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Any impact of blips and low-level viraemia episodes among HIV-infected patients with sustained virological suppression on ART?

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Abstract

Objectives. The objective of this study was to evaluate the prevalence of blips and risk of virological failure (VF) among HIV-infected patients with sustained virological suppression (HIV-RNA <50 copies/mL) on ART.

Methods. Newly diagnosed (2004–13) HIV-infected patients with sustained virological suppression on ART (minimum follow-up of 3 months) were identified. Risk of VF was evaluated according to different plasma HIV-RNA quantification values based on the limits of quantification/detection of current commercial assays (20 copies/mL). Kaplan–Meier and Cox proportional hazards models were used to compare the cumulative incidence of VF.

Results. A total of 565 newly diagnosed HIV-infected patients were identified: 453 started ART and 354 achieved virological suppression. Prevalence of blips (isolated HIV-RNA ranging from 50 to 200 copies/mL) and VF (HIV-RNA ≥50 copies/mL) was 22.7% and 8.8%, respectively (mean follow-up of 42 months). Multivariate analysis identified differences between HIV-RNA values as an independent predictor of VF (P=0.008); risk of VF was higher for patients with blips [HR 2.500 (95% CI 0.524–11.926)] and for those with at least three consecutive detected, but not quantified, HIV-RNA determinations (HIV-RNA <20 copies/mL) [HR 3.813 (95% CI 0.675–21.535)]. Moreover, only HIV-infected patients with at least three consecutive detected, but not quantified, HIV-RNA determinations showed a higher probability of virological rebound with >200 copies/mL [33.7% at 24 and 60 months versus <5% for other HIV-RNA values; HR 6.943 (0.728–66.261), P=0.092].

Conclusions. Blips are frequent (22.7%) among HIV-infected patients with sustained virological suppression on ART. HIV patients with blips and at least three consecutive detected, but not quantified, HIV-RNA determinations (<20 copies/mL) had a higher risk of VF. These findings highlight the relevance of maintaining HIV-RNA levels below the limits of quantification of current assays (<20 copies/mL).

Introduction

The current goal of ART is HIV-RNA suppression <50 copies/mL. However, intermittent episodes of detectable low-level viraemia (50–1000 copies/mL) have been described among HIV patients who achieved virological suppression.[1,2] Blips do not always lead to negative clinical outcomes, although they may occasionally anticipate virological failure (VF) and the selection of drug-resistant HIV variants.[1–4] Moreover, there is evidence for residual low-level viraemia <50 copies/mL in patients on stable suppressive ART.[5–7]

Several studies have suggested a potential role of blips and low-level viraemia events in the likelihood of VF. However, their clinical relevance is not yet clearly established because of the controversial results obtained, probably due to heterogeneity among the different studies (i.e. baseline patient characteristics and definitions of blips and VF).

In this context, the aim of the study was to evaluate the incidence of blips and low-level viraemia in a cohort of newly diagnosed HIV-infected patients on suppressive ART and to identify those factors associated with VF.

Methods

All HIV-infected patients newly diagnosed from 1 January 2004 to 31 December 2013 in a reference hospital in north-west Spain were recorded. Those who started ART and reached HIV-RNA levels <50 copies/mL were identified and followed until 1 January 2015. Epidemiological, clinical and immunovirological characteristics at diagnosis were recorded. The research protocol was approved by the regional ethics committee ('Comité Ético de Investigación Clínica de Galicia', register code 2013/247).

Plasma viral load was determined using the Roche COBAS TaqMan test version 2.0 (limit of quantification=20 copies/mL). HIV-RNA quantification was interpreted as follows: detectable (HIV-RNA >20 copies/mL); not detectable (ND); and detectable, but not quantified (<20 copies/mL) (DNQ).

For subsequent analysis, HIV-infected patients with virological suppression under ART were assigned to the following groups: (i) all determinations ND; (ii) one to two determinations DNQ; (iii) at least three consecutive episodes of DNQ determinations; (iv) intermittent low-level viraemia (ILLV) episodes, i.e. HIV-RNA values of 20–50 copies/mL preceded and followed by ND or DNQ viraemia; and (v) blips, i.e. HIV-RNA value of 50–200 copies/mL preceded and followed by another value <50 copies/mL, according to the definition of the Spanish AIDS Study Group (GeSIDA) guidelines.[8]

VF was defined as HIV-RNA ≥50 copies/mL at two consecutive visits according to GeSIDA and European AIDS Clinical Society guidelines.[8,9] Finally, virological rebound was defined as confirmed HIV-RNA ≥200 copies/mL (VR200) according to the US Department of Health and Human Services guidelines.[10] Adherence was indirectly evaluated by determining the rate of lost pharmaceutical consultation, a delay of ≥15 days after programmed consultation, which might have increased the risk of having no ART medication.

Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS 19.0, Chicago, IL, USA). Categorical variables were presented as the number of cases or percentage and compared by the χ^2 test or Fisher's exact test when appropriate. Continuous variables were expressed as the mean±SD and compared by the non-parametric Mann–Whitney or Kruskal–Wallis test when appropriate. Kaplan–Meier curves were used to evaluate the probability of both VF and VR200 during follow-up and differences between groups were assessed by the log-

rank test and Cox regression analysis, calculating the HR and 95% CI. A *P* value <0.05 was considered statistically significant.

Results

A total of 565 newly diagnosed HIV-infected patients were identified during the study period. Overall, 453 (80.2%) patients started ART and 354 (78.1%) of them achieved suppressed viraemia (HIV-RNA <50 copies/mL). Only patients with a minimum period of follow-up of 3 months, with at least two viral load determinations available after achieving suppressed viraemia, were examined. A total of 326 patients were considered for analysis with a mean time of follow-up of 42±29 months. The rate of blips and ILLV was 22.7% and 24.8%, respectively. Epidemiological, clinical and immunovirological characteristics are shown in Table 1.

Table 1. Baseline characteristics of the study population

	Blips (*n*=74)	ILLV (*n*=81)	≥3 consecutive DNQ (*n*=27)	DNQ viraemia (*n*=95)	ND viraemia (*n*=49)	*P*[a]
Male, %	82.4	79	88.9	81.1	75.5	0.680
Age (years), mean±SD	38.7±9.3	37.3±12	42.8±12.3	37.6±9.2	37.5±10.6	0.190
Route of transmission, %						
MSM	35.6	27.6	33.3	36.3	35.6	
heterosexual	52.1	50	66.7	41.8	55.6	
IVDU	12.3	22.5	0	22	8.9	
AIDS at diagnosis, %	50	46.9	30.8	33.7	20.4	0.005
CD4+ <200 cells/mm^3 at diagnosis, %	43.2	43.2	30.8	28	18.4	0.012
Viral load >100000 copies/mL at diagnosis	73.6	63.8	61.5	33.3	26.5	<0.001
Positive for hepatitis B virus surface antigen, %	5.5	2.5	0	2.1	6.1	
Positive for hepatitis C virus antibody, %	15.1	23.8	11.5	23.9	16.7	0.377
HIV-1 subtype, %						
B	67.9	62.5	72.2	66	69.2	
F	25	28.1	22.2	22.6	17.9	
other subtype	7.1	9.4	5.6	11.3	12.8	
ART regimen (%)						0.542[b]
two NRTIs+NNRTI	42.5	47.5	51.9	50	58.3	
two NRTIs+PI	52.1	45	44.4	41.5	37.5	
two NRTIs+integrase inhibitor	4.1	3.8	3.7	5.3	2.1	
other regimen	1.4	3.8	0	3.2	2.1	
Time to suppressed viraemia (HIV-RNA <50 copies/mL) (weeks), mean±SD	40.4±27.1	43.5±44.9	41.6±44.1	31.1±35.3	29.±21.6	0.006
Lost pharmaceutical consultation (≥15 days)[c], mean±SD	5.4±4.8	7.9±8.9	4.5±5.5	6.4±7.8	4.7±7.3	0.017

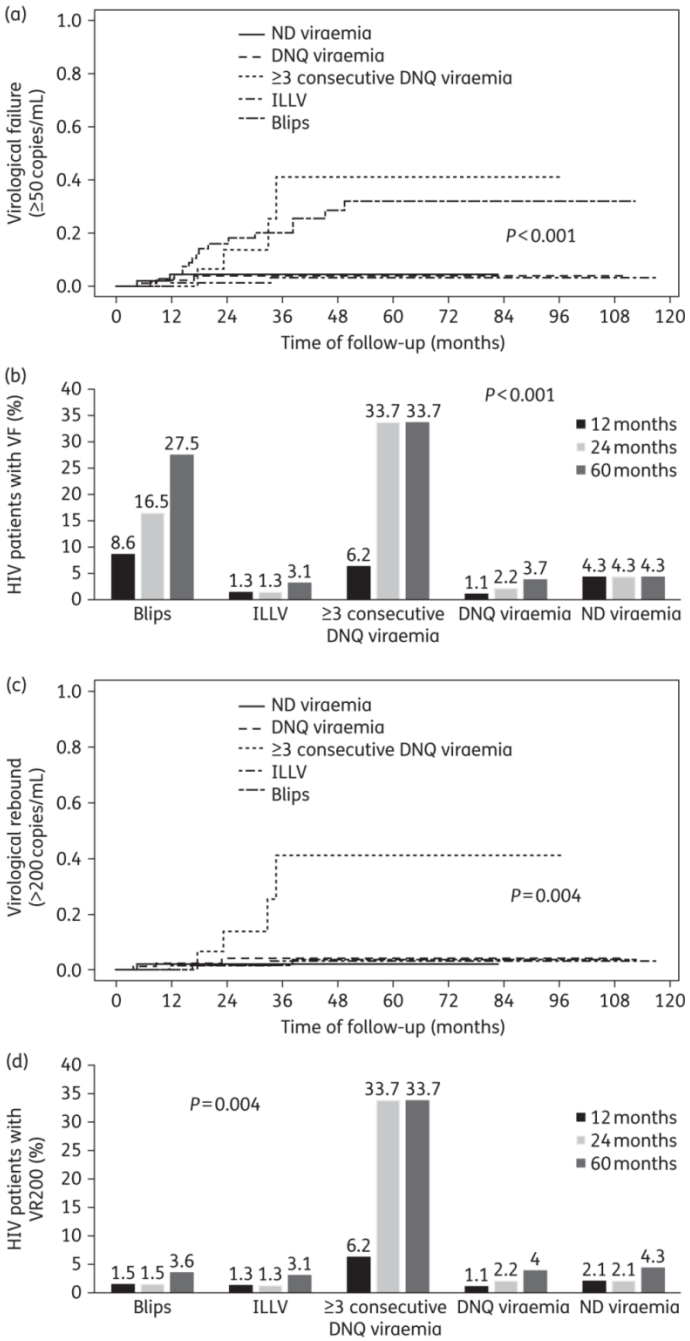
Blips, HIV-RNA value ranging from 50 to 200 copies/mL preceded and followed by another value <50 copies/mL; ILLV, intermittent low-level viraemia episodes, i.e. HIV-RNA value ranging from 20 to 50 copies/mL preceded and followed by another value <20 copies/mL; ≥3 consecutive DNQ viraemia, three or more consecutive detected, but not quantified, HIV-RNA determinations <20 copies/mL; DNQ viraemia, patients with one or two detected, but not quantified, HIV-RNA determinations <20 copies/mL; ND viraemia, all HIV-RNA determinations were not detected.

[a] Univariate analysis.

[b] Comparing NNRTI versus PI regimen.

[c] Lost pharmaceutical consultation (>15 days) means the percentage of lost pharmaceutical consultation with a delay of ≥15 days after programmed consultation and, therefore, with potential, but not mandatory, risk of having no ART medication.

After achieving HIV-RNA suppression, 8.8% (*n*=27) of patients experienced VF and 3.6% (*n*=12) experienced VR200. Time to VF and VR200 according to the presence of blips, ILLV, DNQ viraemia or ND viraemia are shown in Figure 1, with differences between groups ($P<0.001$ for VF and $P=0.004$ for VR200).



Rates of VF (confirmed HIV-RNA ≥50 copies/mL) after achieving HIV-RNA <50 copies/mL according to the following viraemic groups: ND viraemia, all HIV-RNA determinations were not detected; DNQ viraemia, patients with one or two detected, but not quantified, HIV-RNA determinations <20 copies/mL; ≥3 consecutive DNQ viraemia, three or more consecutive detected, but not quantified, HIV-RNA determinations <20 copies/mL; ILLV, intermittent low-level viraemia episodes, i.e. HIV-RNA value ranging from 20 to 50 copies/mL preceded and followed by another value <20 copies/mL; and blips, HIV-RNA value ranging from 50 to 200 copies/mL preceded and followed by another value <50 copies/mL. (a) Time to VF according to Kaplan–Meier analysis. (b) Proportion of HIV patients on ART with VF at 12, 24 and 60 months after achieving virological suppression. (c) Time to VR200 according to Kaplan–Meier analysis. (d) Proportion of HIV patients on ART with VR200 at 12, 24 and 60 months after achieving virological suppression.

Viral load values >100000 copies/mL at diagnosis (P=0.013) and adherence to treatment (proportion of lost pharmaceutical consultation ≥5%) (P=0.044) were predictors of VF in the Kaplan–Meier univariate analysis. Conversely, none of the following variables was associated with VF: route of transmission (P=0.445); AIDS-defining criteria at diagnosis (P=0.539); CD4+ count <200 cells/mm^3 at diagnosis (P=0.491); HIV-1 genetic subtype (P=0.703);[11] presence of transmitted drug resistance mutations (P=0.487);[12] and ART regimen (NNRTI- versus PI-based treatment) (P=0.756).

Multivariate analysis, adjusted by CD4+ <200 cells/mm^3, HIV-RNA >100000 copies/mL and AIDS-defining disease at diagnosis, identified adherence [HR 2.409 (95% CI 1.073–5.411), P=0.033] and different values of HIV-RNA quantification and/or detection (P=0.008) as predictors of VF. Patients with blips [HR 2.500 (95% CI 0.524–11.926), P=0.250] and at least three consecutive DNQ HIV-RNA determinations [HR 3.813 (95% CI 0.675–21.535), P=0.130] were more likely to have VF. Similarly, multivariate analysis for VR200 shows that different viraemic groups (P=0.008) and adherence [HR 4.146 (95% CI 1.095–15.696), P=0.036] were independent predictors of VR200, after adjusting by the same variables. The presence of at least three consecutive DNQ HIV-RNA determinations shows a higher probability of VR200 compared with those patients with ND determinations [HR 6.943 (95% CI 0.728–66.261), P=0.092].

Discussion

The clinical consequences for patients experiencing blips or low-level viraemia remain unclear. This study suggests that HIV-infected patients who achieved viral load suppression under ART, but have at least three consecutive DNQ viral load determinations below the limit of quantification (<20 copies/mL) or have blips, are more likely to have VF. Indeed, only those patients with at least three consecutive DNQ HIV-RNA determinations have shown a higher risk of VR200.

Virological suppression had been historically defined considering the limits of detection of the commercial assays available, which have been improving from 400 copies/mL for first-generation assays, then 50 copies/mL and, currently, 20 copies/mL.[13,14] According to current HIV guidelines, the goal of ART is to maintain virological suppression <50 copies/mL.[8,9] However, in patients on stable suppressive ART, persistent or transient residual viraemia below the cut-off of 50 copies/mL is observed.[5,6] The source of this residual viraemia remains controversial and could be explained by virus release from activation of latently infected CD4+ T cells or viral replication as a consequence of suboptimal therapy with the consequent risk of selection of resistance.[5,15]

The clinical relevance of low-level viraemia <50 copies/mL or even of HIV-RNA detection below the limit of quantification (<20 copies/mL) of current commercial assays remains uncertain.[16] This scenario makes it necessary to determine the frequency of these events and their impact in the clinical success of ART in HIV-infected patients.

In this study, the prevalence of blips was 22.7%, similar to other studies, which have shown a prevalence ranging from 20% to 40%.[2–4] Many studies agree that occasional blips are common and do not reflect viral replication nor predict VF,[4,17] but others have found an association between their presence and subsequent VF.[1,16,18,19] Likewise, this study shows an association between the presence of blips and VF (8.6%, 16.5% and 27.5% at 12, 24 and 60 months, respectively). Of note, HIV patients with at least three consecutive DNQ HIV-RNA determinations were more likely to have VF (Figure 1). Interestingly, only the presence of at least three consecutive DNQ viral load determinations was associated with a higher risk of VR200 with rates of 33.7% at 24 and 60 months compared with rates of <5% for other viraemic groups. Multivariate analysis (adjusted by CD4+ <200 cells/mm^3, HIV-RNA >100000 copies/mL and AIDS-defining disease at diagnosis) identified the presence of at least three consecutive DNQ viral load determinations and treatment adherence as independent predictors of both VF and VR200. Doyle and Geretti,[5] Doyle *et al.*[6] and Calcagno *et al.*[7] have also recognized that among HIV-infected patients under ART, HIV-RNA

values between 40 and 49 copies/mL and, to a lesser extent, <40 copies/mL predict VF considering two definitions, >50 and >400 copies/mL, independently of other recognized determinants (i.e. adherence and ART regimen).

Altogether, these findings suggest that a prolonged and continuous presence of residual viral replication might favour a subsequent VF more than isolated blips of viraemia, which could be the result of different factors (i.e. adherence and intra-assay variability among determinations). This scenario would increase the potential risk of developing resistance mutations and, consequently, treatment failure.[20] Therefore, clinical monitoring of these patients, focused on adherence, evaluation of potential drug–drug interactions with concomitant medications, appropriate food intake when indicated and detailed evaluation of the presence of drug resistance mutations, is recommended.

The present study has some limitations. There is a low number of VF that might explain the lack of statistical significance for the association between VF and the presence of blips or at least three consecutive DNQ HIV-RNA determinations compared to the group of ND viraemia. However, the association becomes significant when we analyse different viraemic groups together. Second, the impact of VF on the risk of developing drug resistance mutations has not been evaluated, as most cases of VF had HIV-RNA <200 copies/mL.

In conclusion, this study provides new data for a better understanding of the clinical impact of low-level viraemia events among HIV-infected patients under stable suppressive ART. The main finding was the identification of the presence of at least three consecutive DNQ HIV-RNA determinations below the limit of quantification of current commercial assays (<20 copies/mL) as being associated with a higher risk of VF. Therefore, the goal of ART should be revised to a lower cut-off of 50 copies/mL considering the limit of detection/quantification of current available assays. Meanwhile, close clinical monitoring would be advisable for these patients with special emphasis on treatment adherence.

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Transparency declarations
None to declare.

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