on the time to virological rebound (first of two consecutive viraemia values >50 copies/ml after virological success) were evaluated by Kaplan–Meier curves and Cox regression analyses.

Results: Median pre-HAART viraemia was 5.1 $\log_{10}$ copies/ml (IQR 4.5–5.5), and 53% of patients had viraemia >100,000 copies/ml. By week 48, the prevalence of patients reaching virological success was >90% in all pre-HAART viraemia ranges, with the only exception of range >500,000 copies/ml (virological success =83%; *P*<0.001). Higher pre-HAART viraemia was tightly correlated with longer median time to achieve virological success. Cox multivariable estimates confirmed this result: patients with pre-HAART viraemia >500,000 copies/ml showed the lowest hazard of virological undetectability after adjusting for age, gender, pre-HAART CD4⁺ T-cell count, transmitted drug resistance, calendar year and third drug administered (adjusted hazard ratio [95% CI]: 0.27 [0.21, 0.35]; *P*<0.001). Pre-HAART viraemia >500,000 copies/ml was also associated with higher probability of virological rebound compared with patients belonging to lower viraemia strata at weeks 4, 12 and 24 (*P*=0.050).

Conclusions: At the time of modern HAART, and even though an average >90% of virological success, high pre-HAART viraemia remains an independent factor associated with delayed and decreased virological success. Patients starting HAART with >500,000 copies/ml represent a significant population that may deserve special attention.

Introduction

HAART has significantly extended the time to development of AIDS and to death in HIV-infected individuals [1,2]. Its efficacy in suppression of plasma HIV-1 RNA to undetectable levels, and in increasing CD4⁺ T-cell count, is well documented in several clinical trials [3–6].

Despite years of great progress in treating AIDS, however, in some patients starting their first treatment; the effectiveness of HAART is still not sufficient, with consequent virological failures [7–9]. These failures can be caused by several factors, such as drug potency, drug

exposure, adherence, drug resistance, age and, above all, viral dynamics, pretreatment CD4+ T-cell count and viraemia level. Regarding viral dynamics, findings show that evaluation of changes in virus load 1–12 weeks after the start of treatment can serve as a prognostic indicator for longer term virological responses [10–12]. Currently, the measurement of viral load before starting and during HAART treatment is recommended by guidelines for the treatment of HIV-infected patients [5,6].

Indeed, plasma HIV RNA level has been considered for many years as a surrogate marker for treatment response and survival [2,13–16]. In particular, patients starting HAART with HIV-1 viral load higher than 100,000 copies/ml and/or CD4+ T-cell count fewer than 200 cells/μl have a higher risk of clinical progression and lower virological response than those with lower HIV-1 viral load or higher CD4+ T-cell count [2,12,17–21]. To minimize the risk of disease progression, these two thresholds are recommended by current guidelines to favour an early initiation of therapy [5,6]. However, so far, the role of a very high viral load on the efficacy of antiviral therapy, and on the progression of the disease, in a time of modern therapies still remains to be fully elucidated.

It should also be noted that a viral load threshold of 100,000 copies/ml (that currently defines in practice the 'high viral load') includes values ranging from 100,000 copies/ml to >10,000,000 copies/ml (the latter measurements made possible by the new HIV RNA detection methods based on real-time PCR) [22,23]. Thus, a better definition of pre-HAART viraemia (especially in the context of viraemia values >100,000 copies/ml) may help in providing explanations about the delay in the achievement of virological suppression and/or of virological rebounds observed in some individuals during HAART.

For these reasons, in the present study, we tested whether pre-HAART viral load can affect viral decay and, thus, the achievement and maintenance of virological response in a large cohort of HIV-1-infected patients starting modern first-line therapies.

Methods

Patients

Patients starting a first-line regimen in several clinical centres of North and Central Italy were selected on the basis of the following criteria: 1) first-line therapy, resistance test tailored, based on ≥3 drugs; 2) year of treatment ≥2000; 3) age ≥18 years; 4) pre-HAART viral load and CD4+ T-cell count measurements in the time-window from 3 months before to 1 week after HAART initiation; 5) viral load at the time of starting therapy >500 copies/ml; 6) at least two viral load measurements available during the first 6 months of therapy; and 7) therapy duration ≥6 months. To characterize the role of pre-HAART viral load on virological response, the analysis was performed by stratifying the population study in different pre-HAART viraemia ranges. A stratification that reflects a progressive increase of 0.5 log (which is generally the minimal change used for virological monitoring on response) has been used, as follows: ≤30,000, 30,001–100,000, 100,001–300,000, 300,001–500,000 and >500,000 copies/ml. Because of a limited number of patients with pre-HAART viraemia >1,000,000 copies/ml (73 patients), and a non-negligible proportion of patients with the upper detection limit of 500,000 copies/ml (87 patients), we decided *a priori* not to stratify further values >500,000 copies/ml.

HIV RNA quantification

Depending on methodologies available at the different clinical centres participating in this study, plasma viraemia was determined using three different assays: the Roche Cobas CA/CTM v2.0 (Mannheim, Germany), the Abbott RealTime HIV-1 (Chicago, IL, USA) and the bDNA version 3.0 (Bayer Corporation, Diagnostics Division, Tarrytown, NY, USA). These assays can quantitate HIV-1 RNA over the range of 20–10,000,000 copies/ml, 40–10,000,000 copies/ml and 50–500,000 copies/ml, respectively. Previous studies demonstrated that, even if there was not a uniform approach regarding the HIV-1 viral load detection, the results obtained by these assays correlated very well, with a difference of >0.5 $\log_{10}$ copies per ml for only few samples [22,23].

Genotyping

Sequencing of *pol* gene (containing the entire protease and the first 240/335 amino acids of the reverse transcriptase open reading frame) was performed in plasma samples collected from the patients before their first-line therapy. Approximately 88% (n=1,254) of tests used in this analysis were performed by means of a commercially available kit (ViroSeq HIV-1 Genotyping System; Abbott Molecular, Des Plains, IL, USA) according to the manufacturer's recommendations, as described above [24]. The remaining 176 tests were performed by means of the Trugene-HIV-1 Genotyping-Kit (TG HIV-1; Bayer HealthCare LLC, Tarrytown, NY, USA) [25]. Subtype has been determined by using a phylogenetic approach, as previously described [26].

To estimate the prevalence of transmitted drug resistance at starting HAART, the list of mutations reported by Bennett *et al*. [27] was used. The genotypic susceptibility score (GSS) for optimized therapy was also calculated according to Rega algorithm (version 8.0.2) based on the sum of genotype sensitivities to all drugs prescribed in the HAART. GSS for single drugs was scored as 0 (resistant virus), 0.5 (virus with intermediate resistance) and 1 (susceptible virus).

Statistical analyses

All the analyses were performed using the statistical R open source software (version 11.0.) and the software package SPSS (version 17.0) for Windows (SPSS Inc., Chicago, IL, USA).

Patient characteristics

χ^2 Test for trend or Fisher's exact test (for categorical variables) and Kruskal–Wallis test (for continuous variables) were used, when appropriate, to compare the baseline characteristics of the different pre-HAART viraemia patients' groups.

Survival analyses: viral load undetectability

To estimate the time and probability to achieve viral load undetectability (defined as the first viral load value ≤50 copies/ml from HAART initiation), Kaplan–Meier curves were used. To estimate the predictive impact of pre-HAART viraemia on virological response, Cox proportional hazard models were used.

Survival analyses were performed both on the set of patients who did not change or discontinue therapy before undetectability (on-treatment [OT] population approach) and on the full set of patients, independently by the therapy change or interruptions.

In the multivariable Cox proportional hazard models, the following variables were used as potential confounders: age, gender, pre-HAART CD4$^+$ T-cell count, presence of transmitted drug resistance [27], calendar year and third drug administered (non-nucleoside reverse transcriptase inhibitor [NNRTI] versus ritonavir-boosted protease inhibitor [PI/r]). The models were built on the subset of patients with complete information on the variables used as potential confounders.

Survival analyses: virological rebound

Survival analyses were also used to estimate the probability of having virological rebound over week 24 of treatment in patients who achieved undetectability and for whom a subsequent follow-up of at least two viraemia values was available. The event of virological rebound was defined as the first of two consecutive viral load measurements >50 copies/ml. A simplified stratification was used (ranges: ≤30,000, 30,001–500,000, >500,000 copies/ml) due to a low number of observed events.

Survival analyses were performed by an OT approach in the same way as described in the previous paragraph.

Results

Study population at HAART initiation

Overall, 1,430 patients satisfying all criteria were included in the present analysis. Baseline characteristics, further stratified by pre-HAART HIV-1 viral load, are summarized in Table 1. The median pre-HAART HIV-1 viral load was 5.1 (IQR 4.5–5.5) $\log_{10}$ copies/ml. Overall, patients started their first antiretroviral regimen around 2008 (median [IQR] year: 2008 [2006–2009]); 99.7% were treated with two nucleoside reverse transcriptase inhibitors (NRTIs) plus either an NNRTI (*n*=638; 86% with EFV) or a PI, always ritonavir-boosted (*n*=796; 71% with lopinavir, 16% with atazanavir, 6% with darunavir and 6% with fosamprenavir). The most used initial NRTI backbone was emtricitabine plus tenofovir (971 patients; 67.8%), followed by lamivudine plus zidovudine (263 patients; 18.4%). Therefore, nearly all patients analysed in the study were treated with a modern HAART based on therapeutic approaches currently recommended.

Regarding the third drug class, a progressive increase of PI/r usage was observed by increasing pre-HAART viraemia values; the proportion of patients with pre-HAART viraemia ≤30,000 copies/ml taking PI/r or NNRTI was similar (153 [47.2%] versus 172 [53.1%]), while the PI/r drug was the most common class used for patients with pre-HAART viraemia >500,000 copies/ml (138 [65.7%] with PI/r versus 72 [34.3%] with NNRTI).

Regarding the specific third drug used, darunavir was administered mostly to patients with pre-HAART viraemia >500,000 copies/ml in comparison with other patients (8.0% versus 2.2%; *P*<0.001), likewise enfuvirtide (5.2% versus 0.9%; *P*<0.001). Differently, efavirenz was less frequently administered in this range of pre-HAART viraemia (>500,000, 29.2% versus ≤500,000, 39.0%; *P*=0.004).

Finally, treatment with >3 drugs was higher in patients with pre-HAART viraemia >500,000 copies/ml in comparison with the other patients (9.5% versus 2.1%; *P*<0.001, by Fisher's exact test). Transmitted drug resistance was approximately 10% overall, without any significant difference in the five pre-HAART viraemia ranges analysed in the study. Nearly all patients (99%) have been treated with effective therapy with GSS≥3.

In total, 974 patients were included in the OT population according to the criteria listed in *Methods*. No differences in the characteristics shown in Table 1 were found in patients included in the OT group in comparison with the other 456 patients excluded as a result of the missing values for confounder variables.

Survival analyses: viral load undetectability

Of the overall population, the median (IQR) time to the first viral load measurement after starting HAART was 4.0 (3.5–5.8) weeks, while the median (IQR) intervals between subsequent viral load measurements were 2.9 (1.7–4.1) weeks (Table 1).

To estimate the time and probability to achieve HIV RNA undetectability, survival analyses were performed both on the OT population and on the full set of patients.

By OT analysis, the overall probability of achieving the undetectability was 72.7% by 24 weeks, and 94.7%

by week 48, with a median time to achieve undetectability of 16 weeks (95% CI 16, 17).

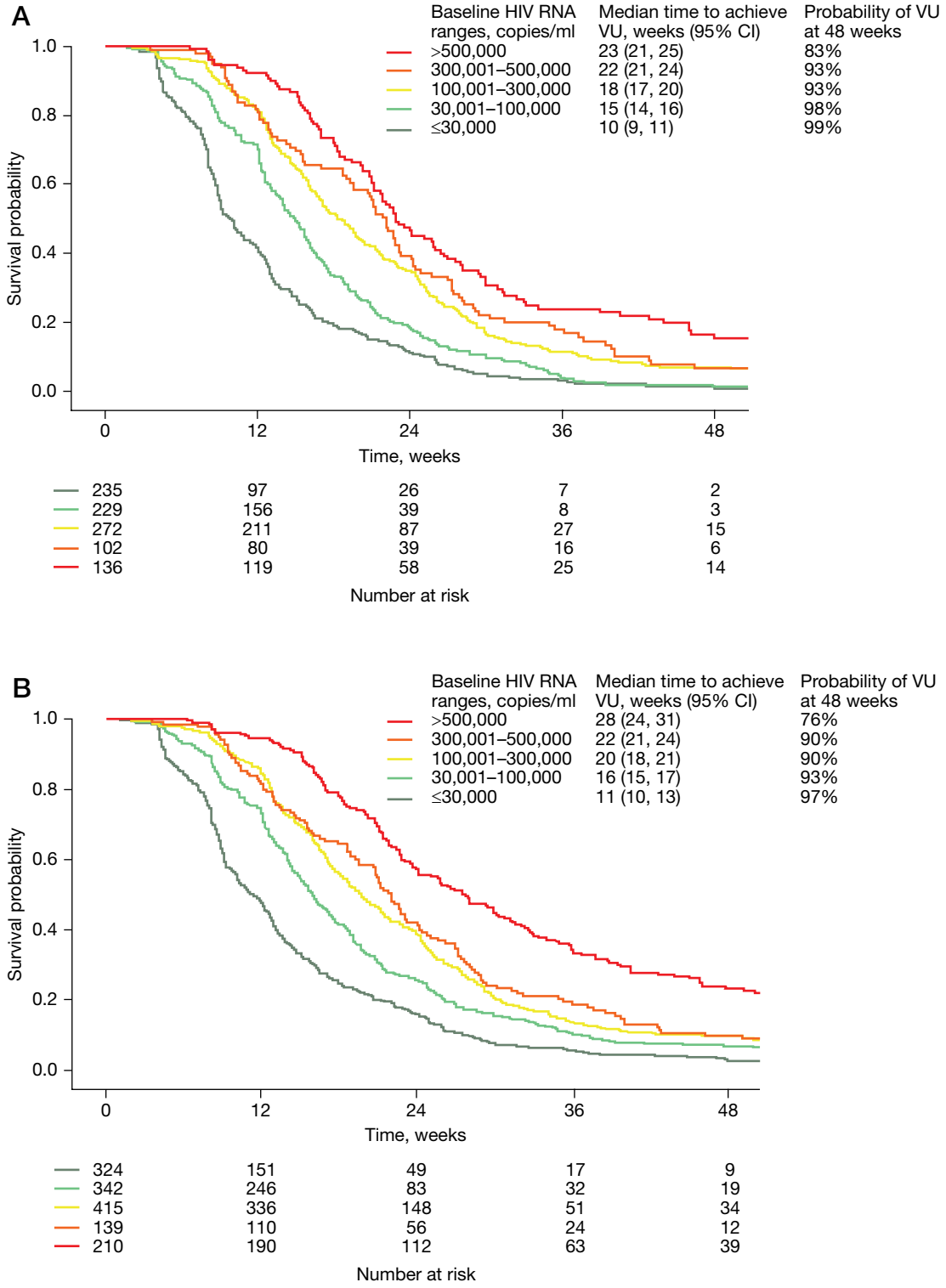
Stratifying patients by pre-HAART viraemia ranges, the rates of virological undetectability over time significantly decreased by increasing the pre-HAART viral load (Figure 1A). In particular, at 48 weeks, the prevalence of patients reaching virological undetectability was >90% in all pre-HAART viraemia ranges, with the only exception of the range >500,000 copies/ml, for which the prevalence of patients reaching virological success was only 83% ($P<0.001$, by log-rank test; Figure 1A). Similarly, the median time-to-achieve virological undectability significantly increased by increasing the pre-HAART viral load. In particular, the median time to achieve virological success ranged from 10 weeks (95% CI 9, 11), in patients with a baseline viral load ≤30,000 copies/ml, to 23 weeks (95% CI 21, 25), in patients with >500,000 copies/ml (Figure 1A). Similar findings were

Table 1. Characteristics of 1,430 drug-naive HIV-1-infected patients starting therapy stratified for pre-HAART viraemia

		Viraemia ranges, copies/ml					
Variable	Overall (*n*=1,430[a])	≤30,000 (*n*=324)	30,001–100,000 (*n*=342)	100,001–300,000 (*n*=415)	300,001–500,000 (*n*=139)	>500,000 (*n*=210)	*P*-value[b]
Male, *n* (%)	1,071 (75.8)	215 (67.0)	247 (73.7)	337 (82.2)	107 (78.1)	165 (78.6)	<0.001
Median age, years (IQR)	39 (33–46)	39 (33–45)	38 (33–45)	40 (34–46)	39 (34–48)	39 (33–46)	0.28
Median pre-HAART CD4$^+$ T-cells, cells/mm^3 (IQR)	202 (80–309)	266 (172–352)	226 (123–309)	205 (87–306)	124 (39–256)	80 (30–201)	<0.001
Risk factor							
Heterosexual, *n* (%)	376 (39.0)	98 (43.0)	82 (36.0)	96 (33.8)	43 (43.4)	57 (45.6)	0.62
Homosexual, *n* (%)	362 (37.6)	84 (36.8)	78 (34.2)	126 (44.4)	36 (36.4)	38 (30.4)	0.67
IDU, *n* (%)	120 (12.5)	22 (9.6)	35 (15.3)	35 (12.3)	10 (10.1)	18 (14.4)	0.51
Sexual, *n* (%)	93 (9.6)	20 (8.8)	28 (12.3)	25 (8.8)	9 (9.1)	11 (8.8)	0.69
Other, *n* (%)	13 (1.3)	4 (1.8)	5 (2.2)	2 (0.7)	1 (1.0)	1 (0.8)	0.24
CDC C stage, *n* (%)	73 (15.0)	13 (9.6)	16 (12.6)	21 (15.2)	12 (26.1)	11 (26.8)	0.001
TDR, *n* (%)	153 (10.7)	38 (11.6)	35 (10.2)	43 (10.4)	15 (10.8)	22 (10.4)	0.97
Subtype							
B, *n* (%)	1,003 (71.5)	228 (72.6)	256 (76.2)	286 (70.4)	96 (70.1)	137 (65.6)	0.027
C, *n* (%)	64 (4.6)	17 (5.4)	13 (3.9)	15 (3.7)	6 (4.4)	13 (6.3)	0.72
CRF02_AG, *n* (%)	67 (4.8)	19 (6.1)	14 (4.2)	8 (2.0)	10 (7.3)	16 (7.7)	0.366
F, *n* (%)	45 (3.2)	6 (1.9)	12 (3.6)	19 (4.7)	3 (2.2)	5 (2.4)	0.81
Other, *n* (%)	223 (15.9)	44 (14.0)	41 (12.2)	79 (19.4)	22 (16.1)	37 (17.8)	0.060
Median year of HAART initiation, (IQR)	2008 (2006–2009)	2008 (2006–2009)	2008 (2006–2009)	2008 (2006–2009)	2007 (2006–2009)	2008 (2006–2009)	0.41
Third drug							
NNRTI, *n* (%)	638 (44.6)	172 (53.1)	154 (45.0)	195 (47.0)	45 (32.4)	72 (34.3)	<0.001
Ritonavir-boosted PI, *n* (%)	793 (55.4)	153 (47.2)	188 (55.0)	220 (53.0)	94 (67.6)	138 (65.7)	<0.001
>3 drugs, *n* (%)	46 (3.2)	6 (1.9)	5 (1.5)	11 (2.7)	4 (2.9)	20 (9.5)	<0.001
Median number of viral load measurements per patient (IQR)	9 (5–16)	10 (5–16)	10 (5–16)	8 (5–16)	11 (5–16)	9 (5–16)	0.054
Median time to the first viral load measurement after starting HAART, weeks (IQR)	4.0 (3.5–5.8)	4.1 (3.7–6.5)	4.1 (3.6–6.0)	4.0 (3.6–5.6)	4.0 (2.6–5.5)	4.0 (3.7–5.0)	0.68
Median time between two consecutive viral load measurements, weeks (IQR)	2.9 (1.7–4.1)	2.8 (1.5–4.0)	3.0 (2.0–4.2)	3.0 (1.7–4.1)	3.0 (1.7–4.1)	2.6 (1.4–4.0)	0.12
Median time of follow-up from starting HAART, weeks (IQR)	173 (105–265)	174 (107–269)	176 (102–250)	166 (102–268)	185 (119–273)	173 (99–280)	0.641

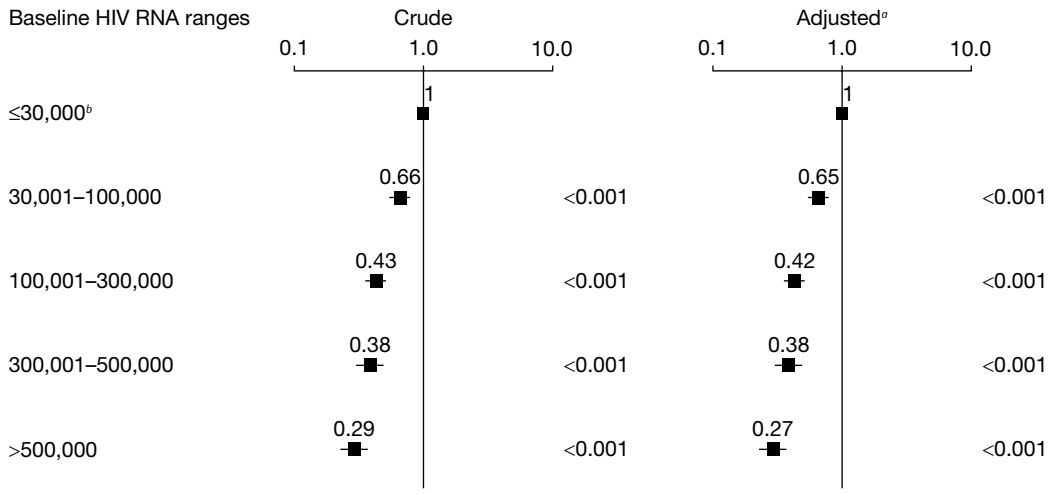
[a]Except for Male where *n*=1,413; age where *n*=1,388; pre-HAART CD4$^+$ T-cells where *n*=1,406; risk factor where *n*=964; CDC C stage where *n*=487; transmitted drug resistance (TDR) where *n*=1,430; subtype where *n*=1,402; and third drug where *n*=1,430. [b]*P*-value was calculated by χ^2 test for trend for categorical variables and by Kruskal–Wallis test for continuous variables. IDU, intravenous drug user; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Figure 1. Kaplan-Meier estimates of probability of virological undectability (HIV RNA<50 copies/ml) according to pre-HAART viraemia by 48 weeks



P<0.001 at log-rank test. **(A)** Estimation by on-treatment approach (number of patients analysed =974). **(B)** Estimation by full dataset analysis, regardless of therapy switches (number of patients analysed =1,430). VU, virological undectability.

Figure 2. Predictive value of pre-HAART viraemia on the achievement of virological undetectability (plasma HIV-1 RNA<50 copies/ml) after starting first-line regimen



Forest plots represent the relative hazard (with 95% CI) to achieve plasma HIV RNA<50 copies/ml after first-line regimen starting. Cox univariable and multivariable analyses are reported on the right and on the left of the figure, respectively. The analyses were performed on 974 patients by on-treatment approach. [a]Adjusted for gender, age, pre-HAART CD4+ T-cell count, transmitted drug resistance, calendar year and third drug (protease inhibitor versus non-nucleoside reverse transcriptase inhibitor). [b]Reference range.

obtained by analysing the full dataset (Figure 1B). Thus, the regimens administered to these patients achieved a very high rate of virological success, confirming that today's high antiviral efficacy is the result of good clinical practice.

Cox models show that the relative hazard to achieving virological suppression significantly decreased by increasing the pre-HAART viral load (Figure 2). In particular, patients having pre-HAART viraemia >500,000 copies/ml had the lowest relative hazard in comparison to other ones, also after adjusting for gender, age, pre-HAART CD4+ T-cell count, transmitted drug resistance, calendar year and third drug administered (PI/r versus NNRTI; relative adjusted hazard ratio [95% CI] 0.27 [0.21, 0.35]; $P<0.001$).

Survival analyses: virological rebound

Survival analyses were also used to estimate the probability of virological rebound after achieving virological undetectability. Overall, the median (IQR) time of follow-up from starting HAART was 173 weeks (105–265; Table 1).

Among the 869 patients achieving undetectable viraemia in OT analysis, viral load follow-up values were available for 794 patients after the achievement of virological undetectability. By 24 weeks after achieving undetectability, 38 (5.2%) episodes of viral rebound were documented. An increasing rate of virological rebound was found at week 4, 12 and 24 by increasing pre-HAART viraemia ($P=0.050$; Figure 3). Patients having pre-HAART viraemia >500,000 copies/ml had the highest relative hazard of virological rebound at univariable analysis (relative hazard ratio [95% CI] 2.1 [1.1, 4.0]; $P=0.025$; Additional file 1), though not confirmed by multivariable analysis.

Discussion

The present study reports data on a large cohort (nearly 1,500 patients) and shows that, at the time of modern HAART, more than 90% of HIV-1-infected patients achieve virological undetectability within 48 weeks after starting their first-line regimen. This high rate of virological success is in agreement with recently obtained findings [21,28], and confirms that these results are consistent with an accurate viral load monitoring (as shown by the high number of viraemia measurements) and an appropriate use of new drugs and new regimens in the framework of good clinical practice. It should be highlighted that all patients analysed in this study started their first-line regimen based on genotypic resistance testing; indeed, even if approximately 10% of the overall population has been infected by a resistant virus, the probability of achieving virological response at any time was similar in patients with transmitted drug resistance (and treated with resistance

test-driven therapy) in comparison with those infected by a wild-type virus (data not shown). Similar results were obtained recently, when patients with transmitted drug resistance were treated with a complete fully active regimen [29]. Moreover, by Cox multivariable analysis, transmitted drug resistance itself did not affect as a confounder the probability of virological response in our study. This confirms the relevant role of a genotypic resistance test in the initial treatment choice for the HIV-1 drug-naive patients. Indeed, nearly all patients (99%) had a GSS of at least 3, highlighting the full activity of all the administered drugs.

Besides this high rate of success, pre-HAART viraemia played a relevant role in this frame. Indeed, patients with high pre-HAART viraemia (>500,000 copies/ml) were characterized by having a lower and delayed probability to achieve virological success.

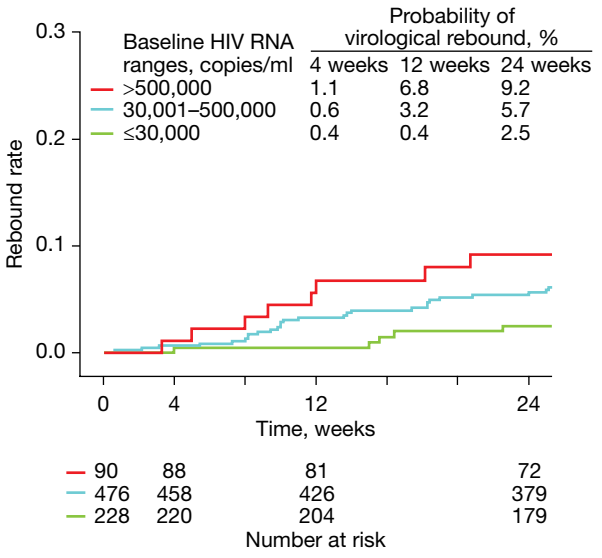
Nearly all patients with pre-therapy viraemia <100,000 copies/ml reached undetectability at 48 weeks of therapy. Patients with pre-HAART viraemia between 100,000 and 500,000 copies/ml also reached remarkable rates of success (>93%), thus supporting the potency and efficacy of modern therapies, even outside the context of randomized prospective studies. However, patients with very high viral load (>500,000 copies/ml) showed a different profile: only 50% and 83% reached undetectability at 24 and 48 weeks of therapy, respectively.

Cox multivariable estimates also confirmed that pre-HAART viraemia >500,000 copies/ml was an independent factor associated with delayed virological success after adjusting for parameters such as pre-HAART CD4$^+$ T-cell-count, calendar year and third drug administered (PI versus NNRTI).

To our knowledge, so far, very little information is available about the response to treatment of patients with very high pre-HAART viraemia (>500,000 copies/ml) [30–34]. The current definition of high viral load is >100,000 copies/ml, and nearly all studies, and guidelines, tend to adapt their analyses and statements to this threshold [2,6,12,17–21]. For instance, Haubrich *et al*. [12] observed a greater viral decay in patients with pre-HAART viraemia >100,000 copies/ml than in those with pre-HAART viraemia <100,000 copies/ml after approximately 2 weeks of HAART. A potential explanation of this phenomenon is the presence of a larger infected cell population in patients with higher viraemia before starting treatment, predominantly done by long-lived productively infected cells [12]. Whether this interesting result (with obvious therapeutic consequences) is driven by patients with very high viraemia (>500,000 copies/ml) or by all those with >100,000 copies/ml is not defined in that study.

Recent and commonly used diagnostic methods, based on real-time PCR, are able to quantify today,

Figure 3. Kaplan-Meier estimates of probability of virological rebound[a] according to pre-HAART viraemia by 24 weeks



P=0.050 at log-rank test. The analysis was performed on 794 patients by on-treatment approach. [a]As two consecutive viral load measurements >50 copies/ml after achieving undetectability.

with remarkable precision, HIV RNA levels up to 10,000,000 copies/ml [35]. This would suggest revision of the concept of 'high viral load' today arbitrarily set at the threshold of 100,000 copies/ml, also considering that, in our studied population, 15% of drug-naive patients had viral loads >500,000 copies/ml before starting antiviral therapy.

Noteworthy, a recent study comparing the efficacy of rilpivirine versus efavirenz showed significant differences in the rate of virological success at 96 weeks between the two treatment groups only when pre-HAART viraemia was >500,000 copies/ml. Differently, a similar efficacy was observed in patients with pre-HAART viraemia <100,000 and 100,000–500,000 copies/ml [34].

Another question raised by these data is whether detectable viral load at 24 weeks in patients with pre-HAART high viraemia always represents an early sign of failure that consequently requires rapid therapeutic switches to prevent the development of resistance or may suggest that some patients just require more time to reach undetectability, thus avoiding inappropriate therapeutic switches to second-line therapies. A study dedicated to this issue, performed on a larger cohort of patients with a longer follow up, might provide clear evidence about this important topic.

Taking pre-therapy viraemia into consideration may be relevant also for current guidelines of anti-HIV therapy. Indeed, approximately half of the patients

with pre-therapy viral load >500,000 copies/ml, then reaching virological undectability in the present study, could have been considered as failing after 6 months of treatment (Figure 1) [5,6]. Similar proportions of patients achieving virological undectability at 6 months were obtained in another recent study [36]. Therefore, a flexible definition of the time to virological failure, driven also by pre-therapy viral load, may favour appropriate recommendations for patient follow-up, and a stronger rationale for delicate therapeutic decisions, such as changing drug regimens because of virological failure.

A possible limitation of this study could be the more frequent usage of the PI/r in patients with pre-HAART viraemia >500,000 copies/ml than in patients with pre-HAART viraemia <500,000 copies/ml. Traditionally, PIs are drugs characterized by a high genetic barrier to resistance [37–39], and thus generally associated with a lower emergence of drug resistance at virological failure [39–41]. For this reason, they are preferentially used in patients with high viral load. However, patients taking PIs may have lower adherence as compared with those taking NNRTIs [42,43]. To exclude that the adherence rate driven by the higher use of PIs may have caused the decreased rate of success in the >500,000 range, we repeated the same analysis (OT approach) also on 448/974 patients treated with NNRTI-based regimens (88.4% with efavirenz). After stratifying by pre-HAART viraemia groups, the Kaplan–Meier analysis showed again that the lowest 48 week probability of virological success was estimated for viraemia >500,000 copies/ml ($P<0.001$; data not shown). Finally, the Cox multivariable analyses showed that even after adjusting for the third drug (PI versus NNRTI), the lowest hazard of virological response was still related with the highest pre-HAART viraemia.

Taken together, all these results indicate that high pre-HAART viral load may play an important role in the rate of, and time to, virological success, independently from the third drug used and, presumably, also from the decreased adherence potentially driven by PI-usage. For instance, no clinical trials have, to date, proven the superiority of a PI-based or an NNRTI-based regimen, with respect to virological response, in the case of high pre-therapy viral load [44,45]. However, new retrospective and prospective studies may provide final results about whether PIs or NNRTIs should be selectively used according to pre-therapy viral load (and $CD4^+$ T-cell number).

Results also suggest a potential role of high pre-therapy viral load on the probability of virological rebound, although larger studies (currently ongoing) are still required to confirm this observation.

In conclusion, our findings support an important role of high pre-therapy viral load in the achievement of virological response (at least within the time frame considered for the evaluation of success or failure). This study also suggests the importance of resetting the threshold of high viraemia (also in light of the new viral load tests available) and reinforces the indication in guidelines to consider high viraemia levels as an important parameter in setting both appropriate therapeutic strategies and frequency of viral load monitoring.

Whether patients with very high viraemia deserve special treatment remains to be elucidated. Special studies, designed for this purpose, with adequate methodological support and appropriate recruitment of patients, will provide more definitive answers to this crucial point.

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Additional files

Additional file 1: A graph showing the predictive value of pre-HAART viraemia on the reaching of virological rebound after first line regimen starting can be found at www.intmedpress.com/uploads/documents/AVT-12-OA-2753_Santoro_Add_file_1.pdf

Additional file 2: A list of individuals who helped with the study can be found at www.intmedpress.com/uploads/documents/AVT-12-OA-2753_Santoro_Add_file_2.pdf

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