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Integrated analysis of viral blips, residual viremia, and associated factors in people with HIV: Results from a retrospective cohort study

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Abstract

The etiology of viral blips is not yet fully elucidated. One of the hypotheses is that blips reflect variations in residual viremia (RV) near the detectability threshold. In this study, we evaluated whether RV is associated with viral blips and which factors are associated with RV. All treatment regimens in 2010–2020 consisting of two nucleos(-t)ide reverse transcriptase inhibitors and one anchor (integrase strand transfer inhibitor [INSTI], non-nucleoside reverse transcriptase inhibitor [NNRTI], or protease inhibitor [PI]) in people with HIV (PWH) were evaluated for RV (detectable viremia <50 cp/mL) and blips (isolated viral loads [VLs] 50–499 cp/mL between measurements <50 cp/mL). All medical records were reviewed and regimens in which a VL ≥ 50 cp/mL was deemed to result from non-adherence (based on the documented conclusion by the treating physician) were excluded. Factors associated with blips and RV were identified using generalized linear mixed models. In total, 24 518 VLs from 1658 PWH were analyzed. VLs were measured during INSTI- (n = 5119; 20.9%), PI- (n = 8935; 36.4%), and NNRTI-use (n = 10 464; 42.7%). VLs were categorized as blips in 1.4% (n = 332). The 24,186 non-blip VLs were RNA^{neg} (no RV) (n = 15 326; 63.4%), 1–19 cp/mL (n = 6318; 26.1%), 20–49 cp/mL (n = 1620; 6.7%), or <50 cp/mL with an unknown RV level (n = 922; 3.8%). In 193/1658 PWH (11.6%), the RV level was RNA^{neg} in all VLs assessed. RV 1–19 cp/mL and 20–49 cp/mL (vs. RNA^{neg}) were significantly associated with subsequent viral blips (respective odds ratio 2.66 and 4.90 [95% confidence intervals: 1.98–3.58 and 3.41–7.04]). Zenith VL and use of PIs (vs. INSTIs/NNRTIs) were associated with higher RV and blip odds. This large cohort study showed that blips were associated with higher preceding RV. Both the anchor type and factors previously linked to the latent viral reservoir were associated with RV, suggesting blips having a multifactorial origin.

Patrick G. A. Oomen and Suzan Dijkstra contributed equally to this work.

KEYWORDS
combination antiretroviral therapy, HIV, integrase strand transfer inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, residual viremia, viral blips

1 | INTRODUCTION

Most people with HIV (PWH) on combination antiretroviral therapy (cART) receive a treatment regimen consisting of two nucleos(-t)ide reverse transcriptase inhibitors (NRTIs) and a third (anchor) drug: either a non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase strand transfer inhibitor (INSTI). The main goals of antiretroviral treatment are to achieve and maintain suppression of viral replication with resulting immunological recovery, as well as prevention of drug-resistant variant selection and HIV transmission.[1] Nevertheless, even in PWH with optimal treatment adherence and well-suppressed viral replication, temporary elevations of plasma HIV viral load (VL) above the detection limit, referred to as viral blips, are frequently observed.[2] To explain the etiology of this phenomenon, multiple hypotheses have been proposed, mostly related to intermittent virion release from the latent reservoir, differences in assay accuracy, or ongoing viral replication.[3–11]

The occurrence of blips generates uncertainty for both PWH and healthcare providers and has been linked to adverse clinical outcomes, including virologic failure.[12,13] Blip occurrences are associated with non-modifiable factors linked to the viral latent reservoir (e.g., time since ART initiation, zenith (highest VL before cART start) VL and nadir $CD4^+$ count). Previously, we demonstrated that blips resulted in increased clinical burden, with fewer blips observed during INSTI-based regimens (vs. PIs and NNRTIs).[2] However, the question remains by what mechanism cART anchors influence the occurrence of blips. An intuitive explanation may be found in residual viremia (RV): detectable viremia below the commonly used threshold of 50 cp/mL.[14,15] It is conceivable that the RV level may (partly) contribute to the occurrence of blips, since at low RV levels a relatively larger increase in viral replication is required to reach the 50-copy threshold, resulting in a blip. A follow-up question that arises in this context is which factors, both modifiable and non-modifiable, are associated with the magnitude of RV.

To date, few studies have been performed on the relationship between RV and blips, and only for PI- and NNRTI-based regimens, rendering their findings less relevant in the current INSTI era.[16–18] Studies aimed at identifying factors associated with RV, reported conflicting data regarding the effect of different cART anchors. Moreover, all these studies lacked information on treatment adherence,[19–22] a factor that could have majorly impacted RV results. Given the clinical implications of blips, including increased healthcare burden and suggested link with virological failure, it is key to better understand the mechanism behind blips, which may partly lie in higher RV levels. Subsequently, it is important to comprehensively investigate factors influencing RV. Therefore, the aim of this cohort study was twofold: first, to assess whether higher RV levels, below the threshold of 50 cp/mL, were associated with the subsequent occurrence of viral blips. Second, to examine which factors (including cART anchor and factors potentially related to the latent reservoir such as the zenith VL) were associated with RV.

2 | METHODS

2.1 | Study population and follow-up

A retrospective assessment was conducted of VL measurements in adult PWH treated in the University Medical Center Utrecht, The Netherlands, between April first 2010 and 2020. PWH were eligible for the study when treated for HIV-1 with cART consisting of two NRTIs plus one anchor (NNRTI/PI/INSTI, booster allowed) and had achieved virologic suppression, defined as a plasma VL <50 cp/mL, on this regimen. cART composed of only these antiretrovirals was chosen to optimize comparability of the treatment regimens. PWH with a registered objection to data research were excluded. All VLs after the first suppressed VL were analyzed until the ending of the study period. We chose not to include the first suppressed VL after start of cART with the rationale that the presumed RV steady state during that treatment regimen may not have been reached yet. A graphical overview of the study is presented in Figure S1. All VLs ≥50 cp/mL not meeting the blip definition (definition below) were excluded from analysis, as viral rebound was considered to be a different clinical phenomenon that was not part of the objectives of this study. When the last VL before ending of the study period was ≥50 cp/mL, subsequent VLs up to 2020-09-01 were reviewed to determine if a blip had occurred. When PWH had a VL ≥50 cp/mL and were deemed non-adherent to treatment by their treating physician, as based on the conclusion documented in the medical records, the VLs during that treatment regimen were excluded from analysis to reduce the influence of adherence variability on RV levels and blips. Physicians' documentation that was assessed included statements regarding number of missed doses, timing of doses, and adherence to food instructions. Ultimately, the documented conclusion regarding the cause of the VL ≥50 cp/mL was followed. When non-adherence was documented to have existed only in a specific and well-defined time period, only VLs during this specific time period were excluded. The institutional ethical review board judged that the study met the criteria for exemption from formal review.

2.2 | Measurements and study outcomes

Demographic, laboratory, and adherence data were extracted from the medical records. VLs were assayed with the Roche COBAS®

TaqMan® v2.0 during the entire study period. All VL results <50 cp/mL were reported to the treating clinician as "<50 cp/mL," without specifying the level of residual viremia.

The main study outcomes were (1) the odds of viral blip occurrences when comparing different RV levels and (2) the odds of higher RV levels when comparing different cART anchors and multiple virologic factors. VLs <50 cp/mL were categorized as "RNA^{neg}" (no HIV RNA detected), "RV 1–19 cp/mL", and "RV 20–49 cp/mL", consistent with the detection and quantification limits of the assay used (i.e., able to detect but not quantify 1–19 cp/mL and able to quantify 20–49 cp/mL). A blip was defined as a VL of 50–499 cp/mL, preceded and followed by a VL <50 cp/mL without an anchor change. Multiple, consecutive measurements 50–499 cp/mL within 30 days were considered a single blip if preceded and followed by VLs <50 cp/mL without an anchor change.[23] Single VLs 50–499 cp/mL immediately before loss to follow-up were censored, as it was uncertain what the subsequent VL would have been (and thus whether the VL 50–499 cp/mL would have met the blip definition). Similarly, single VLs 50–499 cp/mL immediately before anchor switch were censored, as it was uncertain whether the VL would have been a blip if the original cART had been continued.

2.3 | Statistical analysis

Categorical data were compared using Fisher's exact test or χ^2; and continuous variables were compared using the independent samples *t*-test or Mann–Whitney *U*-test. Correlates of viral blips and the RV level were examined using multivariable generalized linear mixed effect models (GLMMs) for repeated measures with a random intercept and slope per individual. A GLMM logistic regression was used to assess the binomial outcome blips and a backwards continuation ratio model was used for the ordinal outcome RV level (RNA^{neg}/1–19/20–49 cp/mL).[24] For each included VL, both outcomes were assessed, meaning that for the outcome RV level, we assessed the RV level of the *current* VL and for the outcome blip occurrence, we examined whether the individual's *next* VL was a blip. This approach ensured that the analysis of both outcomes used all information from the PWH present up to that point in time but not future information (which is the case when summarizing the RV level of a given cART regimen or time period). The following time-varying (i.e., per VL) covariates, chosen based on existing literature, were included in the analysis for both study outcomes: age, time since study inclusion and ART initiation, and cART anchor. Additionally, for the outcome blip occurrence, the preceding RV level (i.e., the RV level in the VL before the VL assessed for blip occurrences) was used as a time-varying determinant of interest. The time-independent covariates for both outcomes were sex assigned at birth, lowest recorded CD4$^+$ count, Zenith VL, and Fiebig stage at ART initiation. In case of convergence issues, continuous variables were centered to improve convergence. Model diagnostics were performed, including assessment for multicollinearity and the proportion odds assumption.

To minimize potential bias resulting from missing variables (including missing RV values), multi-level multiple imputation (MI) was conducted. Level-1 (i.e., varying within one person) and level-2 (i.e., constant within one person) predictors were imputed. Because of collinearity between time since study inclusion, ART initiation, and age, resulting in non-convergence, only time since study inclusion was included as a predictor for MI, as this covariate was deemed the most important to include as it describes the random slope for time for GLMM regressions. Five imputed datasets were constructed using ten iterations and trace line plots were used to assess MI convergence. Results from MI were pooled using Rubin's Rules.[25] Several sensitivity analyses were performed to assess the robustness of observed associations: analysis including the NRTI backbone as a covariate, complete case analysis (i.e., no multiple imputation, only participants without missing data), analysis within each anchor group including the specific anchor drug as a covariate, and analysis excluding PWH with specific periods of non-adherence completely. Results were expressed as odds ratios (ORs) and their 95% confidence interval (CI) with two-sided $p < 0.05$ being considered statistically significant. All analyses were conducted using RStudio (v1.3.1093).

3 | RESULTS

3.1 | Study population and VL measurements

A total of 2056 PWH with VL measurements in the study period were assessed, of whom 1658 had VL data eligible for analysis (Figure 1). At baseline, the mean age was 43.6 years and 317 (19.1%) were female (Table 1). A median of 14 VLs (range: 1–35) per individual were included in the study. In total, 30 857 individual VLs were assessed for eligibility, of which 6339 were excluded for the reasons listed in Figure 1. Adherence was documented at least once in all but four PWH with VLs stable <50 cp/mL (99.8%). A total of 74/1658 PWH (4.5%) had specific and well-defined periods of non-adherence, resulting in excluded VLs during that non-adherent period. After assessing the physicians' documented conclusion on treatment adherence, VLs <50 cp/mL during PI-based treatment regimens were more frequently excluded based on documented non-adherence than VLs during other regimens (5.4% vs. 1.1% excluded; $p < 0.001$). No difference in documented non-adherence was found between NNRTIs and INSTIs (1.1% vs. 1.0% excluded; $p = 0.89$). Ultimately, a total of 24 518 VLs were analyzed, of which 5119 (20.9%), 8935 (36.4%), and 10 464 (42.7%) were during INSTI-, PI-, and NNRTI-use, respectively (Table S1 for the anchor, backbone and booster specification).

3.2 | Viral blips

Of the 24 518 VLs included, 332 (1.4%) were categorized as blips, occurring in 254 PWH (15.3% of the study population). In 2/332 blips (0.6%), the NRTI backbone was changed after the blip. Of the 332 blips, 52 were found immediately after the first suppressed VL, rendering them ineligible for the analysis of the association with RV, as the RV level of the first suppressed VL was not included. This left 280 blips eligible for

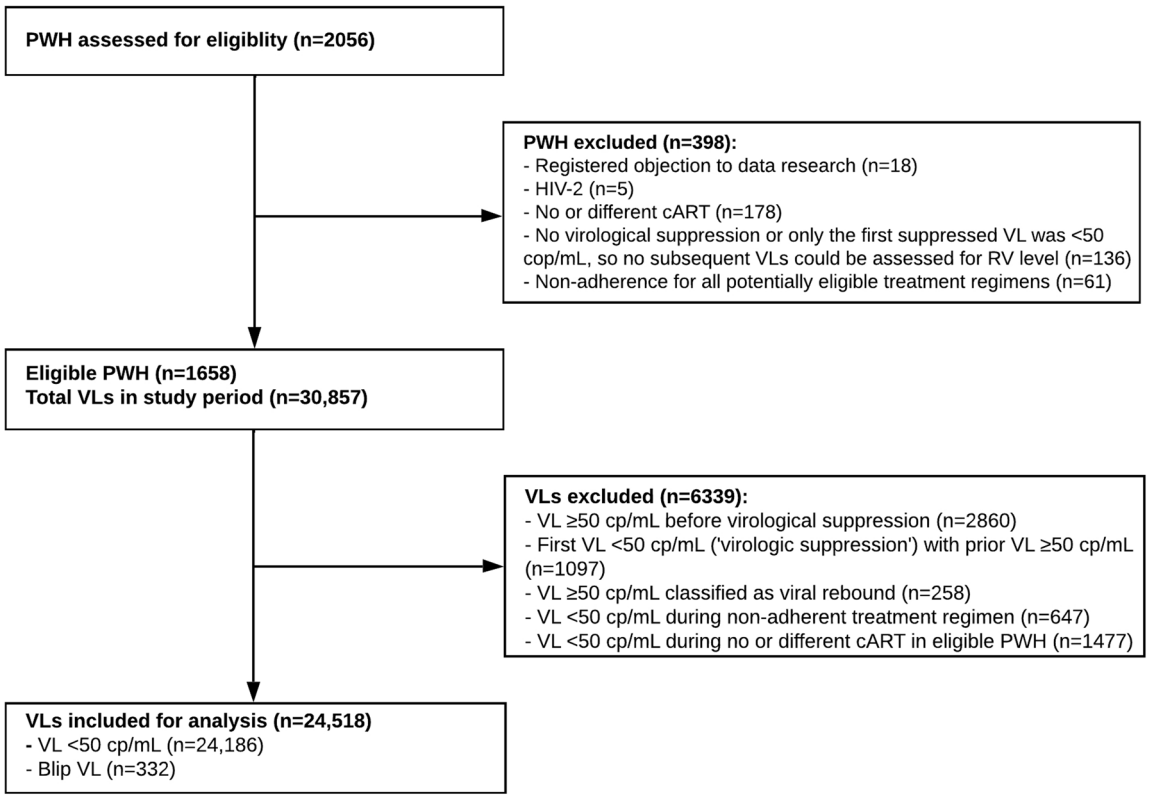

FIGURE 1 Study flow chart. Viral rebound was defined as a VL ≥50 cp/mL after virological suppression not meeting the blip criteria. cART, combination antiretroviral therapy; cp, copies; n, number; PWH, people with HIV; VL, viral load.

regression analysis. The 24 186 non-blip VLs were categorized as RNA^{neg} (n = 15 326; 63.4%), RV 1–19 cp/mL (n = 6318; 26.1%), and RV 20–49 cp/mL (n = 1620; 6.7%). Additionally, 922 VLs (3.8%) were known to be <50 cp/mL, but no RV level was reported and thus considered missing. In 193/1658 PWH (11.6%), the RV level was RNA^{neg} in all VLs assessed. Multivariable GLMM logistic regression (including all 24 518 VLs after multiple imputation) showed that preceding RV 1–19 cp/mL and 20–49 cp/mL (vs. RNA^{neg}) were associated with a 2.66 (95% CI: 1.98–3.58) and 4.90 (95% CI: 3.41–7.04) higher odds of the next VL being a blip (Table 2). Regarding cART anchor, both PIs and NNRTIs were found to have higher odds of blips than INSTIs (OR: 2.28; 95% CI: 1.47–3.54 and OR: 1.79; 95% CI: 1.15–2.79, respectively). Additionally, a zenith VL ≥1 000 000 cp/mL (vs. <10 000) was associated with significantly higher odds of viral blips. Longer times since ART initiation and study inclusion were associated with lower odds of blips.

3.3 | Residual viremia

For the 24 186 non-blip VLs, RV levels were evaluated by anchor type and virologic factors. RNA^{neg} was observed in 66.6% of VLs during INSTI-use, 59.5% during PI-use and 70.9% during NNRTI-use (Figure 2). Multivariable GLMM ordinal regression (including all 24 186 non-blip VLs after multiple imputation) showed that, compared with INSTIs, PIs had significantly higher odds of higher RV levels (OR: 1.28; 95% CI: 1.14–1.44) whereas NNRTIs had lower odds (OR: 0.76; 95% CI: 0.68–0.86) (Table 3). Moreover, shorter time since ART initiation or study inclusion, lower $CD4^+$ counts, Fiebig stage VI (vs. Stage I–V), and a higher zenith VL were associated with higher RV levels.

3.4 | Sensitivity analyses

The sensitivity analysis including the NRTI backbone as covariate and complete case analysis showed regression coefficients similar to the main analysis (Tables S2–S3). The within-anchor group comparisons for the RV level showed no significant within-anchor associations for INSTIs and NNRTIs, but "other" PIs (*vs.* darunavir) were independently associated with less RV (Tables S4–S6). Finally, sensitivity analyses excluding the PWH with periods of non-adherence yielded similar results as the main analyses (Tables S7–S8).

4 | DISCUSSION

In the present study, we analyzed viral blips, subclinical RV, and the associated modifiable and non-modifiable risk factors. In more than 24 000 VLs from adherent PWH treated with contemporary cART regimens, we found that the probability of a blip increased with higher RV levels. The magnitude of RV was strongly associated with

TABLE 1 Characteristics of PWH at baseline.

Demographics	1658 PWH
Age—years	43.6 (±11.9)
Female sex	317 (19.1)
Region of origin	
Europe/North America	1185 (71.5)
Other	473 (28.5)
Clinical characteristics	
Time since HIV diagnosis—years[a]	4.5 (1.7–9.5)
Time since ART initiation—years[a]	2.3 (1.2–7.5)
Mode of transmission	
MSM	937 (56.5)
Heterosexual	337 (20.3)
IVD	33 (2.0)
Other/unknown	351 (21.2)
Co-infections	
$HBsAg^{pos}$ at any point during follow-up	60 (3.6)
HCV RNA^{pos} at any point during follow-up	69 (4.2)
Fiebig stage at ART initiation[a]	
Stage I–V	54 (3.3)
Stage VI	1567 (94.5)
Biochemical characteristics	
Lowest recorded $CD4^+$ count—cells/mm^3	240.0 (113.0–360.0)
Zenith VL cp/mL[a]	
<10 000	103 (6.2)
10 000–99 999	472 (28.5)
100 000–999 999	773 (46.6)
≥1 000 000	72 (4.3)
Study characteristics	
Follow-up duration per individual—years	5.5 (2.5–9.1)
cART regimens per individual during follow-up	2 (1–3)
VLs per individual included in study	14 (7–23)

Note: All categorical data are expressed as number (percentage of total population) and all continuous data are expressed as median (interquartile range) or mean (standard deviation (±)).

Abbreviations: ART, antiretroviral therapy; cp, copies; IVD, intravenous drug use; MSM, men who have sex with men; no., number; PWH, people with HIV; VL, viral load.

[a]Missing data: Time since HIV diagnosis (n = 15, 0.9%); Time since ART initiation (n = 14, 0.8%); Fiebig Stage at ART initiation (n = 44, 2.7%); Zenith VL (n = 238, 14.4%).

the type of cART anchor: the lowest RV levels were seen during NNRTI-use, followed by INSTI- and then PI-use. Moreover, lower RV levels were observed in PWH with low zenith VLs, acute infection at ART initiation, and longer time since ART initiation.

The strong, positive correlation between different RV levels and blips persisted even after adjusting for potential confounders, confirming observations from a small case-control study where RV was assessed up to 1 year before the blip[16] and two observational studies where PWH were stratified as RNA^{neg} or RNA^{pos} based on their baseline VLs.[17,18] In the last two studies, it should be noted that classifying PWH into an RV group on this basis (rather than on individual VL results) reduced the sensitivity of the findings, as a single RNA^{pos} result can already change the classification. The present study majorly extends these previous data by the assessment of three different, ordinal levels of RV, taking repeated measurements over time into account, and including a group of INSTI recipients. Additionally, we showed that the previously demonstrated difference in blip rates for different cART anchors still held true when accounting for RV level.[2] This implies that blips have a multifactorial origin, with cART exerting its effect on blips not solely by altering the RV level.

RV is driven by both modifiable, cART-related factors, as well as non-modifiable, virological characteristics. Regarding the impact of cART anchor type, conflicting results have been reported. One study found that INSTIs, but not NNRTIs or PIs, were significantly associated with RNA^{neg},[20] whereas another reported both NNRTI- and INSTI-based regimens to show significantly lower time spent with RV than PI-based regimens.[21] Yet another study reported no significant differences regarding RV rates between individual drugs within ART classes in ART-naïve PWH.[22] In the largest prior analysis comparing anchor types, 11 045 VLs 1–19 cp/mL were assessed and categorized as either "detectable" or "undetectable," finding that the probability to have a detectable VL was lower during NNRTI- and INSTI-use than during PI-use.[19] However, no difference was found between NNRTIs and INSTIs. In our analysis, that included a time-varying model, an additional RV level, adherence restrictions, and considerably more VLs, significantly lower RV levels were seen for NNRTI- and INSTI-based cART compared to PI-based cART. Moreover, significantly less RV was seen for NNRTIs compared to INSTIs. PIs were found to be associated with both higher RV levels and blips, even in the context of an adherent group of PWH. As PIs, in contrast to NNRTIs and INSTIs, exert their antiretroviral effect after HIV-1 integration and proviral transcription,[26] these steps in the HIV-1 replication cycle could have an influence on blip occurrences. Interestingly, when comparing NNRTIs and INSTIs, less RV but more blips are observed for NNRTIs, despite the strong relationship between RV and blips. Thus, cART appears to influence blips not solely by affecting the RV level. The opposite correlations for RV and blips during NNRTI-use reinforce this idea, as they imply that there are different pathways at play: though the lower RV level "protects" NNRTI recipients against blips, PWH using NNRTIs experience a higher blip frequency through another, yet unidentified, pathway. Although we cannot exclude that certain unaccounted person-specific characteristics or pharmacokinetic properties of the anchor groups (e.g., half-life, penetration into anatomical compartments, time above 90% inhibitory concentration) influenced the results, one would not expect a complete reversal in the effect direction between NNRTIs and INSTIs regarding RV and blips.

TABLE 2 Generalized linear mixed model logistic regression results: associations with viral blip occurrences.

Occurrence of blips				
	Univariable analysis		Multivariable analysis	
Variables	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Time since study inclusion (per year increase)	0.79 (0.69–0.90)	**<0.001**	0.87 (0.78–0.98)	**0.03**
Female sex (vs. male sex)	0.76 (0.51–1.13)	0.17	0.94 (0.62–1.42)	0.77
Age (per year increase)	1.03 (0.88–1.20)	0.75	1.00 (0.86–1.16)	0.99
European/North American region of origin (vs. other)	0.66 (0.46–0.94)	**0.021**	1.33 (0.91–1.93)	0.14
cART anchor				
INSTI	1	–	1	–
PI	2.41 (1.55–3.78)	**<0.001**	2.28 (1.47–3.54)	**<0.001**
NNRTI	1.58 (1.01–2.48)	**0.047**	1.79 (1.15–2.79)	**0.01**
Time since ART initiation (per year increase)	0.93 (0.90–0.97)	**<0.001**	0.97 (0.93–1.00)	**0.04**
Fiebig Stage VI at ART initiation (vs. Stage I–V)	1.27 (0.39–4.18)	0.70	1.78 (0.59–5.35)	0.31
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	1.00 (0.99–1.01)	0.44	1.00 (0.99–1.01)	0.47
Zenith VL cp/mL				
<10 000	1	–	1	–
10 000–99 999	2.77 (0.90–8.58)	0.08	1.97 (0.64–6.00)	0.24
100 000–999 999	3.97 (1.34–11.94)	**0.01**	2.42 (0.80–7.30)	0.12
≥1 000 000	9.30 (2.83–30.57)	**<0.001**	4.64 (1.43–15.05)	**0.01**
Residual viremia level				
RNA^{neg}	1	–	1	–
RV 1–19 cp/mL	3.06 (2.27–4.10)	**<0.001**	2.66 (1.98–3.58)	**<0.001**
RV 20–49 cp/mL	6.11 (4.26–8.67)	**<0.001**	4.90 (3.41–7.04)	**<0.001**

Note: Bold values indicate significant *p*-values. Pooled associations were obtained from the five datasets after multiple imputation of all 24 518 VLs.

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; RV, residual viremia; VL, viral load; vs., versus.

In addition to cART anchor, several non-modifiable factors were associated with the magnitude of RV, such as Fiebig stage at ART initiation, zenith VL, time since ART initiation, and lowest $CD4^+$ count. Interestingly, these factors have previously been linked to the size of the viral reservoir: treatment initiation during acute infection (Fiebig I–V) reduces viral reservoir seeding,[27] pre-treatment VL is related to the reservoir size, even years after treatment initiation,[28] and the reservoir decays slowly over time on ART.[29] Moreover, an inverse relationship between $CD4^+$ nadir and HIV-1 proviral DNA in circulating $CD4^+$ T cells on ART has previously been suggested to either reflect the repopulation of the $CD4^+$ compartment after ART initiation by the relatively highly frequent infected $CD4^+$ T cells harboring HIV-1 (proviral) DNA (resulting in a larger reservoir) or the incomplete suppression of HIV-1 replication in PWH with low $CD4^+$ nadirs.[30] Indeed, some studies into reservoir measures like cell-associated HIV RNA and DNA have shown a correlation between these measures and RV, suggesting that RV is at least partially indicative of the reservoir size.[28,31] In contrast to the association between Fiebig stage and RV, no statistically significant impact of Fiebig stage on blips was found in this study, but both blips and ART initiation in the acute phase of infection were relatively rare, precluding a rigorous assessment of their potential relationship.

The clinical relevance of transient detectable viremia at these very low levels remains a topic of discussion. Though most clinicians agree that isolated blips should not prompt treatment changes,[32] multiple studies have found an association with the emergence of drug resistance[33,34] or virologic failure,[12,13] especially if blips with a higher VL were encountered.[35] Moreover, blips have been shown to lead to extra outpatient visits and laboratory tests.[2] Similar to the much debated relevance of blips, no

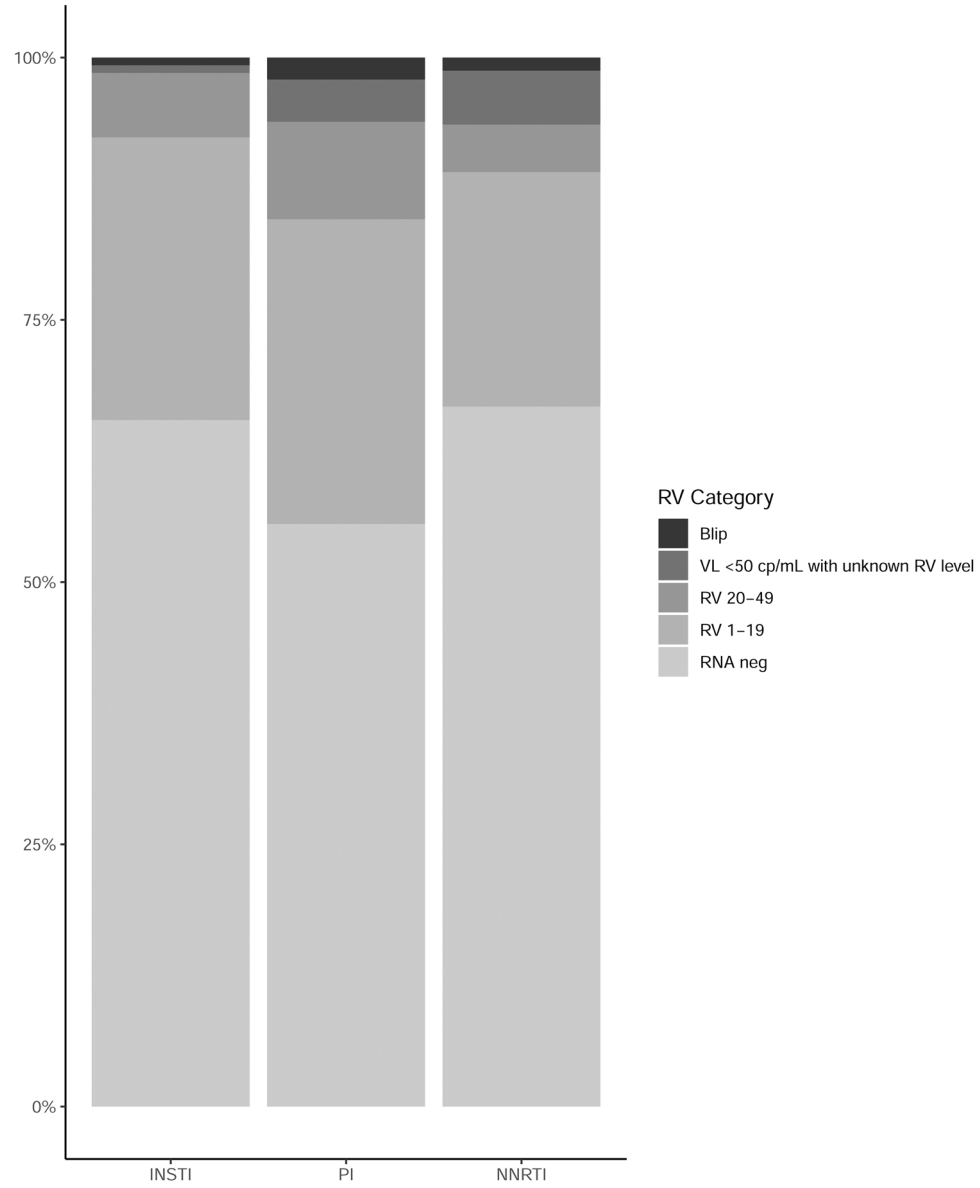

FIGURE 2 Stacked bar plot showing the percentage of VLs per observed VL category by cART anchor group. Distribution of the 24 518 included VLs is shown. Of these, 5119 VLs were during INSTI-use: RNA^{neg} (n = 3358; 65.6%), RV 1–19 (n = 1369; 26.7%), RV 20–49 (n = 315; 6.2%), blip (n = 40; 0.8%) and VL <50 cp/mL with unknown RV level (n = 37; 0.7%). A total of 8935 VLs were during PI-use: RNA^{neg} (n = 5003; 56.0%), RV 1–19 (n = 2596; 29.1%), RV 20–49 (n = 806; 9.0%), blip (n = 163; 1.8%) and VL <50 cp/mL with unknown RV level (n = 367; 4.1%). A total of 10 464 VLs were during NNRTI-use: RNA^{neg} (n = 6965; 66.6%), RV 1–19 (n = 2353; 22.5%), RV 20–49 (n = 499; 4.8%), blip (n = 129; 1.2%) and VL <50 cp/mL with unknown RV level (n = 518; 5.0%). cART, combination antiretroviral therapy; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RV, residual viremia; VL, viral load.

consensus has been reached on the nature and virologic consequences of RV. It is unclear whether RV represents virus released from latently infected cells, ongoing viral replication (with the subsequent risk of selection of resistance), or both. Although studies are highly heterogeneous, several studies have found a higher risk of viral rebound in PWH with RV.[16–18,36,37] Additionally, RV has been associated with low-grade immune activation and chronic inflammation.[38,39] HIV persistence has been suggested to both cause inflammation and be 'fueled' by it when new target cells are generated[19] and to replenish the HIV reservoir.[6] However, much is still unclear, and the long-term clinically relevant health effects of RV and immune activation remain to be elucidated.

To our knowledge, this is the first study that uses an integrated approach to comprehensively study the relationship between RV, blips, all contemporary cART anchors, and virological factors in a large, real-world cohort of PWH. A major strength is the long follow-up period of up to 10 years and the substantial amount of repeated samples allowing us to model RV levels and subsequent blip occurrences over time: the relationship between RV and blips was assessed using only information available at the time of each

TABLE 3 Generalized linear mixed model ordinal regression results: associations with the level of residual viremia.

Level of residual viremia				
	Univariable analysis		Multivariable analysis	
Variables	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Time since study inclusion (per year increase)	0.85 (0.83–0.87)	**<0.001**	0.88 (0.86–0.91)	**<0.001**
Female sex (vs. male sex)	0.81 (0.69–0.96)	**0.01**	0.88 (0.74–1.04)	0.13
Age (per year increase)	1.01 (1.00–1.01)	**0.02**	1.00 (1.00–1.01)	0.18
European/North American region of origin (vs. other)	0.94 (0.82–1.09)	0.42	0.91 (0.78–1.06)	0.25
cART anchor				
INSTI	1	–	1	–
PI	1.36 (1.22–1.53)	<**0.001**	1.28 (1.14–1.44)	<**0.001**
NNRTI	0.79 (0.70–0.89)	<**0.001**	0.76 (0.68–0.86)	<**0.001**
Time since ART initiation (per year increase)	0.96 (0.95–0.97)	**<0.001**	0.95 (0.94–0.96)	**<0.001**
Fiebig stage VI at ART initiation (vs. Stage I–V)	1.17 (0.79–1.72)	0.44	1.51 (1.02–2.25)	**0.04**
Lowest recorded $CD4^+$ count (per 10 cell/mm^3 increase)	0.99 (0.98–0.99)	**<0.001**	0.99 (0.99–1.00)	**0.03**
Zenith VL cp/mL				
<10 000	1	–	1	–
10 000–99 999	2.21 (1.67–2.94)	<**0.001**	2.06 (1.56–2.71)	<**0.001**
100 000–999 999	3.86 (2.90–5.14)	<**0.001**	3.38 (2.54–4.49)	<**0.001**
≥1 000 000	6.20 (4.24–9.06)	<**0.001**	5.30 (3.58–7.86)	<**0.001**

Note: Bold values indicate significant *p*-values. Pooled associations were obtained from the five datasets after multiple imputation of the 24 186 non-blip VLs. RV level, categorized as an ordinal outcome (RNAneg, 1–19 cp/mL, and 20–49 cp/mL), was analyzed using a backwards continuation ratio model for ordinal regression. The odds of 1–19 cp/mL versus RNAneg and 20–49 cp/mL versus 1–19 cp/mL + RNAneg were investigated.

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; cp, copies; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; VL, viral load; vs., versus.

assessed VL, in contrast to other studies incorporating information from the future on predictors not available at the time of the outcome (such as time spent with RV in general).[21] The results are further strengthened by the comprehensive assessment of medical records for information on adherence, as this reduced the influence of adherence variability on RV levels and blips[40] and allowed us to focus on underlying virological factors as a mechanism for RV and blips. Nevertheless, as with any observational study, the potential for confounding by indication existed. As PIs (compared with NNRTIs) traditionally had the highest barrier to resistance before the arrival of the newer INSTIs,[41] physicians may have preferentially prescribed PIs when suspecting poor adherence. Moreover, different anchors (and associated boosters) are associated with different adverse effects and may thus present different tolerability issues. Indeed, VLs <50 cp/mL during PI-based regimens were more likely to be excluded based on documented non-adherence than VLs during other regimens. However, excluding VLs from periods with non-adherence has strengthened our analyses, as the higher rate of non-adherence in PI recipients could potentially have skewed the data toward even more RV and blips during PI-based therapy. Such skewing of results would not be expected for comparisons between NNRTIs and INSTIs, as adherence levels were observed to be similar. Moreover, though residual confounding can never be completely excluded, the risk of confounding bias generally present in observational studies was minimized by controlling for a large number of factors known to be associated with the virological response to cART.

In conclusion, in this large cohort of adherent PWH, we demonstrated that viral blips were strongly associated with higher preceding RV. Rates of both RV and blips were correlated with the type of cART anchor used and several virologic parameters linked to the latent viral reservoir. These findings indicate that blips observed in the clinic have a multifactorial origin, with underlying RV attributable to cART anchor type partially contributing to this phenomenon. Future studies should explore other pathways in which anchor types may lead to higher blip rates and, since the viral reservoir appears to play a role in them, study blips in the context of reservoir measures such as cell-associated HIV RNA and DNA.

AUTHOR CONTRIBUTIONS

Patrick G. A. Oomen, Suzan Dijkstra, and Berend J. van Welzen: designed the study. **Berend J. van Welzen**: wrote the study protocol. **Patrick G. A. Oomen and Suzan Dijkstra**: collected and analyzed the data. **Patrick G. A. Oomen and Suzan Dijkstra**: drafted the manuscript in close collaboration with Berend J. van Welzen. All

authors contributed to the interpretation of the results, critically reviewed the manuscript and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

Berend J. van Welzen: research grant and speaker fees from Gilead Sciences as well as speaker and advisory board fees from ViiV Healthcare: all fees were paid to the institution. Tania Mudrikova: research grant from Gilead Sciences, as well as advisory board (consultancy) fees from Gilead sciences, MSD, ViiV healthcare: all paid to institution. Annemarie M.J. Wensing: investigator-initiated research grant from Gilead sciences, and consultancy fees from ViiV Healthcare/GSK and Gilead sciences: all paid to the institution. Monique M. Nijhuis: consultancy fees from Gilead sciences. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The institutional ethical review board judged that the study met the criteria for exemption from formal review.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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