

ORIGINAL ARTICLE

Predictors of low-level HIV viraemia and virological failure in the era of integrase inhibitors: A Spanish nationwide cohort

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Abstract

Objectives: To pinpoint factors associated with low-level viraemia (LLV) and virological failure (VF) in people living with HIV in the era of high-efficacy antiretroviral treatment (ART) and widespread use of integrase strand transfer inhibitor (INSTIs)-based ART.

Methods: We included adults aged > 18 years starting their first ART between 2015 and 2018 in the Spanish HIV/AIDS Research Network National Cohort (CoRIS). Low-level viraemia was defined as plasma viral load (pVL) of 50–199 copies/mL at weeks 48 and 72 and VF was defined as pVL ≥ 50 copies/mL at week 48 and pVL ≥ 200 copies/mL at week 72. Multivariable logistic regression models assessed the impact on LLV and VF of baseline CD4 T-cell count, CD4/CD8 T-cell ratio and pVL, initial ART classes, age at ART initiation, time between HIV diagnosis and ART initiation, gender and transmission route.

[Correction added on 28 March 2022, after first online publication: the affiliation of Antonio Rivero has been corrected in this version.]

[Correction added on 17 June 2022, after first online publication: the order of author affiliations was updated.]

Eva Poveda and Josep M. Llibre share senior authorship and have contributed equally to this work.

The members of the CoRIS Study Group are given in [the Appendix](#).

Results: Out of 4186 participants, 3120 (76.0%) started INSTIs, 455 (11.1%) started boosted protease inhibitors (bPIs) and 443 (10.8%) started nonnucleoside reverse transcriptase inhibitors (NNRTIs), either of them with two nucleos(t)ide reverse transcriptase inhibitors (NRTIs). Low-level viraemia was met in 2.5% of participants and VF in 4.3%. There were no significant differences throughout the years for both virological outcomes. Baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL was the only consistent predictor of higher risk of LLV [adjusted odds ratio (aOR) = 9.8, 95% confidence interval (CI): 2.0–48.3] and VF (aOR = 5.4, 95% CI: 1.9–15.1), even in participants treated with INSTIs.

Conclusions: The rates of LLV and VF were low but remained steady throughout the years. Baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL showed a persistent association with LLV and VF even in participants receiving INSTIs.

KEYWORDS

antiretroviral treatment-naïve, HIV-1, integrase inhibitors, low-level viraemia, virological failure

INTRODUCTION

Antiretroviral therapy (ART) has brought about a dramatic reduction in HIV-related morbidity and mortality and has undergone continuous fine-tuning with regard to its safety and efficacy [1]. Current ART combinations achieve high and durable rates of HIV suppression. However, long-term efficacy of ART can be jeopardized in subjects with persistent low-level viraemia (LLV) and incomplete HIV suppression. The potential consequences include increased rates of virological failure (VF), HIV resistance selection, suboptimal immune reconstitution, persistent immune activation and latent viral reservoir replenishment [2–8].

The lack of uniformity with regard to definitions of LLV, blips, optimal virological suppression (VS) and VF makes comparisons of results among different studies inconsistent [9–22].

A previous analysis from the Spanish HIV/AIDS Research Network Cohort (CoRIS), with an administrative censoring date at 2015, identified an overall rate of 4.0% of at least one episode of LLV, defined as two consecutive plasma viral load (pVL) readings in the range 50–199 copies/mL, with an additional 2.8% for two consecutive pVLs in the range 200–499 copies/mL. Boosted protease inhibitor (bPI)-containing regimens and baseline pVL $> 5 \log_{10}$ copies/mL were associated with increased risk of LLV [relative risk ratio (RRR) = 1.6, 95% CI: 1.3–2.1 and RRR = 2.4, 95% CI: 1.9–3.1, respectively]. The percentage of individuals treated with integrase strand transfer inhibitors (INSTIs) was low (16.4%), thus limiting the analysis of its impact [17].

Considering the year 2015 as a turning point for starting universal ART in all HIV-positive people and the widespread use of INSTIs as preferred first-line regimens, we aimed to revisit factors associated with LLV and VF defined

following stringent criteria with ART regimens used in more recent times.

METHODS

The HIV/AIDS Research Network Cohort (CoRIS) is an open, prospective, nationwide multicentre cohort launched in 2004, including participants over 13 years old, with confirmed HIV-1 infection, naïve to ART at study entry, and recruited in 44 HIV care units of the Spanish Public Health System. Each participant signs an informed consent form and is followed periodically in accordance with routine clinical practice. A complete description of CoRIS has been published previously [17,23]. CoRIS uses the HIV Cohorts Data Exchange Protocol (HICDEP) for data collection (details at <https://hicdep.org/>).

Adults aged > 18 years were included in the current analysis if they had initiated ART from January 2015 to November 2018 and had laboratory controls at weeks 24, 48 and 72; the administrative censoring date was 29 November 2019. Considering 24 and 48 weeks as standardized follow-up time points, an additional supplementary time point at 72 weeks was defined to reinforce the results. Additional pVL assessments between the established time points were not systematically available for all the participants, and were excluded from the analyses. Baseline was defined as the last date recorded before initiating ART. Participants with unavailable values of pVL at any time point beyond the baseline were included for analysis, but pVLs at these time points were considered missing values. A window of ± 12 weeks was allowed for data collection in the defined time points. The variables

collected included: demographics (age, gender, ethnicity), body mass index, HIV transmission route, year of diagnosis of HIV infection, CDC category (1993), initial ART (classes, regimen, number of drugs), and virological (plasma HIV-1 RNA) and immunological markers (CD4 T-cell count, CD8 T-cell count, CD4/CD8 T-cell ratio).

Statistical methods

The outcomes were based on an intention-to-treat-exposed (ITT-e) analysis including all participants starting their first ART regimen in the defined period.

A descriptive analysis of participants' demographic and immunovirological characteristics at ART initiation was carried out using frequency tables for categorical variables, and median and interquartile range (IQR) for continuous variables. Variables were compared between groups using the chi-square test or Fisher's exact test for categorical variables and Mann-Whitney *U*-test for continuous variables.

Multivariable logistic regression models assessed the impact on response variables (dependent, dichotomous variables) such as virological outcomes (LLV and VF) of multiple independent predictor or explanatory variables, including those with significance level $\alpha < 0.1$ in univariable analysis or variables with potential clinical impact. Multicollinearity analyses between the variables considered for inclusion in the regression model were performed using variance inflation factor (VIF) values. None of the VIF values for the predictors variables was > 5 , which indicates no multicollinearity and there was no need for correction, as variables did not provide redundant information about the response. Predictor variables included (modelled as categorical variables) were: baseline CD4 count (≤ 200 , 201–499, ≥ 500 cells/ μL), CD4/CD8 T-cell ratio (per 0.1-unit increase), baseline HIV-1 RNA (≤ 5 , $> 5 \log_{10}$ copies/mL), initial ART classes [two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus either one nonnucleoside reverse transcriptase inhibitor (NNRTI), one bPI or one INSTI], age at ART initiation (< 50 , ≥ 50 years), time between HIV diagnosis and ART initiation (≤ 8 weeks, > 8 weeks), gender (male, female) and transmission route [men who have sex with men/bisexual (MSM), heterosexual, intravenous drug user].

In addition, different sensitivity analyses were performed, including only participants who started treatment with INSTIs and allowing ART changes for any reason.

Associations between the variables were expressed as adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Statistical analysis was performed using SPSS v.19.0. All tests were two-tailed, and a significance level α was set at 0.05.

Virological outcomes

Virological outcomes were defined as the following pVL ranges beyond week 24 after initiating ART, over the number of participants with available values of pVL at all time points and with no treatment change. Virological suppression (VS) was defined as pVL < 50 copies/mL at any time (at week 48 or 72) or pVL < 50 copies/mL at weeks 48 and 72 (confirmed values). Low-level viraemia (LLV) was defined as pVL between 50 and 199 copies/mL at weeks 48 and 72 (consecutive values that rule out isolated blips). Virological failure was defined as pVL ≥ 50 copies/mL at week 48 and pVL ≥ 200 copies/mL at week 72 [as defined in most phase III randomized clinical trials of first-line ART approved by the drug regulatory agencies (European Medicines Agency and US Food and Drug Administration)]. The threshold of 200 copies/mL eliminates most cases of apparent viraemia caused by pVL blips or technical assay variability [24].

In addition, ART change for any reason was considered at any time at 48 weeks, both within-class and between-class (NRTI, NNRTI, PI or INSTI). A change of the booster (ritonavir to cobicistat) or switch between different forms of the same component, such as tenofovir disoproxil fumarate to tenofovir alafenamide, were not considered as treatment changes, as no differences in virological efficacy have been reported in randomized studies comparing these single-drug changes within the follow-up period considered in the current analysis [25,26]. An additional analysis of virological outcomes was carried out including the subset of participants undergoing ART changes.

RESULTS

Out of 4627 participants who started ART in the defined study period, 441 were excluded from the analysis: 399 of them due to errors in data gathering or lack of baseline pVL; seven due to being < 18 years old; and 35 due to absence of treatment initiation in real life (estimated through pVL values similar to those at baseline in all available time points).

Demographic characteristics

Out of 4186 eligible ART-naïve participants in CoRIS, the majority were male ($n = 3694$, 88.2%), of Caucasian ethnicity ($n = 4039$, 96.5%), MSM as transmission route ($n = 2874$, 68.7%) and clinical category CDC A ($n = 2679$, 64%). The median age was 35 years [interquartile range (IQR: 29–44)] and 534 (12.7%) were ≥ 50 years old. The number of participants aged > 65 years was low ($n = 65$,

1.5%) with the lowest number reported in 2018 ($n = 7$, 0.8%). Only 23.0% ($n = 964$) of them had been diagnosed with HIV before the period defined for the current analysis (2015–2018). The estimated median time between HIV diagnosis and ART initiation was 62 (IQR: 27–232) days, with a progressive decrease throughout the years from 104 (IQR: 41–578) days in 2015 to 37 (IQR: 18–82) days in 2018 ($p < 0.001$) (Table 1).

ART classes

In all, 4034 (96.3%) participants started three drug-based regimens and 48 (1.1%) started two drug-based ones: 3120 (76.0%) started INSTIs, 455 (11.1%) bPIs and 443 (10.8%) NNRTIs, all them with two NRTIs (Table 1).

Among the INSTI-based regimens, dolutegravir/lamivudine/abacavir was the most frequent regimen overall over the 4-year study period ($n = 1276$, 30.5%) with an increasing trend from 2015 (22.2%) to 2018 (30.2%) ($p < 0.001$). Overall, elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide was the second most frequent regimen during the study period ($n = 669$, 16.0%) with a progressive increase over the years ($p < 0.001$) at the expense of a decrease in elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate ($p < 0.001$) in such a way that none started this latter regimen in 2018. Among the bPI-based regimens, boosted-darunavir plus emtricitabine/tenofovir disoproxil fumarate was the most frequent regimen ($n = 295$, 7.0%) with a progressive decrease over the years ($p < 0.001$) in favour of an increase in co-formulated darunavir/cobicistat/emtricitabine/tenofovir alafenamide ($p < 0.001$). Overall, NNRTI-based regimens were the least used and the most frequent regimen was rilpivirine/emtricitabine/tenofovir disoproxil fumarate ($n = 307$, 7.3%) with a significant decrease over the years ($p < 0.001$).

Almost one-quarter of participants had changed their ART at week 48 ($n = 954$, 22.8%), with a significant increase in more recent years ($p < 0.001$). The regimens most frequently switched to were dolutegravir/lamivudine/abacavir ($n = 422$, 44.2%), elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide ($n = 181$, 19.0%), dolutegravir/lamivudine ($n = 38$, 4.0%), rilpivirine/emtricitabine/tenofovir disoproxil fumarate ($n = 38$, 4.0%) and bictegravir/emtricitabine/tenofovir alafenamide ($n = 36$, 3.8%). The clinical reasons for these treatment changes were not available. It is worth noting that the definition of changing ART in this study did not include a change of the booster drug component or a switch between individual components of tenofovir. These single-drug substitutions involved 167 participants (4%) and should have no impact on virological efficacy, as mentioned earlier.

TABLE 1 Demographic and immunovirological characteristics in the Spanish HIV/AIDS Research Network National Cohort (CoRIS), 2015–2018

<i>n</i> = 4186	
Gender [<i>n</i> (%)]	
Male	3694 (88.2)
Female	492 (11.8)
Age at ART initiation (years)	
Median (IQR)	35 (29–44)
≥ 50 [<i>n</i> (%)]	534 (12.7)
> 65 [<i>n</i> (%)]	65 (1.5)
Ethnicity [<i>n</i> (%)]	
Caucasian	4039 (96.5)
Black	147 (3.5)
BMI (kg/m ²) [median (IQR)]	23.5 (21.6–25.9)
Transmission route [<i>n</i> (%)]	
MSM/bisexual	2874 (68.7)
Heterosexual	1024 (24.5)
IDU	99 (2.4)
Missing	189 (4.5)
Year of diagnosis of HIV infection [<i>n</i> (%)]	
1980–2014	964 (23.0)
2015–2018	3222 (77.0)
Clinical category (CDC 1993) [<i>n</i> (%)]	
A	2679 (64.0)
B	284 (6.8)
C	336 (8.0)
Unknown	887 (21.2)
Time between HIV diagnosis and ART initiation (days) [median (IQR)]	62 (27–232)
Initial ART	
Classes [<i>n</i> (%)]	
Two NRTI + NNRTI	443 (10.8)
Two NRTI + bPI	455 (11.1)
Two NRTI + INSTI	3120 (76.0)
Other	85 (2.1)
Non-specified	83 (2.0)
Regimen (the most frequent) [<i>n</i> (%)]	
DTG/3TC/ABC	1276 (30.5)
EVG/c/FTC/TAF	669 (16.0)
DTG + FTC/TDF	492 (11.8)
EVG/c/FTC/TDF	378 (9.0)
RPV/FTC/TDF	307 (7.3)
DRV/r + FTC/TDF	182 (4.3)
RAL + FTC/TDF	157 (3.8)
DRV/c + FTC/TDF	113 (2.7)
Other [<i>n</i> (%)]	
DRV/c/FTC/TAF	78 (1.9)

TABLE 1 (Continued)

	n = 4186
DOR + FTC/TDF	16 (0.4)
RPV/FTC/TAF	10 (0.2)
BIC/FTC/TAF	7 (0.2)
DTG/3TC	37 (0.9)
Number of drugs [n (%)]	
Three	4034 (96.3)
Two	48 (1.1)
Four	17 (0.4)
Other	87 (2.0)
ART changing [n (%)]	954 (22.8)
DTG/3TC/ABC	422 (44.2)
EVG/c/FTC/TAF	181 (19.0)
DTG/3TC	38 (4.0)
RPV/FTC/TDF	38 (4.0)
BIC/FTC/TAF	36 (3.8)
EVG/c/FTC/TDF	34 (3.6)
Baseline CD4 count (cells/ μ L)	
Median (IQR)	407 (232–580)
≤ 200 [n (%)]	866 (21.2)
< 350 [n (%)]	1678 (41.1)
Baseline ratio CD4/CD8 [median (IQR)]	0.4 (0.2–0.6)
Baseline HIV-1 RNA ($\log_{10}$ copies/mL) [median (IQR)]	4.7 (4.2–5.3)
Baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL [n (%)]	1621 (38.8)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral treatment; BIC, bictegravir; BMI, body mass index; DOR, doravirine; DRV/c, darunavir/cobicistat; DRV/r, darunavir/ritonavir; DTG, dolutegravir; EVG/c, elvitegravir/cobicistat; FTC, emtricitabine; IDU, intravenous drug user; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor; RAL, raltegravir; RPV, rilpivirine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Immunological characteristics and outcomes

Median baseline CD4 count was 407 cells/ μ L (IQR: 232–580) (Table 1). Overall, 1678 participants (41.1%) were late presenters, defined as baseline CD4+ T cells < 350 cells/ μ L; the percentage of late presenters showed a non-significant trend towards increasing throughout every particular year ($p = 0.053$). Overall, 866 participants (21.2%) had severe immunosuppression, defined as baseline CD4 count ≤ 200 cells/ μ L, with a significant increase in more recent years, 2017 ($n = 250$, 23.0%) and 2018 ($n = 210$, 24.3%) ($p = 0.004$). The increases in median CD4 count after treatment initiation at week 48 and week 72 remained steady throughout the years [overall, +237 (IQR: 124–379) and +272 (IQR: 155–421) cells/ μ L, respectively].

Median baseline CD4/CD8 T-cell ratio was 0.4 (IQR: 0.2–0.6), and remained similar over the years. The median CD4/CD8 T-cell ratio increases at weeks 48 and 72 were also maintained.

Virological outcomes

Median baseline HIV-1 RNA was 4.7 (IQR: 4.2–5.3) $\log_{10}$ copies/mL (Table 1), with an increase in recent years, 4.8 (IQR: 4.2–5.3) $\log_{10}$ copies/mL in 2017 and 4.9 (IQR: 4.3–5.5) $\log_{10}$ copies/mL in 2018 ($p < 0.001$). A total of 1621 participants (38.8%) had baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL.

Analyses were performed both excluding changes in ART and allowing changes in ART.

When changes in ART were not allowed, overall 1364 (89.6%) and 1104 (91.2%) participants achieved VS defined as pVL < 50 copies/mL at weeks 48 and 72, respectively. Confirmed VS (pVL < 50 copies/mL at both week 48 and 72) was achieved in 664 participants (74.2%). There were no significant differences throughout the years for VS rates. Low-level viraemia was seen in 20 participants (2.5%), and 34 (4.3%) experienced VF. There were also no statistically significant differences throughout the years for LLV and VF rates (Figure 1).

In a sensitivity analysis, when changes in ART were allowed, overall 1787 (88.7%) and 1421 (90.9%) participants achieved VS, defined as pVL < 50 copies/mL at weeks 48 or 72, respectively. Confirmed VS (pVL < 50 copies/mL at both weeks 48 and 72) was achieved in 858 participants (72.9%). There were no significant differences for VS rates over the years. LLV was seen in 26 participants (2.5%), and 48 (4.6%) experienced VF. There were also no significant differences in LLV and VF rates over the years (Figure 1).

In the multivariable logistic regression model adjusted for baseline CD4 count, CD4/CD8 T-cell ratio, HIV RNA, initial ART classes, gender and transmission route, when ART changes were excluded, baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL was an independent predictor of higher risk of LLV (aOR = 9.8, 95% CI: 2.0–48.3, $p = 0.005$) and VF (aOR = 5.4, 95% CI: 1.9–15.1, $p = 0.001$). In a sensitivity analysis, allowing ART changes in this model, HIV-1 RNA $> 5 \log_{10}$ copies/mL remained as a predictor of higher risk of LLV (aOR = 3.2, 95% CI: 1.1–9.6, $p = 0.035$) and VF (aOR = 3.1, 95% CI: 1.4–6.8, $p = 0.006$).

This association remained consistent in a sensitivity analysis considering only participants treated with INSTIs both for LLV [both allowing and not allowing ART changes (aOR = 3.4, 95% CI: 1.0–11.3, $p = 0.043$ and aOR = 15.7, 95% CI: 1.9–128.3, $p = 0.010$, respectively)] and for VF [both allowing and not allowing ART changes (aOR = 2.9, 95% CI: 1.2–7.4, $p = 0.020$ and aOR = 6.2, 95% CI: 1.7–23.2, $p = 0.006$, respectively)].

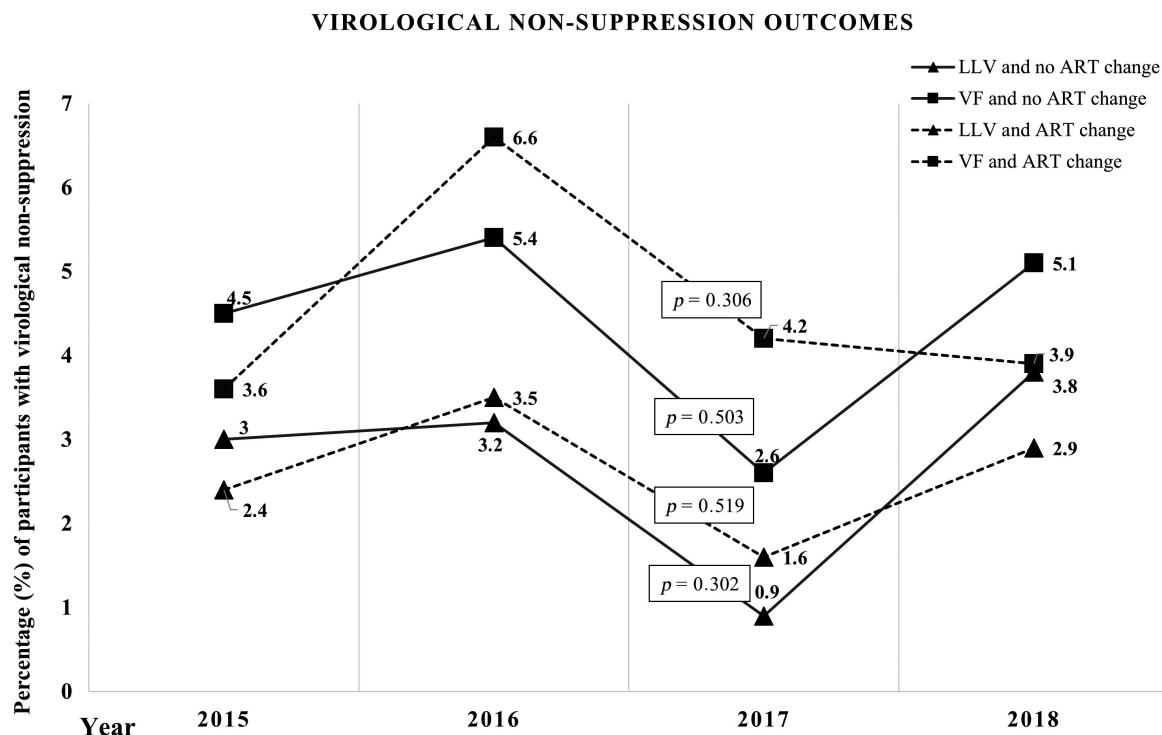


FIGURE 1 Virological non-suppression outcomes within 72 weeks in the Spanish HIV/AIDS Research Network National Cohort (CoRIS), 2015–2018. LLV, low-level viraemia; VF, virological failure

When age at ART initiation (threshold at 50 years old) and time between HIV diagnosis and ART initiation (threshold at 8 weeks) were included in the logistic regression model (fully adjusted model) and ART changes were not allowed, baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL remained as an independent predictor of higher risk of LLV (aOR = 8.9, 95% CI: 1.8–44.2, $p = 0.007$) and VF (aOR = 5.6, 95% CI: 2.0–15.9, $p = 0.001$). This association remained in the subset of participants treated with INSTIs either for LLV (aOR = 13.8, 95% CI: 1.7–112.9, $p = 0.014$) or VF (aOR = 6.4, 95% CI: 1.7–23.7, $p = 0.005$).

In a sensitivity analysis allowing ART changes in this fully adjusted model, HIV-1 RNA $> 5 \log_{10}$ copies/mL remained as an independent predictor of LLV (aOR = 3.2, 95% CI: 1.1–9.5, $p = 0.035$) and age ≥ 50 years at ART initiation emerged as a predictor of higher risk of LLV (aOR = 2.9, 95% CI: 1.1–7.6, $p = 0.028$). Moreover, HIV-1 RNA levels $> 5 \log_{10}$ copies/mL remained as an independent predictor of VF (aOR = 3.3, 95% CI: 1.5–7.4, $p = 0.004$) and baseline CD4/CD8 T-cell ratio was inversely related to the risk of VF (aOR = 0.8, 95% CI: 0.6–0.9, $p = 0.030$) (Table 2).

In the subset of participants treated with INSTIs, HIV-1 RNA $> 5 \log_{10}$ copies/mL and age ≥ 50 years at ART initiation remained consistent predictors of LLV (aOR = 3.3, 95% CI: 1.0–10.9, $p = 0.049$ and aOR = 3.5, 95% CI: 1.2–10.3, $p = 0.020$, respectively). In addition, HIV-1 RNA $> 5 \log_{10}$ copies/mL remained as a predictor of VF (aOR

= 2.9, 95% CI: 1.2–7.4, $p = 0.020$), but the association with CD4/CD8 T-cell ratio was lost.

For all the analyses, baseline CD4 count, initial ART classes, time between HIV diagnosis and ART initiation, gender and transmission route were not associated with a higher risk of LLV or VF.

DISCUSSION

In this analysis of ART-naïve participants initiating contemporary ART in the prospective Nationwide Spanish HIV/AIDS Research Network Cohort (CoRIS) during 2015–2018, and using strict definitions for LLV or VF, the rates of these two virological non-suppression events remained steady over the years. Low-level viraemia was recognized in 2.5% of participants, and 4.3% evolved into VF. Likewise, the massive use of INSTI-based ART has not resulted in more reduced rates of LLV or VF.

Higher baseline HIV-1 RNA was consistently associated with increased rates of LLV and VF, and these associations persisted in sensitivity analyses allowing ART changes or including only participants initiating treatment with INSTIs. This result is in agreement with previously reported studies and suggests that high baseline HIV-1 RNA is an intrinsic determinant of suboptimal virological suppression with LLV and VF, independently of the ART received [9,10,16–18,21].

TABLE 2 Multivariable logistic regression analysis of factors associated with low-level viraemia (LLV) and virological failure (VF), allowing antiretroviral treatment (ART) changes in the definition of virological outcomes, in the Spanish HIV/AIDS Research Network National Cohort (CoRIS), 2015–2018

Year of ART initiation: overall, 2015–2018	LLV (<i>n</i> = 23) ^a		VF (<i>n</i> = 42) ^a	
	Adjusted OR (95% CI)	<i>p</i> -value	Adjusted OR (95% CI)	<i>p</i> -value
Baseline CD4 count (cells/μL)				
≤ 200	1.0 (Ref.)		1.0 (Ref.)	
201–499	1.3 (0.4–4.2)	0.624	1.0 (0.4–2.4)	0.979
≥ 500	1.6 (0.3–7.3)	0.524	0.8 (0.2–2.7)	0.677
Baseline CD4/CD8 (per 0.1-unit increase)	0.8 (0.6–1.1)	0.176	0.8 (0.6–0.9)	0.030
Baseline HIV-1 RNA (log ₁₀ copies/mL)				
≤ 5	1.0 (Ref.)		1.0 (Ref.)	
> 5	3.2 (1.1–9.5)	0.035	3.3 (1.5–7.4)	0.004
Initial ART (classes)				
2 NRTI + 1 NNRTI	1.0 (Ref.)		1.0 (Ref.)	
2 NRTI + 1 PI	1.5 (0.1–15.5)	0.726	0.9 (0.2–3.8)	0.870
2 NRTI + 1 INSTI	1.3 (0.1–11.1)	0.797	0.7 (0.2–2.7)	0.635
Age at ART initiation (years)				
< 50	1.0 (Ref.)		1.0 (Ref.)	
≥ 50	2.9 (1.1–7.6)	0.028	1.5 (0.7–3.4)	0.327
Time between HIV diagnosis and ART initiation (weeks)				
≤ 8	1.0 (Ref.)		1.0 (Ref.)	
> 8	0.8 (0.3–2.2)	0.743	1.6 (0.8–3.3)	0.175
Gender				
Male	1.0 (Ref.)		1.0 (Ref.)	
Female	0.2 (0.0–2.1)	0.198	0.5 (0.1–2.1)	0.384
Transmission route				
MSM/bisexual	1.0 (Ref.)		1.0 (Ref.)	
Heterosexual	1.6 (0.6–4.2)	0.318	1.1 (0.5–2.4)	0.852
IDU	—	—	0.7 (0.1–5.6)	0.726

Abbreviations: ART, antiretroviral treatment; IDU, intravenous drug user; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor.

^a*n* included in multivariable model, allowing ART changes.

Bold values highlight the significant results (*p* < 0.05).

In our analysis fully adjusted for baseline CD4 count, CD4/CD8 T-cell ratio, HIV-1 RNA, initial ART classes, gender, transmission route, age at ART initiation, and time between HIV diagnosis and ART initiation and allowing ART changes in the definition of virological outcomes, higher baseline CD4/CD8 T-cell ratio was identified as an independent predictor of lower risk of VF. This association was nonetheless lost in the subset of participants who started INSTIs. Notably, in a revealing study, a strong inverse correlation was observed between detectable levels of ongoing viral replication in reservoirs (evaluated through CD4 T cells carrying HIV-1 proviral DNA) and immunological parameters, such as the CD4/CD8 T-cell ratio [27].

Higher CD4 count recovery has been reported in those who initiate ART closer to the time of HIV infection [28].

Data are lacking regarding incomplete virological suppression and early initiation of ART. In this regard, we considered it intriguing to clarify whether persistent viraemia could be associated with timing of ART initiation. In our analysis, time between HIV diagnosis (as estimated HIV infection date) and ART initiation (irrespective of whether this was < or > 8 weeks) was not associated with LLV or VF.

Initial ART classes, gender and transmission route did not show an association with any one of the virological non-suppression outcomes analysed. Interestingly, in the fully adjusted model and when ART changes were allowed, age ≥ 50 years at ART initiation appeared as a predictor of higher risk of LLV and this association remained in participants who started INSTIs.

It is worth noting that the median time between HIV diagnosis and ART initiation over the study period showed a progressive and significant decrease throughout the years, from 104 (IQR: 41–577) days in 2015 to 37 (IQR: 18–82) days in 2018 ($p < 0.001$). However, in spite of early ART being recommended for all individuals with HIV infection regardless of CD4 count, almost half of participants in the cohort were late presenters (CD4 count < 350 cells/ μ L), with no significant change over the years. Quite unexpectedly, one-fifth of participants were severely immunosuppressed (CD4 count ≤ 200 cells/ μ L) at ART initiation, with a significant increasing trend in recent years (23.0% in 2017, 24.3% in 2018, $p = 0.004$). Therefore, despite shortening the time from HIV diagnosis to ART initiation, baseline CD4 count at diagnosis has not increased over the time (2015–2018) and the percentage of late presenters remains unacceptably high (41.1%). This burden of late presentation is slightly lower than that reported from the COHERE and EuroSIDA cohorts (48.4%), including newly diagnosed individuals across Europe during 2010–2016 [29]. An inappropriate delay in diagnosing HIV can jeopardize the clinical benefit of starting ART earlier. In Spain, both ART and access to the health-care system are free, and therefore these data suggest that proactive strategies to promote early HIV diagnosis in the population need to be seriously improved.

Quite unexpectedly, our data show a progressive increase in median baseline HIV-1 RNA in recent years ($p < 0.001$). Our study was not designed to identify the potential underlying causes for this.

Notably, almost one-quarter of participants changed the ART regimen at week 48 (22.8%) for any reason, except for booster change or switching from tenofovir disoproxil fumarate to tenofovir alafenamide. A significant increase in changes to treatment was observed in recent years ($p < 0.001$).

The definition of LLV and VF has been inconsistent in studies to date [9–22]. The main caveat is that most studies included samples with up to 999 HIV-RNA copies/mL, which are actually confirmed cases of VF and introduce uncertainty into the analyses. Our study aimed to identify factors associated with LLV and VF, defined following very stringent criteria.

Our study has limitations. In a real-life cohort study, errors in data gathering and recording cannot be ruled out. The limited sampling intervals did not allow for more detailed characterizations of viraemia dynamics. We did not have information on the HIV subtypes. Non-B subtypes, particularly subtype F, have been associated with longer time to achieve VS [30,31].

HIV-RNA laboratory assessment was not centralized. Likewise, different assays for pVL determination during the study period were time- and centre-dependent (the commercial assays used for HIV-RNA quantification for each centre were not available). However, the detection threshold

of 50 copies/mL was used consistently for the virological outcomes analysis, despite some centres using thresholds at 40 or even 20 copies/mL. Genotyping and resistance data were not available in samples at CoRIS Biobank.

CONCLUSIONS

The rates of LLV and VF were low but remained steady throughout the study years despite using high-efficacy ART regimens and, specifically, INSTIs. A baseline HIV-1 RNA $> 5 \log_{10}$ copies/mL was the only consistent predictor of higher risk of LLV and VF and this association persisted in participants starting treatment with INSTIs.

Therefore, LLV and VF seem to be intrinsically related to baseline pVL, despite the progressive improvement over time in the efficacy and safety of ART regimens. The mechanism underlying this long-lasting memory effect of baseline HIV-1 RNA on incomplete virological suppression, even in the integrase inhibitor era, merits further investigation.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest in relation to the content of the manuscript.

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APPENDIX

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