Intermittent HIV-1 Viremia (Blips) and Virological Failure in a Cohort of People Living with HIV from São Paulo, Brazil

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Dear Editor:

We estimated the incidence of blip (intermittent viremia) and virological failure (VF) in a Brazilian cohort of patients followed up for 78 weeks under stable antiretroviral therapy (ART) and investigated factors associated with these outcomes. HIV-infected patients under ART, who presented two consecutive undetectable plasma viral loads (lower limit of detection = 50 copies of HIV-RNA/mL), during follow-up at the HIV Clinic, School of Medicine, University of São Paulo, Brazil, were enrolled between July 2005 and July 2007. Viral blips were defined as plasma HIV-RNA loads above 50 copies/mL of a nonviremic patient, followed by an assessment below 50 copies/mL. If patients presented more than one blip, only the first episode was considered for analysis. VF was defined as two consecutive plasma viral loads above 50 HIV-RNA copies/mL.

At baseline, we collected data regarding demographic characteristics, history of previous ART regimens, presumed mode of HIV acquisition, use of and adherence to antiretrovirals (having missed at least one intake in the previous week was defined as nonadherence), co-morbidities, and use of alcohol and illicit drugs. At 3-month interval visits, we collected information on presumed predictors of study outcomes, including occurrence of concurrent diseases, immunizations, and risky sexual behaviour (self-reported episode of unprotected sex in the previous month), and blood samples were drawn for assessment of HIV viral loads and CD4+ T-cell counts. At time of occurrence of the study outcomes, HIV genotyping was performed in search of drug resistance mutations (DRMs) for protease inhibitors (PI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and nucleoside/nucleotide reverse transcriptase inhibitors (NRTI), according to International AIDS Society (IAS) algorithms.¹

The study cohort comprised 350 patients, and 250 (71.5%) were men. CD4+ T-cell counts at baseline ranged from 112 to 1869/mm³ (median 571). 264 (75.4%) subjects exhibited undetectable HIV plasma viral loads throughout the study, whereas 24 patients did not complete follow-up. Out of these, 22 remained nonviremic during the time they were seen, and two others presented a detectable HIV viral load at

the last visit. As their outcome could not be classified, the latter were excluded from analysis. Forty-four individuals developed viral blips (12.6%), and 21 (6.0%) presented VF. The overall incidences of blip and VF were 9.4 and 4.2/100 person-years, respectively. Three individuals presented VF 9 months after having experienced a blip episode. Baseline characteristics of the study participants are presented in Table 1. Predictors of blip and VF were initially investigated in bivariate analysis. For that, subjects who presented a blip episode were compared with nonviremic individuals, whereas those who exhibited VF were compared with nonviremic individuals and those who presented blip together (Table 2). In a multiple logistic regression model, we identified nonadherence to safer sex measures in the previous month and history of multiple exposures to antiretroviral regimens as independent predictors of both study outcomes (Table 2). It is important to highlight that blips did not predict the occurrence of VF.

Out of 44 patients who presented viral blips, 22 could undergo HIV genotyping assays. Six were successfully sequenced in both genes, whereas in the other 11, results were obtained for the *Prot* gene only. No patient presented primary mutations in the *Prot* gene, but secondary mutations were detected in most. Three out of six patients exhibited mutations in the *RT* gene that contributed to antiretroviral resistance: two of them to NRTI and another to NNRTI. Taken together, 9 out of 13 (69.2%) patients showed sensitivity to the ARV regimen they used at the time of a blip episode.

As for VF, all 21 patients that developed this outcome underwent genotyping assays at time of failure. Twenty accomplished successful amplification of the *Prot* gene, 13 (65%) of whom were sensitive to PI. It must be pointed out that half of them were not using such antiretrovirals. Seven subjects presented major mutations in the *Prot* gene: M46L, I50L, I54V, V82A/F, I84V, and L90M, with L90M being the most frequent, followed by I54V. In contrast, 12 (85.7%) of the isolates had DRMs in the *RT* gene, nine (75%) exhibiting DRMs to both classes of RTIs. The most frequent DRMs were M184V, followed by T215D/Y/F to NTRIs and K103N to NNTRIs, mutations that are able to confer phenotypical resistance themselves. In four cases, M184V was found in association

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TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS WITH SUSTAINED UNDETECTABLE VIRAL LOAD, VIRAL BLIP, AND VIROLOGICAL FAILURE, AIDS CLINIC, SÃO PAULO, BRAZIL, 2005–2007

Variable	Suppressed (n = 286)		Viral blip (n = 44)		p	Suppressed + viral blip (n = 327)		Virological failure (n = 21)		p
	N	%	N	%		N	%	N	%	
Median age [years]	42		40		0.055*	42		42		0.084*
Sex					0.363"					0.631"
Male	202	85.6	34	14.4		234	94.4	14	5.6	
Female	84	89.4	10	10.6		93	93.0	7	7.0	
Skin color					0.451#					0.010#
White	201	87.0	30	13.0		229	92.7	18	7.3	
Black	15	93.8	1	6.3		16	100.0	–	–	
Yellow	4	100.0	–	–		4	66.7	2	33.3	
Other	66	83.5	13	16.5		78	98.7	1	1.3	
Primary risk behavior					0.540#					0.526#
Homosexual	97	85.8	16	14.2		112	94.1	7	5.9	
Bisexual	30	88.2	4	11.8		34	100.0	–	–	
Heterosexual (male)	52	85.2	9	14.8		61	92.4	5	7.6	
Heterosexual (female)	77	89.5	9	10.5		85	93.4	6	6.6	
IVDU	14	87.5	2	12.5		16	94.1	1	5.9	
Blood transfusion	16	84.2	3	15.8		18	90.0	2	10.0	
Other	–	–	1	100.0		1	100.0	–	–	
Schooling					0.134"					0.134"
Elementary school	91	87.5	13	12.5		103	97.2	3	2.8	
High school	100	87.0	15	13.0		115	94.3	7	5.7	
College	95	85.6	16	14.4		109	90.8	11	9.2	
Baseline CD4+ cell count [cells/mm ³] (median)	574		642.5		0.108	598		481		0.027

*2-sided Student *t* test; "chi-squared test; #equal variance; §Mann-Whitney *U* test.

with A62V and Q151M, thus conferring phenotypical resistance to all NTRIs.

At the occurrence of any of the study outcomes, we also detected many polymorphisms in the *RT* and *Prot* genes. In the study cohort, 35 (94.6%) out of 37 successfully sequenced samples belonged to subtype B and two to subtype F1.

A blip is a common phenomenon, occurring in 9–52% of patients who achieve viral suppression.^{2–6} In our cohort, intermittent viremia occurred in 12.6% of patients, in accordance with previously reported prospective studies,^{6,7} whereas VF was prospectively identified in 6% during follow-up, a result that is also consistent with some previous studies.^{2,4} The occurrence of blip was not shown predictive of VF, supporting findings from previous reports suggesting that ART regimens do not need to be switched following a blip episode.^{2,4,7,8} The overall incidence of 9.4 blips/100 person-years we found is lower than that from previous reports, based on similar viral load assessment techniques, including the one by Sungkanuparph et al.,⁸ using a definition of blip and a time of follow-up that were similar to ours, and by Greub et al.,⁵ who included patients with sustained low-level viremia with different blip definition criteria.

Nonadherence to safer sex measures in the previous month was an independent predictor with the occurrence of blip and VF. This could be explained based on the findings of Willberg et al.,⁹ who evaluated the impact of continued exposure to HIV-1 on anti-HIV-1 T-cell responses in HIV-infected individuals. According to them, infected individuals regularly exposed to an HIV-1 viremic long-term sexual partner, exhibited higher

HIV-1-specific CD4+ and CD8+ T-cell responses, leading to transient viral replication, as compared with subjects with nonviremic partners. Alternatively, risky sexual behavior (i.e., nonadherence to safer sex practices) may be a proxy of nonadherence to HIV treatment, highlighting the need for a comprehensive approach when intervening with people living with HIV/AIDS. In fact, Kalichman et al.^{10,11} found that people, who had been nonadherent to ART, also reported significantly more sexual partners and less frequent protected sexual practices, regardless of the partner's serostatus. Even though our findings did not show an association between viral blips or VF with nonadherence to ARV, this may have been because over 90% of our study subjects reported being adherent to therapy.

Our data suggest that longer ART experience is associated with higher risk of developing both an HIV blip and VF, what may be a consequence of a longer duration of HIV infection and/or of lower adherence to ART previous regimens. This finding is concordant with our observation that exposure to a higher number of ART regimens predicts the occurrence of viral blips or VF, as previously described.^{4,8}

Our results demonstrate that viral blips should not be taken necessarily as an indication of resistance to ART. Few patients who developed blip presented DRMs in the *RT* gene (M41L/T215Y) or secondary mutations only in the *Prot* gene. In addition, they did not present VF after the transient rebound of viremia, during follow-up, in spite of exhibiting DRMs that could have led to ART failure. This could be because HIV strains with multiple DRMs to each antiretroviral may still be phenotypically sensitive to a combination of these drugs. In

TABLE 2. BIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION FOR PREDICTORS OF VIRAL BLIP AND VIROLOGIC FAILURE; AIDS CLINIC, SAO PAULO, BRAZIL, 2005–2007

	<i>Bivariate analysis</i>			<i>Multivariate analysis</i>				
	<i>Viral blip</i> OR (95%CI)	<i>p Value</i>	<i>Virological failure</i> OR (95%CI)	<i>p Value</i>	<i>Viral blip</i> adjOR (95%CI)	<i>p Value</i>	<i>Virological failure</i> adjOR (95%CI)	<i>p</i>
Median age [years]	0.97 (0.93–1.01)	0.094	0.99 (0.93–1.05)	0.719	–		–	
Underlying disease								
No	1.00		1.00		–		–	
Yes	0.68 (0.35–1.35)	0.269	0.35 (0.10–1.23)	0.102	–		–	
History of substance abuse								
No	1.00		1.00		–		–	
Yes	0.37 (0.11–1.22)	0.103	0.86 (0.11–6.76)	>0.999	–		–	
Adherence to safer sex behavior								
No	26.98 (4.22–172.66)	0.001	22.38 (3.59–139.61)	0.001	24.64 (4.40–137.88)	<0.001	24.69 (4.20–145.18)	<0.001
Yes	1.00		1.00		1.00		1.00	
Antiretroviral regimen								
NRTI+NNRTI	1.00		1.00		–		–	
NRTI+PI	1.70 (0.78–3.70)	0.178	1.28 (0.52–3.19)		–		–	
other	1.16 (0.20–6.54)	0.869	1.24 (0.15–10.31)	0.864	–		–	
Previous ART [median number]	1.56 (0.98–2.48)	0.059	1.57 (0.86–2.86)	0.139	1.82 (1.41–2.36)	<0.001	1.67 (1.19–2.35)	0.003
ART changes due to virologic failure [median number]	1.02 (0.68–1.53)	0.939	1.12 (0.66–1.91)	0.673	–		–	
Time since HIV infection [months]	1.01 (1.00–1.02)	0.132	–		–		–	

adjOR, adjusted odds ratio; ART, antiretroviral therapy; 95%CI, 95% confidence interval; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor.

our cohort, at the time of a blip episode, successfully sequenced viruses were sensitive to PI. This means that, for these individuals, ART regimens most probably retained enough activity to suppress HIV-RNA levels. Furthermore, multidrug resistant variants are known to present a compromised capacity to replicate, what may have rendered their detection less probable in our patients. As long as patients with blips adhere strictly to ART, VF may be deferred. However, new blips may progressively drive the selection of variants less susceptible to ART. In contrast, most participants who presented VF, exhibited genotypic evidence of resistance to all classes of antiretroviral drugs, including the regimen they received during the study, which could have accounted for the observed rebound. As for viral genetic variability, we found most strains belonged to subtype B. This variant is recognised as the most frequent in Brazil (80%), followed by subtypes F.¹²

Limitations of our study should be considered, including the fact that a complete evaluation of the association between resistance-conferring HIV mutations and the occurrence of the study outcomes was not possible; blood samples were not available for genotyping at different time points for all patients. In addition, technical difficulties reduced the number of successful amplifications of the RT gene. Moreover, we have not accessed sexual behavior thoroughly.

Our analysis suggests that blips are significantly associated with patients' attitudes. Risky sexual behavior and impaired adherence to therapy, elements that may be interrelated, were

identified as predictors of blip. Furthermore, no association was found between the occurrence of blips and subsequent VF and development of drug resistance. However, a detectable blip should highlight the need for the clinician to address issues such as adherence to ART and to safer-sex practices. Caution is also warranted before taking the blip episode as an ART regimen failure that requires changing therapies.

Acknowledgments

The authors are grateful to São Paulo State Research Funding Agency (FAPESP) [Grant number 024/05] for financial support. We would also like to thank Bruno Vita Ricci for assistance in data management; Maria Carolina Alamos Bernal, Anderson Molina Nascimento Filho, and Ana Carolina Mamana Fernandes de Souza for technical laboratory assistance, as well as Rogério Ruscitto do Prado for statistical consultancy.

Author Disclosure Statement

No competing financial interests exist.

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