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MAJOR ARTICLE

Virological Outcome Measures During Analytical Treatment Interruptions in Chronic HIV-1-Infected Patients

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Background. Analytical treatment interruptions (ATIs) are essential in research on HIV cure. However, the heterogeneity of virological outcome measures used in different trials hinders the interpretation of the efficacy of different strategies.

Methods. We conducted a retrospective analysis of viral load (VL) evolution in 334 ATI episodes in chronic HIV-1-infected patients collected from 11 prospective studies. Quantitative (baseline VL, set point, delta set point, VL, and delta VL at given weeks after ATI, peak VL, delta peak VL, and area under the rebound curve) and temporal parameters (time to rebound [TtR], set point, peak, and certain absolute and relative VL thresholds) were described. Pairwise correlations between parameters were analyzed, and potential confounding factors (sex, age, time of known HIV infection, time on ART, and immunological interventions) were evaluated.

Results. The set point was lower than baseline VL (median delta set point, −0.26; $P < .001$). This difference was >1 log10 copies/mL in 13.9% of the cases. The median TtR was 2 weeks; no patients had an undetectable VL at week 12. The median time to set point was 8 weeks: by week 12, 97.4% of the patients had reached the set point. TtR and baseline VL were correlated with most temporal and quantitative parameters. The variables independently associated with TtR were baseline VL and the use of immunological interventions.

Conclusions. TtR could be an optimal surrogate marker of response in HIV cure strategies. Our results underline the importance of taking into account baseline VL and other confounding factors in the design and interpretation of these studies.

Keywords. HIV-1; immune-based therapy; STI; vaccine; viral load.

Analytical treatment interruptions (ATIs) form an inherent part of the design of studies on HIV cure [1]. As currently there are no adequate surrogate markers of treatment efficacy [1, 2], the direct assessment of viral control during ATI is the recommended method to evaluate these novel interventions [2]. The imperative use of ATI in this field has been, however, the subject of debate and criticism in recent years [3]—especially since the publication of the SMART study [4]—as it raises important ethical and safety issues. There have been proposed potential adverse events associated with ATI, encompassing clinical [4–6], virological [7, 8], and epidemiological [9] risks. Although there is recently increasing evidence that short-term treatment interruptions are essentially safe [10], the debate is far from over [11]. To minimize the potential risks of ATI, HIV remission studies are advised to contain only a small number of participants [2], and they frequently dispense with an adequate comparator group [12]. This, in turn, leads to a loss of statistical power and entails the possibility of biased conclusions [13].

In the absence of consensus about a "gold standard" virological outcome measure, different studies use different virological end points (time to rebound, viral set point, etc.), which makes the adequate comparison of their results highly cumbersome, if not impossible [1]. Intensively monitored antiretroviral pause (MAP) [2] involves prompt treatment reintroduction after viral rebound. It is gaining popularity as an alternative of ATI, because the relatively short time the participants have to remain off antiretroviral therapy (ART)

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confers an enhanced safety profile to this strategy. However, the only virological end point that can be recorded in a MAP study is time to rebound (TtR), and—according to the current evidence—TtR might not be extrapolated to predict other important outcome measures, such as the set point [1, 14]. Moreover, increasing evidence suggests that VL rebound is followed by a significant drop in viremia in some patients, and this pattern could not be detected in the MAP strategy [13].

There are only a few available publications [14] that directly address the expected values and the possible correlations between different virological end points measured during ATI. Our aims were to perform a thorough description of the dynamics of viral rebound in a big retrospective cohort of ATI episodes in chronic HIV-1-infected patients, to establish the correlations between different rebound parameters, to propose a "resuming parameter," and to identify possible confounding factors for some of the most important parameters (TtR, set point, peak, and area under the curve [AUC]) that should be taken into consideration in the design and interpretation of future studies on HIV cure.

METHODS

Data on weekly VL evolution during ATI episodes were extracted from 11 prospective studies with similar inclusion criteria. All of these studies were performed and/or coordinated by our group: 10 of them have been previously published [15–24], and 1 is currently underway (ClinicalTrials.gov number NCT02767193). Four were structured treatment interruption studies with no additional intervention [15–17, 21], 4 were therapeutic vaccine studies (NCT02767193, [20, 23, 24]), 2 included an intervention arm with a cytostatic drug [18, 19], and 1 evaluated the effect of a 12-month vaccination schedule on the dynamics of viral rebound [22]. All the studies were approved by institutional ethical review boards and by the Spanish Regulatory Authorities. The present study was also evaluated and approved by the institutional ethical board of the Hospital Clinic of Barcelona (HCB/2018/0740); the procedures followed in the study were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Cases were excluded if they did not fulfill the following criteria: available VL data, undetectable VL (according to the detectability threshold used in the original study) at the time of treatment interruption, at least 1 detectable follow-up VL determination before ART reinitiation, an ATI of at least 12 weeks or documented viral rebound in cases with ART reinitiation before week 12. Cases with largely incomplete or nonverifiable data were also excluded from the analysis. The VL data available for the analysis corresponded to weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 after treatment interruption, the last VL value considered in each case being the value before either treatment reinitiation or end of follow-up.

The parameters used for the analysis were categorized as quantitative if they were principally related to the magnitude of VL and temporal if they described a time-related variable. The quantitative parameters analyzed were the following: (1) baseline VL, (2) set point, (3) delta set point, (4) VL at a given week after treatment interruption, (5) delta VL, (6) peak VL, (7) delta peak VL, and (8) AUC. For set point and delta set point, sensitivity analyses were also performed (set point "forward" and delta set point "forward"). All temporal parameters were determined in weeks: (1) TtR, (2) time to set point, (3) time until certain absolute VL thresholds (200, 1000, and 10 000 copies/mL), (4) time until relative thresholds (0.5 and 1 log10 copies/mL), and (5) time to peak VL. (For the definitions of the analyzed parameters, see Supplementary Table 1.)

The detectability threshold was defined as 50 copies/mL. A clinically relevant difference between VL values was defined as >0.5 log10 copies/mL. All analyses were carried out on the overall study population and also on the subset of cases without immunological intervention (cytostatic drug or therapeutic vaccine).

All analyses were carried out in R (version 3.4.1; R Foundation for Statistical Computing, Vienna, Austria) using RStudio (version 1.0.143; RStudio Inc., Boston, MA, USA). Continuous and discrete variables were expressed in median (interquartile range [IQR]) and in absolute number and percentage, respectively. Confidence intervals of proportions were calculated with the Clopper-Pearson method. Comparisons were performed using the Student *t*, Mann-Whitney *U*, Kruskal-Wallis, Wilcoxon signed rank, chi-square, and Fisher exact tests according to data type. The associations between virological parameters were determined using Pearson and Spearman correlations. Confounding factors independently associated with TtR were identified by multiple linear regression analysis on variables significantly associated with TtR in the univariate analysis. Survival curves were compared by the log-rank test. Statistical significance was defined as a *P* value <.05.

RESULTS

Demographics of the Study Population

There were 334 ATI episodes analyzed, belonging to 249 chronic HIV-1-infected patients (from 63 patients, ≥2 ATI episodes were included in the study). The median age (IQR) was 39.5 (35.0 to 45.7) years, and 26.3% of the episodes were registered in women. The median durations of known HIV infection and ART (IQR) were 6.8 (4.1 to 11.5) and 3.6 (2.1 to 5.6) years, respectively. All patients started ART in the chronic phase of HIV infection. An immunological intervention (cytostatic drug or therapeutic vaccine) accompanied 62 ATI episodes (18.6%). The median length of follow-up before ART reinitiation (IQR) was 12 (10 to 12) weeks.

Description of Virological Outcome Measures

The observed values of the analyzed parameters in the overall study population and in the subset of cases without intervention are summarized in Table 1. Figure 1A shows the evolution of viral loads during ATI of all analyzed cases, and the weekly distribution of VL is depicted in Figure 1B.

Quantitative Parameters

The set point was lower than the baseline VL in 68.2% of the cases (95% confidence interval [CI], 60.1%–75.5%). This difference was not clinically relevant in 65.6%, but it was >0.5 log10 copies/mL in 34.4% (95% CI, 26.9%–42.6%) and >1 log10 copies/mL in 13.9% of the cases (95% CI, 8.8%–20.5%). Similar results were obtained in the sensitivity analysis and in the analysis of the subset without immunological interventions (Supplementary Table 2), and these proportions did not vary significantly when we limited the analysis to the 215 first ATI episodes (data not shown).

The VL was <1000 copies/mL in around 10% of the cases at all time points between week 6 and week 24 (Figure 1C). Delta VL was >0.5 copies/mL in >25% of the cases at any follow-up week (Figure 1D). For more details, see also Supplementary Figure 1, which shows VL values at different weeks of ATI as compared with baseline VL in the intervention-free subset, and Supplementary Table 3, which shows the proportion of cases in whom the VL remained below given thresholds throughout follow-up.

During ATI, 2 different forms of viral load rebound kinetics are expected: A peak VL may precede the set point or coincide with it (Supplementary Figure 2). Peak VL preceded the set point in 37.7% of the cases where the set point could be determined (95% CI, 30.0%–45.8%). The set point (IQR) was significantly lower in cases where it was preceded by a peak, but

Table 1. Observed Values of the Explored End Point Parameters in the Study Population

	Overall Study Population (n = 334)		ATIs Without Intervention (n = 272)			Overall Study Population (n = 334)		ATIs Without Intervention (n = 272)	
	Median (IQR)	No.	Median (IQR)	No.		Median (IQR)	No.	Median (IQR)	No.
Quantitative parameters									
Baseline VL, log10 copies/mL	4.43 (4.08 to 4.95)	312	4.43 (4.08 to 4.91)	252	AUC, log10 copies/mL	–0.36 (–0.74 to –0.00)	312	–0.28 (–0.65 to 0.09)	252
Set point, log10 copies/mL	4.33 (3.79 to 4.81)	154	4.37 (3.99 to 4.91)	106	Delta set point, log10 copies/mL	–0.26 (–0.64 to 0.18)	151	–0.20 (–0.58 to 0.25)	105
Set point "forward," log10 copies/mL	4.35 (3.79 to 4.87)	334	4.37 (3.85 to 4.91)	272	Delta set point "forward," log10 copies/mL	–0.22 (–0.66 to 0.27)	312	–0.17 (–0.62 to 0.30)	252
Peak VL, log10 copies/mL	4.65 (4.15 to 5.15)	154	4.72 (4.21 to 5.17)	106	Delta peak VL, log10 copies/mL	0.06 (–0.44 to 0.70)	151	0.17 (–0.34 to 0.85)	105
VL post-ATI, log10 copies/mL					Delta VL, log10 copies/mL				
Week 1	1.57 (1.30 to 2.30)	88	1.57 (1.30 to 2.30)	80	Week 1	–2.44 (–3.01 to –1.87)	88	–2.WW54 (–3.03 to –1.88)	80
Week 2	2.28 (1.57 to 3.33)	167	2.57 (1.57 to 3.66)	112	Week 2	–2.06 (–2.67 to –1.06)	164	–1.73 (–2.51 to –0.81)	111
Week 3	3.55 (2.17 to 4.63)	73	3.69 (2.30 to 4.71)	68	Week 3	–0.86 (–1.73 to –0.06)	73	–0.79 (–1.65 to –0.06)	68
Week 4	4.16 (3.36 to 4.88)	208	4.32 (3.52 to 5.04)	149	Week 4	–0.39 (–1.10 to 0.45)	205	–0.20 (–0.90 to 0.60)	148
Week 5	4.26 (3.13 to 5.04)	36	4.57 (3.73 to 5.06)	31	Week 5	0.31 (–0.51 to 0.77)	36	0.37 (–0.26 to 0.77)	31
Week 6	4.36 (3.79 to 4.78)	88	4.42 (3.89 to 4.94)	65	Week 6	0.03 (–0.81 to 0.50)	88	0.17 (–0.64 to 0.56)	65
Week 8	4.29 (3.66 to 4.76)	160	4.35 (3.72 to 4.86)	103	Week 8	–0.30 (–0.77 to 0.20)	157	–0.22 (–0.74 to 0.23)	102
Week 10	4.32 (3.65 to 4.60)	50	4.32 (3.82 to 4.58)	37	Week 10	–0.09 (–0.64 to 0.28)	50	–0.18 (–0.46 to 0.06)	37
Week 12	4.28 (3.77 to 4.73)	242	4.34 (3.80 to 4.86)	183	Week 12	–0.30 (–0.79 to 0.17)	220	–0.21 (–0.68 to 0.25)	163
Week 24	4.16 (3.75 to 4.64)	83	4.16 (3.72 to 4.86)	44	Week 24	–0.46 (–0.84 to –0.10)	81	–0.42 (–0.79 to –0.04)	43
Week 48	4.22 (3.71 to 4.51)	36	4.04 (3.69 to 4.47)	12	Week 48	–0.52 (–0.86 to –0.16)	36	–0.46 (–0.71 to –0.12)	12
Temporal parameters									
Time to rebound, wk	2 (2 to 4)	170	2 (2 to 3)	122	Time to peak VL, wk	4 (4 to 8)	154	4 (4 to 6)	106
Time to set point, wk	8 (4 to 8)	154	6 (4 to 8)	106	Time to 200-copies/mL threshold, wk	2 (2 to 4)	164	2 (2 to 4)	116
Time to 1000-copies/mL threshold, wk	4 (2 to 4)	159	3 (2 to 4)	113	Time to 10 000-copies/mL threshold, wk	4 (3 to 4)	134	4 (2.75 to 4)	96
Time to delta 0.5-log10 copies/mL threshold, wk	4 (3 to 4)	130	4 (2.25 to 4)	98	Time to delta 1-log10 copies/mL threshold, wk	4 (2 to 4)	147	3 (2 to 4)	107

"n" indicates the total number of cases; "No." indicates the number of cases with available information for each category.

Abbreviations: ATI, analytical treatment interruption; AUC, area under the curve; IQR, interquartile range; VL, viral load.

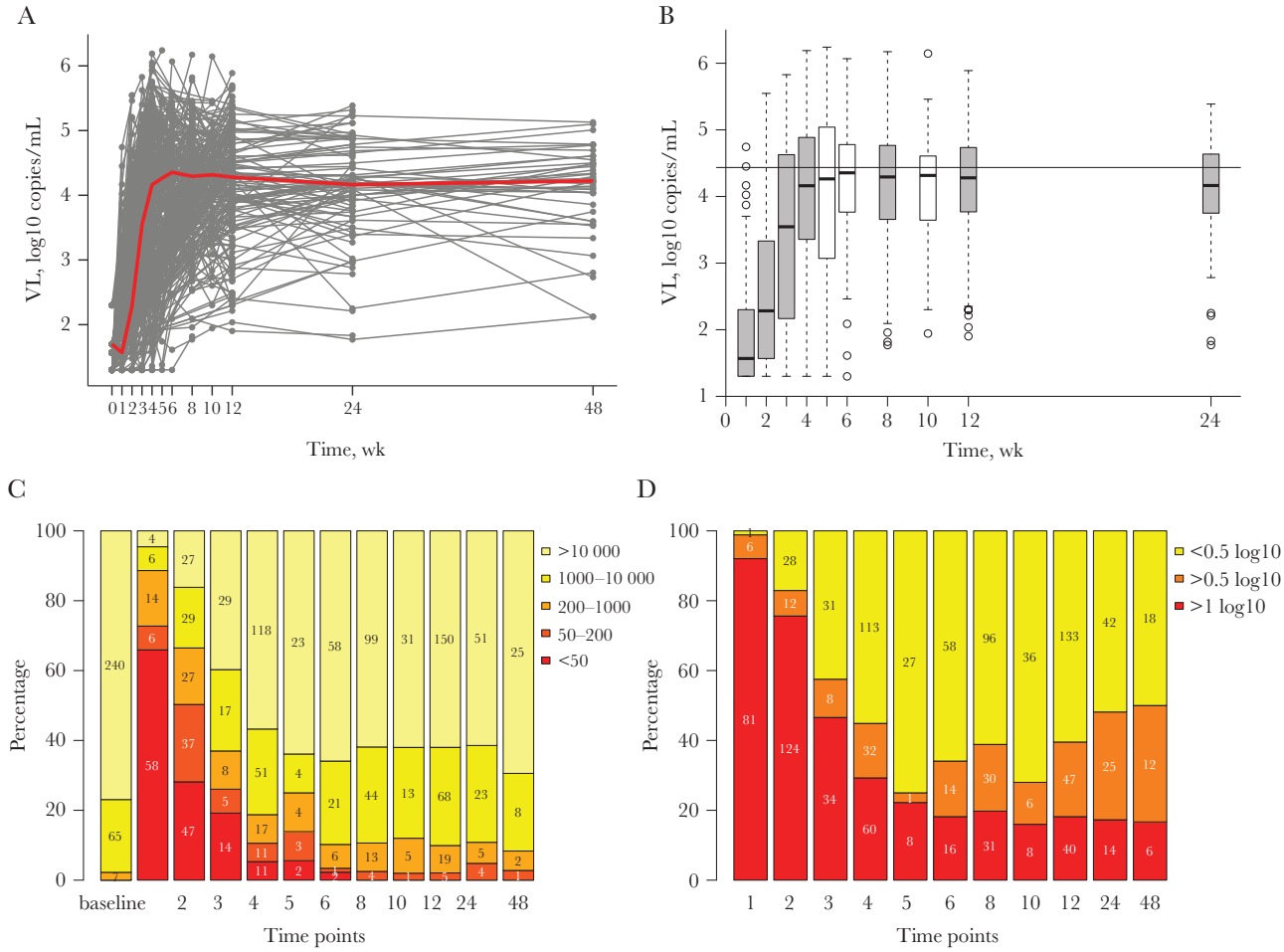

Figure 1. The evolution of viral load (VL) according to time after treatment interruption and its comparison with the baseline VL in the overall study population. The numbers within the boxes and bars represent the number of episodes in each category. A, Spaghetti plot of the evolution of VL in all analytical treatment interruption episodes included in the study. The red line corresponds to the median value. B, The distribution of VL measured at each time point after treatment interruption until week 24. The numbers within the boxes indicate the sample size. The horizontal line marks the median baseline VL values (4.43 log10 copies/mL). Gray coloring of boxes indicates a significant difference in baseline VL (Mann-Whitney *U* test). C, The proportions of cases with VL values within ranges of the thresholds 50, 200, 1000, and 10 000 copies/mL at baseline and throughout the follow-up weeks. D, The proportions of cases with delta-VL values >1, between 0.5 and 1, and <0.5 log10 copies/mL throughout the follow-up weeks.

this difference was not clinically relevant (4.13 [3.60 to 4.49] vs 4.40 [4.00 to 4.91] log10 copies/mL; $P = .003$). The set point was <200 copies/mL in 2 cases in both groups (2/58 [3.4%] vs 2/96 [2.1%]; $P = .673$), all 4 episodes belonging to different patients. There was no statistically significant difference in baseline parameters (demographics, baseline VL) or TtR between cases with different peak VL patterns.

The median AUC (IQR) was −0.36 (−0.74 to −0.00) log10 copies/mL, and the absolute AUC was >0.5 log10 copies/mL in 42.0% (95% CI, 36.4%–47.7%) (Supplementary Table 2).

Temporal Parameters

In all the ATI episodes but 1, VL was detectable by week 6. The median TtR (IQR; range) was 2 weeks in both the overall population (2 to 4; 1–8) and the cases without intervention (2 to 3; 1–8 weeks). The proportion of patients with an undetectable VL at week 12 was 0% in both the overall study population (upper 95% CI, 1.5%) and the cases without intervention (upper 95% CI, 2.0%).

The median time until reaching the set point (IQR; range) was 8 (4 to 8; 1–24) weeks in the overall population and 6 (4 to 8; 1–24) weeks in the intervention-free subset. By week 12, 97.4% (95% CI, 93.5%–99.3%) of the patients had reached the set point.

Correlations Between Outcome Measures

To find a safe and easy-to-assess "resuming measure" of VL rebound, we analyzed the correlations between different rebound parameters.

Figure 2 resumes the pairwise Spearman correlations between the main explored parameters. An overview of pairwise Spearman and Pearson correlations between all parameters can be observed in Supplementary Figure 3. Baseline VL was significantly correlated to VL at all follow-up weeks, to set point, to peak VL, to the AUC, and also to some of the temporal

parameters including TtR (Figure 3A). TtR was positively correlated to all other temporal parameters and showed a significant negative correlation to most of the quantitative parameters, including set point (Figure 3B), peak VL, and AUC (Supplementary Figure 4).

Confounding Factors

We assessed the effect of 5 potential confounding variables—sex, age, time of known HIV infection, time on ART, and immunological interventions—on the dynamics of viral rebound.

Baseline VL, set point, and peak VL (IQR) were significantly lower in women (4.26 [3.79 to 4.72] vs 4.60 [4.17 to 5.03] log10 copies/mL; $P < .001$; 4.03 [3.28 to 4.42] vs 4.41 [4.06 to 4.91] log10 copies/mL; $P = .005$; and 4.30 [4.01 to 4.74] vs 4.83 [4.20 to 5.30] log10 copies/mL; $P = .003$; respectively). A higher set point was observed in older patients (Spearman's *rho* = .25; $P = .005$), and a smaller AUC was found in cases with a longer known duration of HIV infection (Spearman's *rho* = .18; $P = .002$). Immunological interventions significantly affected the magnitude of most quantitative and temporal parameters, except baseline VL (Supplementary Table 4).

In the univariate analysis age, the known duration of HIV and ART, the use of immunological interventions, and baseline VL were significantly associated with TtR. The variables independently associated with TtR according to the multivariate analysis were baseline VL (beta = –.32; $P < .001$), duration of ART (beta = .42; $P = .002$), and interventions (beta = .26; $P < .001$) (Table 2).

DISCUSSION

In this study, we have described the dynamics of viral rebound in a cohort of patients undergoing ATI, established correlations between the most frequently used virological outcome measures, and identified certain confounders that should be taken into consideration in the evaluation of ATI studies.

Our group previously reported that the set point after 3 ATIs in a cohort of 45 chronic HIV-1-infected patients was significantly lower than the baseline VL [25]. In accordance with this, our present results support the recent finding of Treasure et al. [14] claiming that the new set point is lower than the baseline VL in >60% of ATIs. Moreover, in our study, in one-third of the cases, this difference was >0.5 log10 copies/mL, and in 10% of the episodes, it was >1 log10 copies/mL. However, this finding may correspond only to a temporary decrease in VL, as some studies with longer follow-up reported a slow but steady increase of VL after ATI until becoming virtually identical to baseline VL values [26, 27].

We observed that the set point was lower than the peak VL in more than one-third of the ATI episodes and that the VL descended below 200 copies/mL in 3.4% of these cases—a proportion similar to the 4%–10% of post-treatment controllers reported by other authors in chronic HIV-1-infected patients [28, 29]. Additionally, a peak preceded half of the cases in our cohort with a set point <200 copies/mL (2/4), which is in accordance with previous observations [30] and supports the theory that viral rebound does not exclude the possibility of subsequent control of viral load [13], although in our cohort

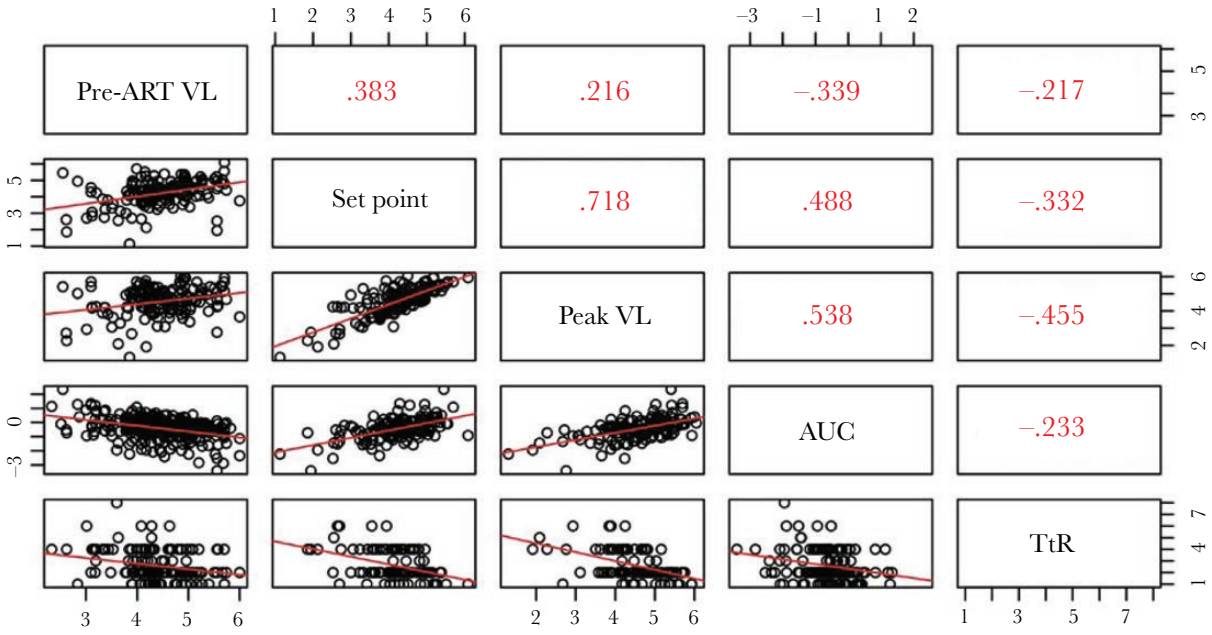

Figure 2. Correlation matrix of the most commonly used virological outcome measures observed in the study cohort. Spearman's *rho* coefficients are represented in the upper panel (all *P* values < .005); the corresponding scatter plots with trend lines are shown in the lower panel. Abbreviations: ART, antiretroviral therapy; AUC, area under the curve; TtR, time to rebound; VL, viral load.

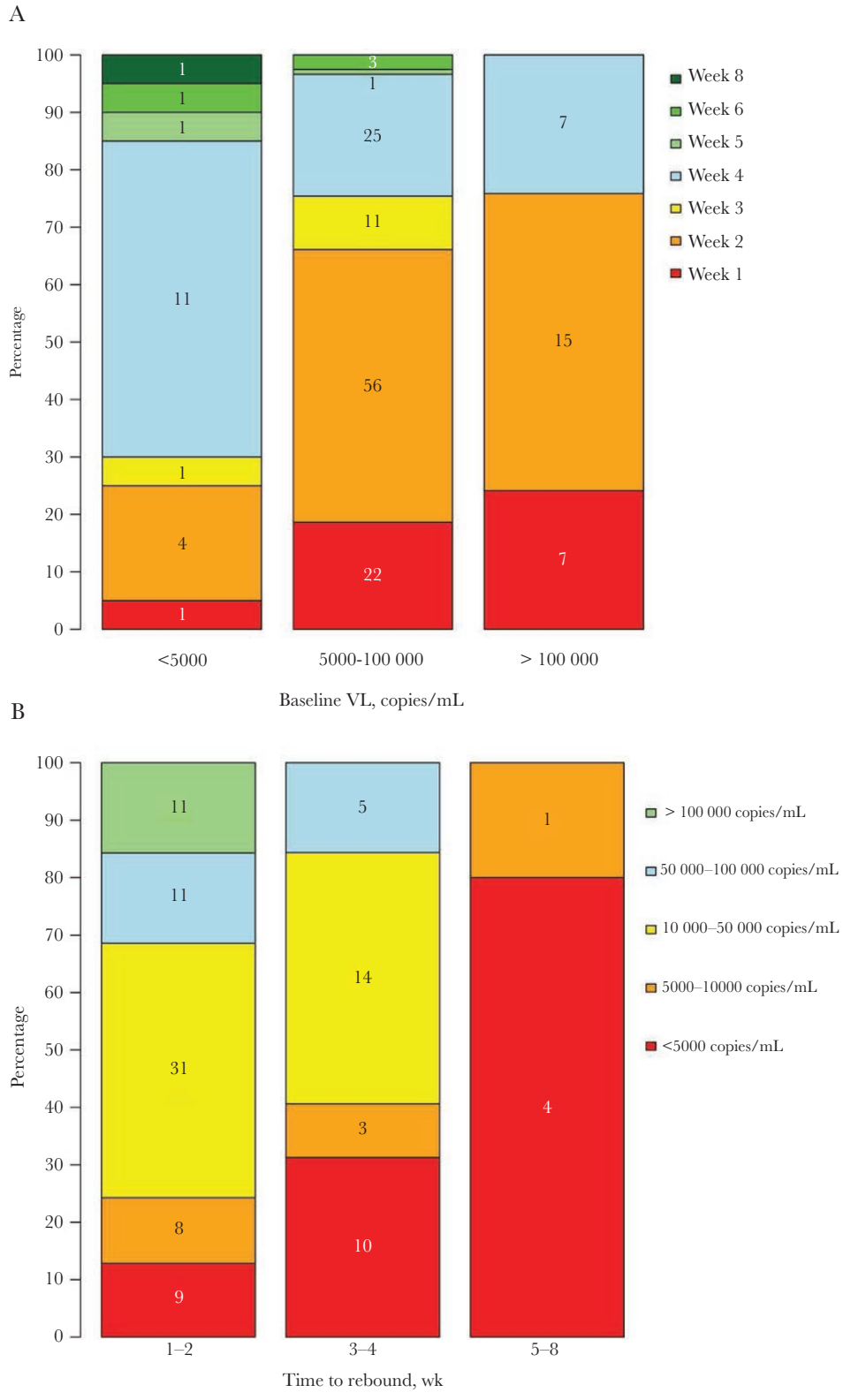

Figure 3. Predictive patterns of early rebound parameters. Numbers in the bars represent the number of episodes in each category. B, Time to rebound according to different strata of baseline viral load. Kruskal-Wallis test $P < .001$. C, Set point according to different strata of time to viral rebound. Kruskal-Wallis test $P = .002$.

the difference in set point was not clinically relevant between cases with and without a preceding peak. Neither TtR nor any other early assessable parameter predicted the presence of a peak in the rebound curve.

Table 2. Univariate and Multivariate Analysis of Possible Confounders of TtR

	Univariate Analysis		Multivariate Analysis	
Variable	Comparison TtR	*P* Value	Standardized (Beta) Coefficient	*P* Value
Sex, median (IQR), wk		.854		.702
Male	2 (2 to 4)		Reference	
Female	2 (1 to 4)		.030	
Age	Spearman's *rho* = .24	.004	.105	.238
HIV duration	Spearman's *rho* = .22	.009	–.151	.250
ART duration	Spearman's *rho* = .32	<.001	.419	.002
Time between HIV diagnosis and ART initiation	Spearman's *rho* = –.03	.719	-	-
Intervention, median (IQR), wk		<.001		<.001
No intervention	2 (2 to 3)		Reference	
Intervention	2.5 (2 to 4)		.260	
Baseline VL	Spearman's *rho* = –.22	.005	–.319	<.001

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; TtR, time to rebound; VL, viral load.

Previous reports have found a weak or no association between TtR and set point or other commonly utilized virological end points [1, 14, 31]. Based on these results, experts recommend avoiding MAP designs in studies assessing immunological interventions [2]. However, in the current study, we have found significant correlations between the TtR and the majority of other important end points that can only be measured at a later time point, including the set point, the peak VL, and the AUC. Our results indicate that it may be possible to estimate the expected value of these late parameters based on TtR: We demonstrated that clinically significant virus control is mainly to be expected in patients with longer TtR. This observation opens the possibility of improving the safety profile of these studies: Patients with an early viral rebound should be put back on ART without further delay, whereas only participants with longer TtR would be exposed to prolonged ATIs. These data should be used with caution, as TtR has certain limitations. Although our study suggests that TtR is correlated with all the other quantitative and temporary outcomes, with our data it is difficult to determine a TtR cutoff that predicts a very low risk of control of viral load.

The effect of baseline VL on the characteristics of viral rebound dynamics has also been suggested by previous publications. In a study with repeated treatment interruptions, the patients with a baseline VL >50 000 copies/mL were significantly more likely to reach VL peaks >50 000 copies/mL during ATIs than the ones with lower baseline VL [32]. Other researchers found that a baseline VL <100 000 copies/mL was significantly associated with the probability of maintaining a VL <5000 copies/mL 24 weeks after treatment interruption [33]. However, in contrast with our findings, other authors have not found an association between baseline VL and TtR [34]. Further studies are needed to confirm our results.

In our cohort, we identified some important confounding factors that may affect viral rebound dynamics. Similar to other reports, gender [35–37], age [38, 39], and previous HIV and ART duration [37] significantly influenced certain rebound parameters. In a multivariate analysis, the factors independently associated with TtR were baseline VL, previous duration of ART, and immunological interventions.

Our study has a number of limitations. First, it is a retrospective study. Second, the included studies were heterogeneous to some extent. Third, VL data were largely unavailable at certain weeks (eg, weeks 5, 10, and 48). Fourth, some of the analyzed parameters were not possible to determine in an important proportion of the cohort: Set point was only available in 154 cases (46.1%), and delta set point in 151 cases (45.2%). For this reason, we carried out a sensitivity analysis with these data. Fifth, the ART regimens the patients were receiving by the time of treatment interruption were not available for the analysis, although this may be a factor affecting rebound dynamics [40]. Sixth, most patients recruited in the source studies were from Catalonia (a geographical region of Spain), which may affect the generalizability of our results to other populations. Seventh, one-third of the ATI episodes were preceded by previous ATIs that may have influenced the measured parameters and the correlations between them. However, in a subgroup analysis of the 215 first ATI episodes, we did not find any significant differences with respect to the overall study population. Finally, our data could not be adjusted to the magnitude of other unavailable parameters, such as viral reservoir or nadir CD4.

In conclusion, our study provides a detailed description of the dynamics of viral rebound after ART interruption, based on a retrospective cohort of considerable size. We believe that these data may be useful in the evaluation of the outcomes of future ATI studies without a control arm. In addition, we have demonstrated that there are significant correlations between most of the virological end points assessed. If confirmed by independent prospective studies, these observations could be helpful to design the duration of ATIs or the threshold to reintroduce ART in future HIV cure clinical trials. For example, a short TtR could discriminate those patients who should reinitiate ART sooner.

Additionally, our results underline the importance of taking into account some potential confounding factors in the interpretation of these studies.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. C.F., L.L., P.A., and F.G. designed the study. C.F., L.L., M.P., N.C., A.C.G., E.M., P.C., V.D.B., B.M., J.C.L.B.Q., and F.G. performed data acquisition. C.F. did data analysis. L.L., J.M.G., P.A., and F.G. contributed to the interpretation of data. C.F., P.A., and F.G. wrote the manuscript, and all other authors revised it for intellectual content. All authors gave their approval of the final version of the manuscript.

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