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# Effect of HIV-1 low-level viraemia during antiretroviral therapy on treatment outcomes in WHO-guided South African treatment programmes: a multicentre cohort study

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## Summary

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See [Comment](#) page 130

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**Background** Antiretroviral therapy (ART) that enables suppression of HIV replication has been successfully rolled out at large scale to HIV-positive patients in low-income and middle-income countries. WHO guidelines for these regions define failure of ART with a lenient threshold of viraemia (HIV RNA viral load  $\geq 1000$  copies per mL). We investigated the occurrence of detectable viraemia during ART below this threshold and its effect on treatment outcomes in a large South African cohort.

**Methods** In this observational cohort study, we included HIV-positive adults registered between Jan 1, 2007, and May 1, 2016, at 57 clinical sites in South Africa, who were receiving WHO-recommended ART regimens and viral load monitoring. Low-level viraemia was defined as the occurrence of at least one viral load measurement of 51–999 copies per mL during ART. Outcomes were WHO-defined virological failure (one or more viral load measurement of  $\geq 1000$  copies per mL) and switch to second-line ART. Risks were estimated with Cox proportional hazard models.

**Findings** 70 930 patients were included in the analysis, of whom 67 644 received first-line ART, 1476 received second-line ART, and 1810 received both. Median duration of follow-up was 124 weeks (IQR 56–221) for patients on first-line ART and 101 weeks (IQR 51–178) for patients on second-line ART. Low-level viraemia occurred in 16 013 (23%) of 69 454 patients, with an incidence of 11.5 per 100 person-years of follow-up (95% CI 11.4–11.7), during first-line ART. Virological failure during follow-up occurred in 14 380 (22%) of 69 454 patients on first-line ART. Low-level viraemia was associated with increased hazards of virological failure (hazard ratio [HR] 2.6, 95% CI 2.5–2.8;  $p < 0.0001$ ) and switch to second-line ART (HR 5.2, 4.4–6.1;  $p < 0.0001$ ) compared with virological suppression of less than 50 copies per mL. Risk of virological failure increased further with higher ranges and persistence of low-level viraemia.

**Interpretation** In this large cohort, low-level viraemia occurred frequently and increased the risk of virological failure and switch to second-line ART. Strategies for management of low-level viraemia need to be incorporated into WHO guidelines to meet UNAIDS-defined targets aimed at halting the global HIV epidemic.

**Funding** None.

## Introduction

In a global effort to halt the HIV epidemic, UNAIDS has set ambitious targets for expansion of access to HIV testing and antiretroviral therapy (ART), and for high treatment success rates in patients on ART.<sup>1</sup> Access to ART has expanded substantially and is currently reaching approximately 18 million HIV-infected patients, of whom more than 14 million reside in low-income and middle-income countries.<sup>2</sup> Although large-scale roll out of ART in these countries is accompanied by concerns about sustained adherence to treatment and retention in care, increasing rates of transmitted drug resistance to first-line ART, and growing uptake of second-line ART,<sup>3–7</sup> treatment programmes in low-income and middle-income countries generally report durable success rates of ART and low on-treatment rates of virological failure.<sup>3,8</sup>

The definition of virological failure differs around the world. Substantial differences exist between guidelines

in high-income countries, which use HIV RNA load (viral load) thresholds of 50–200 copies per mL to define virological failure, and WHO guidelines for low-income and middle-income countries, which apply a more lenient threshold of 1000 copies per mL.<sup>9–11</sup> Low-level viraemia refers to detectable viraemia during ART between these thresholds (50–999 copies per mL).<sup>12,13</sup> In high-income countries, clinical interventions are initiated upon detection of viral loads higher than 50 copies per mL. This approach is based on associations in this setting between persistent low-level viraemia and suboptimal adherence to ART,<sup>14,15</sup> selection of resistance to some ART regimens with a low genetic barrier to resistance,<sup>16–18</sup> and subsequent virological failure.<sup>12,19–25</sup>

Current WHO guidelines do not advise interventions in monitoring or treatment interventions even after repeated measurements of low-level viraemia, resulting in patients being kept on a failing first-line ART regimen with a low genetic barrier to resistance. The incidence of

## Research in context

### Evidence before this study

We searched PubMed for studies published between Jan 1, 2000, and July 1, 2017, on the effect of HIV low-level viraemia, defined as a detectable viral load between 50 and 1000 copies per mL during antiretroviral therapy (ART), on subsequent failure of ART. We used the search terms “HIV”, “low-level viraemia”, and “antiretroviral therapy”, and common synonyms. We identified studies in adult patients that reported virological failure as an outcome. We did not review studies that exclusively reported on viral blips (ie, detectable viraemia immediately followed by suppression of <50 copies per mL), reports of cohorts treated with outdated ART regimens, or studies without a control group.

We identified three multicentre observational cohort studies. In a combined European and North American cohort, a significantly increased risk for virological failure was seen in HIV-infected patients with repeated measurements of low-level viraemia of 200–499 copies per mL during ART (hazard ratio [HR] 3.97, 95% CI 3.05–5.17), but not in those with low-level viraemia of 51–199 copies per mL (HR 1.38, 0.96–2.00). By contrast, in a French cohort, low-level viraemia of 51–199 copies per mL was associated with significantly increased risk for virological failure (HR 2.30, 1.65–3.20), but this study did not assess low-level viraemia of more than 200 copies per mL. A British–German cohort showed a significantly increased risk for virological failure after unstratified low-level viraemia between 50 and 400 copies per mL (risk ratio [RR] 2.18, 95% CI 1.15–4.10).

Taken together, the effect of low-range low-level viraemia (51–199 copies per mL) remains unclear, although both studies that assessed middle-range low-level viraemia (200–500 copies per mL) showed increased rates of virological failure in patients with viral loads within this range. However, these two studies set thresholds for virological failure at 400 copies per mL and 500 copies per mL, respectively, precluding the study of high-range low-level viraemia (400–1000 copies per mL).

We identified two single-centre studies that used a threshold for virological failure of 1000 copies per mL. Of these, one study from the USA reported that patients with low-level viraemia of 50–999 copies per mL had a significantly increased risk for virological failure (HR 3.8, 2.2–6.4), whereas in a Canadian cohort, a significant risk for virological failure was seen in 165 patients with repeated measurements of low-level viraemia of 51–199 copies per mL (HR 2.22, 1.60–3.09), 200–499 copies per mL (HR 2.15, 1.46–3.17), or 500–999 copies per mL (HR 4.85, 3.16–7.45). Identified studies reported prevalence figures of low-level viraemia ranging between 6.2% and 25.5%.

All studies were done in high-income countries where frequent viral load monitoring is performed. In these settings, upon

detection of raised viral loads higher than 50 copies per mL, interventions such as adherence counselling, intensified monitoring, resistance testing, pharmacokinetic measurement, and switch of ART regimen might already be initiated. Therefore, the available evidence might not apply to treatment programmes in low-income and middle-income countries, where WHO guidelines recommend that annual viral load testing and interventions are only advised if viraemia exceeds the threshold of 1000 copies per mL. Furthermore, available studies included patients on a range of ART regimens from the earliest phase of combination therapy onwards, and do not reflect the situation in low-income and middle-income countries, where first-line ART with a low genetic barrier to resistance is provided to all patients.

On the basis of the available evidence from high-income countries, no conclusions can be drawn regarding the prevalence of low-level viraemia or its effect on virological failure in low-income and middle-income countries. Considering that this evidence was collected in settings where strict monitoring is applied, the threat of low-level viraemia to treatment success might be even more pronounced in low-income and middle-income countries, where the majority of the global population of HIV-positive patients on ART reside.

### Added value of this study

To our knowledge, our study is the first to analyse the occurrence of low-level viraemia and its effect on treatment failure in low-income and middle-income countries. It is also the largest analysis on this topic to date, including nearly 71 000 patients from 57 South African clinics. Our results support available evidence of an increased risk of virological failure even after a single occurrence of low-level viraemia of the lowest range. Our study is also the first to show clinical consequences of low-level viraemia—namely, the increased risk of switching to second-line ART. Compared with previous studies from high-income settings, the observed rates of low-level viraemia and virological failure in this setting were higher and the risk of virological failure after low-level viraemia was equal to or more pronounced, indicating that low-level viraemia is a serious threat to treatment programmes in low-income and middle-income countries.

### Implications of all the available evidence

Active clinical follow-up of raised viral loads should be prioritised and strategies for specific management of low-level viraemia should be included in WHO guidelines to mitigate the risk of subsequent virological failure. The high threshold for virological failure currently used in low-income and middle-income countries should be reconsidered.

low-level viraemia during ART and its effect on outcomes of ART in treatment programmes in low-income and middle-income countries have not been studied. We aimed to describe the effect of low-level

viraemia on outcomes of ART in a large multicentre rural-urban South African cohort of HIV-positive adults undergoing treatment and monitoring according to WHO guidelines.

## Methods

### Study design and participants

We did a multicentre observational study of a cohort of South African HIV-positive patients receiving ART. Patient data were collected, captured, and verified by dedicated data management teams as part of operational monitoring and assessment of HIV treatment programmes and laboratory service providers. This study received ethical approval from the University of Witwatersrand human research ethics committee (Johannesburg, South Africa) and the research ethics committee of the Faculty of Health Sciences, University of Pretoria (South Africa). Because of the observational nature of this study using previously collected and anonymised data, individual informed consent was not required.

HIV-positive adults ( $\geq 18$  years) on ART attending 57 clinical sites located in four provinces in South Africa were included. Clinics are located in inner city Johannesburg (Gauteng province), in an urban-rural mixed environment in Dr Kenneth Kaunda District (North West province), and in rural settings in Sekhukhune district (Limpopo province) and Ehlanzeni district (Mpumalanga province). All clinics provide ART in the framework of the South African national ART programme, which provides HIV-positive patients with free-of-charge treatment using WHO-aligned ART regimens and virological monitoring. First-line ART consists of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI), and second-line ART

consists of two NRTIs and a ritonavir-boosted protease inhibitor (PI). Virological monitoring during ART consists of a first viral load measurement at 6 months and 12 months after initiation of treatment, and 12-monthly measurements thereafter. Virological failure is defined as a viral load of 1000 copies per mL or more, confirmed by a second viral load within 3 months, although the timing of repeat testing may vary between settings. Most viral load results were generated by use of the same assay (appendix p 17). All patients in the cohort with available data were screened for inclusion in separate first-line and second-line ART subcohorts. Inclusion criteria for the first-line subcohort were prescription of NNRTI-based first-line ART, and availability of at least one viral load measurement taken at least 20 weeks after initiation of first-line ART. Inclusion criteria for the second-line subcohort were prescription of boosted PI-based second-line ART, and availability of at least one viral load measurement taken at least 20 weeks after initiation of second-line ART. If patients switched from first-line to second-line ART and met inclusion criteria for both subcohorts, they were included separately in each subcohort for the duration of each treatment episode. Treatment episodes with insufficiently potent ART (ie, monotherapy, dual therapy) or third-line ART (ie, triple-class ART, darunavir-containing ART) were censored from the analysis. Patients with missing data for critical parameters (ie, age, sex) were excluded from the analysis.

### Data analysis and outcomes

Medical record data collected during regular clinical care, consisting of an anonymous unique identifier, age, sex, ART prescription history, viral load results, and CD4 T-lymphocyte counts, were extracted from electronic medical databases. To account for missing laboratory data, patient records were cross-referenced to National Health Laboratory Services records using a probability matching algorithm. Anonymised database records were subject to quality control, including removal of duplicate data entries, outliers in continuous and date parameters, and ambiguous or erroneous entries in categorical and text parameters. Verification of extracted records with source data was done in a randomly selected subset of ten patients from each centre. After quality control, all available records from HIV-1-infected patients registered at the participating facilities between Jan 1, 2007, and May 1, 2016, were screened for inclusion.

The primary endpoint for patients on first-line and second-line ART was virological failure (defined as one or more viral load measurement of  $\geq 1000$  copies per mL). Secondary endpoints for patients on first-line ART were confirmed virological failure (defined as two or more viral load measurements of  $\geq 1000$  copies per mL without resuppression on first-line ART) and switch to second-line ART (defined as switch from NNRTI-containing to PI-containing ART after one or

See Online for appendix

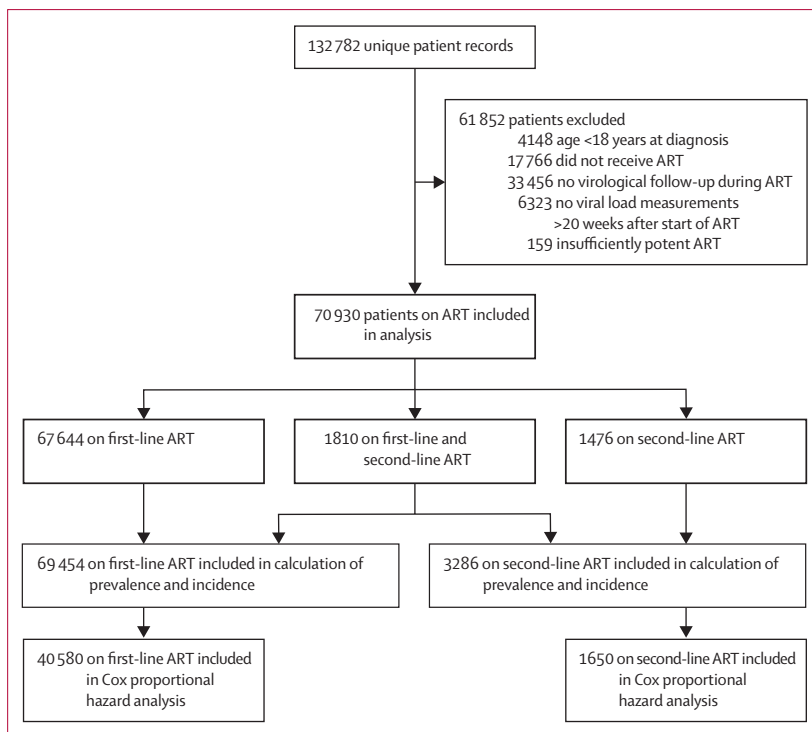


Figure 1: Study profile

Characteristics	First-line ART (n=69 454)	Second-line ART (n=3286)
Province		
Mpumalanga	4691 (7%)	194 (6%)
Gauteng	27 121 (39%)	660 (20%)
North West	33 506 (48%)	1940 (59%)
Limpopo	4136 (6%)	492 (15%)
Sex		
Men	22 311 (32%)	1068 (33%)
Women	47 143 (68%)	2218 (67%)
Age at start of ART (years)*	35.7 (30.2–42.8; 18–98)	37.5 (32.2–44.2; 18–78)
Duration of follow-up on ART (weeks)*	124 (56–221)	101 (51–178)
Range of follow-up on ART* (years)		
<1	14 724 (21%)	856 (26%)
1–3	25 960 (37%)	1408 (43%)
>3	28 770 (41%)	1022 (31%)
Calendar year of start of ART*		
<2010	18 478 (27%)	686 (21%)
2010–13	29 715 (43%)	1304 (40%)
>2013	21 261 (31%)	1296 (39%)
Viral load tests during follow-up†		
1	27 097 (39%)	1570 (48%)
2	14 270 (21%)	702 (21%)
>2	28 087 (40%)	1014 (31%)
NRTI exposure‡		
TDF plus FTC or 3TC	63 763 (92%)	2759 (84%)
ABC plus 3TC	1157 (2%)	269 (8%)
AZT plus 3TC	8663 (12%)	1931 (59%)
Any stavudine-containing regimen	19 147 (28%)	1658 (50%)
Any didanosine-containing regimen	236 (<1%)	286 (9%)
NNRTI‡		
Efavirenz	66 830 (96%)	2611 (79%)
Nevirapine	10 304 (15%)	776 (24%)
PI exposure‡		
Ritonavir-boosted lopinavir	NA	3268 (99%)
Ritonavir-boosted atazanavir	NA	90 (3%)
CD4 count at start of ART (cells per mL)*	186 (101–285)	228 (109–385)
CD4 count at start of ART (cells per µL)*§		
<200	32 118 (55%)	1246 (44%)
200–500	23 850 (41%)	1133 (40%)
500	2690 (5%)	430 (15%)
CD4 count nadir (cells per µL)	181 (96–282)	136 (52–249)
CD4 count recovery (cells per µL)	167 (0–344)	89 (0–265)

(Table 1 continues in next column)

more viral load result of  $\geq 1000$  copies per mL). Low-level viraemia was defined as the occurrence of at least one viral load measurement of 51–999 copies per mL during ART. Instances of low-level viraemia were grouped in

	First-line ART (n=69 454)	Second-line ART (n=3286)
(Continued from previous column)		
<b>Virological status and outcomes</b>		
LLV (51–999 copies per mL)	16 013 (23%)	855 (26%)
LLV range (copies per mL)¶		
51–199	9443 (59%)	458 (54%)
200–399	3171 (20%)	187 (22%)
400–999	3399 (21%)	210 (25%)
Frequency of LLV¶¶		
Single measurement	12 606 (79%)	687 (80%)
2 consecutive measurements	1605 (10%)	83 (10%)
2 intermittent measurements	978 (6%)	39 (5%)
>2 repeated measurements	438 (3%)	27 (3%)
>2 intermittent measurements	386 (2%)	19 (2%)
Virological outcome		
VF (viral load >1000 copies per mL)	14 380 (21%)	1092 (33%)
Initial VF†	9242 (13%)	766 (23%)
VF during follow-up	5138 (7%)	326 (10%)

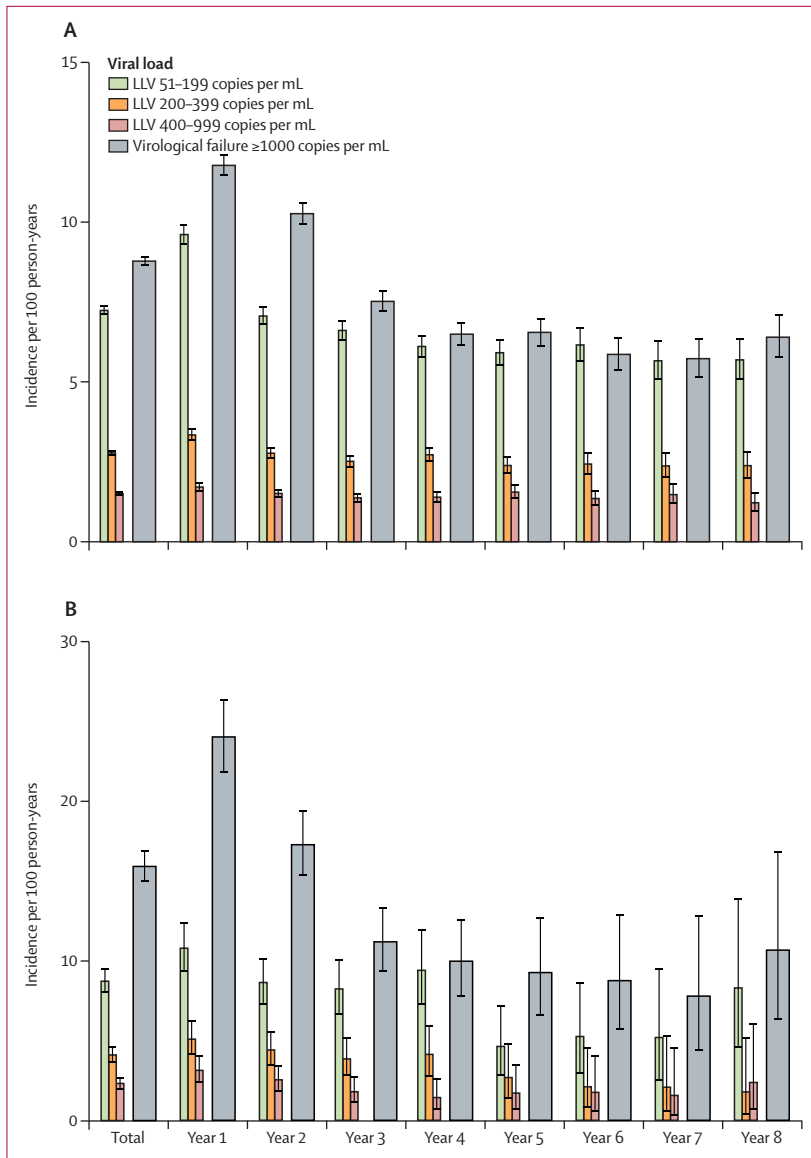
Data are n (%), median (IQR), or median (IQR; range). Due to rounding some percentages do not total 100%. 3TC=lamivudine. ABC=abacavir. ART=antiretroviral therapy. AZT=zidovudine. FTC=emtricitabine. LLV=low-level viraemia. NA=not available. NRTI=nucleos(t)ide reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. PI=protease inhibitor. TDF=tenofovir disoproxil fumarate. VF=virological failure. \*Measured from start of relevant line of ART. †Patients with initial virological failure (first viral load  $\geq 1000$  copies per mL) have one available viral load per definition due to censoring. ‡Measured as cumulative exposure. §First-line ART, n=58 658; second-line ART, n=2809; data were not available for 10 796 patients on first-line ART and 477 patients on second-line ART. ¶As percentage of total number of patients with low-level viraemia (first-line ART, n=16 013; second-line ART, n=855).

Table 1: Characteristics and virological outcomes of included patients

ranges of 51–199 copies per mL, 200–399 copies per mL, and 400–999 copies per mL. Patients with low-level viraemia were further classified as having a single instance of low-level viraemia, multiple instances of low-level viraemia with intermittent virological suppression of less than 50 copies per mL, or multiple consecutive instances. Patients with multiple instances of low-level viraemia were classified in the range of their highest result.

### Statistical analysis

Available CD4-cell counts were analysed as both continuous and categorical variables. Differences between groups were compared with the Student's *t* test for continuous variables and the  $\chi^2$  test for categorical variables. Incidence rates for low-level viraemia and virological failure were calculated by a complete cases only approach in which the first viral load of every year was allowed in order to avoid bias from repeat measurements within 1 year. The analysis allowed for multiple low-level



**Figure 2:** Incidence of low-level viraemia and viraemia higher than 1000 copies per mL in patients on (A) first-line ART and (B) second-line ART. Error bars show 95% CI. ART=antiretroviral therapy. LLV=low-level viraemia.

viraemia events in different years of follow-up and right-censored patients after virological failure, switch of ART line, or at the end of the follow-up year of their last viral load result. Predictors of low-level viraemia were identified by logistic regression and further evaluated using mixed-effect logistic regression models assuming a random effect for each province. Cox proportional hazards models were used to assess the effect of low-level viraemia on study outcomes and included all patients without virological failure at the first viral load test and with at least 1 year of follow-up on ART without virological failure, and with at least two available viral load results. Viral load results were analysed as time-dependent covariates in

these models, enabling separate assessment of each interval between two viral loads, the first viral load representing the predictor status and the last viral load the dichotomous outcome status. Results were reported as hazard ratios (HRs) reflecting the relative risk of the outcome for each range of low-level viraemia compared with virological suppression of less than 50 copies per mL and were displayed as extended Kaplan-Meier estimators, allowing for changing composition of exposure groups over time, in accordance with the most recent viral load result for each patient. These methods and their application in case of repeated measurements of predictor and outcome status are discussed elsewhere.<sup>26,27</sup> Patients not reaching study outcomes were right-censored after their last available viral load.

All Cox proportional hazards models assumed a random effect for each province and were corrected for clinically relevant variables known to affect outcomes of ART—ie, sex, age and CD4-cell count at ART initiation, and calendar year of ART initiation. Proportionality of the hazards assumption was assessed using Schoenfeld residuals. Cox models were iterated in subsets of patients to assess their robustness, including subsets without probability matched laboratory data, subsets of patients exposed to different antiretroviral regimens, and subsets from individual cohorts.

All results were reported with their respective 95% CI. All data parsing, quality control, and statistical analysis procedures were done by the researchers with R version 3.4.1.

#### Role of the funding source

No specific funding was received for the study. Funding sources for the participating centres had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Results

Data from 132 782 HIV-infected patients were obtained, of whom 59 712 attended clinics in Gauteng province (19 sites), 40 961 in North West province (36 sites), 20 664 in Limpopo province (one site), and 11 445 in Mpumalanga province (one site).

After application of inclusion criteria, 70 930 patients were included in the analysis, of whom 67 644 received first-line ART (206 190 patient-years of follow-up), 1476 received second-line ART (4690 patient-years), and 1810 were treated with both lines (3747 patient-years; figure 1). Patients who were excluded from the analysis (n=61 852) had similar clinical and demographic characteristics to included patients (appendix p 1).

Median duration of follow-up was 124 weeks (IQR 56–221) for patients on first-line ART and 101 weeks (IQR 51–178) for patients on second-line ART (table 1). Of 69 454 patients on first-line ART, most were treated with the NNRTI efavirenz (96%) and an NRTI-backbone

	Patients on first-line ART (n=40 580)				Patients on second-line ART (n=1650)			
	Model 1*		Model 2†		Model 1*		Model 2†	
	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value
Virological suppression <50 copies per mL	1 (ref)	..	1 (ref)	..	1 (ref)	..	1 (ref)	..
LLV 51–999 copies per mL	2.6 (2.5–2.8)	<0.0001	NA	NA	3.1 (2.5–4.0)	<0.0001	NA	NA
LLV 51–199 copies per mL	NA	NA	1.9 (1.8–2.1)	<0.0001	NA	NA	2.1 (1.5–2.8)	<0.0001
LLV 200–399 copies per mL	NA	NA	3.2 (2.9–3.5)	<0.0001	NA	NA	3.7 (2.6–5.4)	<0.0001
LLV 400–999 copies per mL	NA	NA	4.7 (4.2–5.2)	<0.0001	NA	NA	6.8 (4.7–9.8)	<0.0001
Sex: male	1.3 (1.2–1.4)	<0.0001	1.3 (1.2–1.4)	<0.0001	1.1 (0.9–1.4)	0.35	1.1 (0.9–1.4)	0.45
Age at start of ART	0.9 (0.9–0.9)	<0.0001	0.9 (0.9–0.9)	<0.0001	0.9 (0.9–1.0)	0.48	0.9 (0.9–1.0)	0.061
CD4 count at start of ART ≥200 cells per µL	0.9 (0.8–0.9)	0.00019	0.9 (0.8–0.9)	0.00014	0.7 (0.5–0.9)	0.0093	0.7 (0.6–0.9)	0.016
Calendar year of start of ART ≥2010	1.0 (0.9–1.1)	0.66	1.0 (1.0–1.1)	0.57	1.1 (0.8–1.4)	0.7	1.0 (0.8–1.4)	0.81

Analysis for patients on first-line ART excluded 9242 patients with initial virological failure, 17 855 patients with one viral load result, and 1777 patients with less than 1 year of follow-up on ART, resulting in 40 580 patients. Analysis for patients on second-line ART excluded 766 patients with initial virological failure, 804 patients with one available viral load, and 66 patients with less than 1 year of available follow-up on ART, resulting in 1650 patients. Virological failure was defined as at least one viral load measurement of 1000 copies per mL or more. All models included a random effect for province. Virological suppression of less than 50 copies per mL was the reference value for low-level viraemia. The increment for age was set at 5 years. ART=antiretroviral therapy. LLV=low-level viraemia. \*Model 1 included LLV as a dichotomous variable. †Model 2 included LLV as a categorical variable.

**Table 2: Cox proportional hazards analysis of the association between low-level viraemia and virological failure**

containing tenofovir (92%). Of 3286 patients on second-line ART, most received ritonavir-boosted lopinavir as a ritonavir-boosted protease inhibitor (99%) with a zidovudine or lamivudine NRTI-backbone (59%).

Low-level viraemia was recorded in 16 013 (23%) patients on first-line ART and 855 (26%) patients on second-line ART (table 1). Of these patients, 3407 (21%) on first-line ART and 168 (20%) on second-line ART had multiple consecutive or intermittent episodes of low-level viraemia. Prevalence was highest for low-level viraemia in the range of 51–199 copies per mL (59% of patients on first-line ART and 54% of patients on second-line ART), compared with 200–399 copies per mL (20% first-line, 22% second-line) and 400–999 copies per mL (21% first-line, 25% second-line). Incidence of low-level viraemia was 11.5 per 100 person-years of follow-up (95% CI 11.4–11.7) in patients on first-line ART, and 15.1 per 100 person-years in patients on second-line ART (14.2–16.1).

Virological failure (one or more viral load measurement of ≥1000 copies per mL) occurred in 14 380 (22%) of 69 454 patients on first-line ART and 1092 (33%) of 3286 patients on second-line ART. Incidence rates of low-level viraemia and virological failure decreased after the first year of ART ( $p=0.0014$  for low-level viraemia in the range of 400–999 copies per mL,  $p<0.0001$  for other ranges of low-level viraemia and virological failure; figure 2). Incidence rates per calendar year and province are listed in the appendix (pp 2–4).

Cox proportional hazards analysis of patients on first-line ART excluded 9242 patients with initial virological failure, 17 855 patients with one viral load result, and 1777 patients with less than 1 year of follow-up on ART, resulting in 40 580 patients. Low-level viraemia was associated with a significantly increased hazard of subsequent virological failure compared with virological suppression of less than

50 copies per mL, with an overall adjusted HR of 2.6 (95% CI 2.5–2.8;  $p<0.0001$ ; table 2). Adjusted HRs increased with increasing range of low-level viraemia, from 51–199 copies per mL to 200–399 copies per mL to 400–999 copies per mL (table 2, figure 3). Similar trends were seen in Cox proportional hazards analysis of 1650 patients on second-line ART, which excluded 766 patients with initial virological failure, 804 patients with one available viral load, and 66 patients with less than 1 year of available follow-up on ART (table 2, figure 3).

For patients on first-line ART, low-level viraemia of each range increased the risk of confirmed virological failure (two or more viral load measurements of ≥1000 copies per mL) as well as the risk of virological failure followed by switch to second-line ART (table 3, figure 3). The hazard for virological failure after consecutive measurements of low-level viraemia (adjusted HR 3.1, 95% CI 2.8–3.5;  $p<0.0001$ ) was higher than that after single measurements (adjusted HR 2.6, 2.4–2.8;  $p<0.0001$ ) or intermittent measurements (adjusted HR 1.5, 1.2–2.0;  $p=0.00054$ ; appendix p 5).

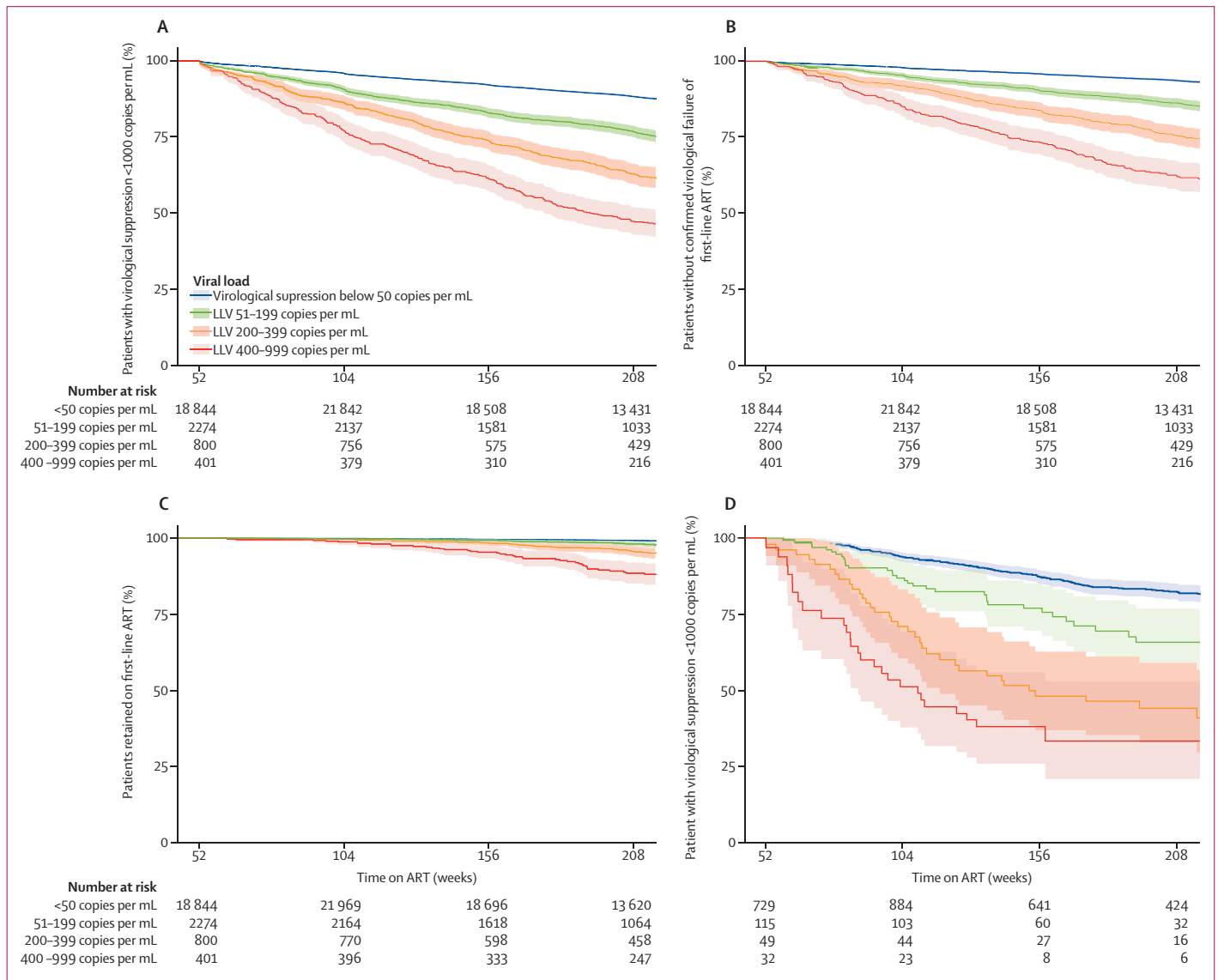
Extensive post-hoc data subset analyses of individual cohorts and patients on currently recommended first-line ART consistently supported the findings of the main analysis (appendix pp 7–14).

Of 14 380 patients on first-line ART who had virological failure during follow-up, 3785 (26%) achieved resuppression to 50 copies per mL on the same regimen. Patients on first-line ART with virological failure directly preceded by low-level viraemia were significantly less likely to achieve resuppression (adjusted odds ratio [aOR] 0.92, 95% CI 0.90–0.95;  $p<0.0001$ ), and significantly more likely to switch to second-line ART (aOR 1.02, 1.00–1.05;  $p=0.044$ ) than were patients with virological failure directly preceded by virological suppression of less than 50 copies per mL (appendix p 6).

Of 3854 patients on first-line ART who met virological eligibility criteria for switch to second-line ART according to WHO guidelines (two consecutive viral load measurements of  $\geq 1000$  copies per mL within a 3-month interval), 2594 (67%) had available follow-up data after the event, of whom 1068 (41%) were switched to second-line ART. Patients were switched after a median of 59.2 weeks of virological failure.

Patients on first-line ART with low-level viraemia were more often men (5360 [33%] of 16013 vs 13605 [31%] of 44199),

older (36.6 years vs 35.6 years), and had lower baseline CD4 counts (172 cells per  $\mu\text{L}$  vs 196 cells per  $\mu\text{L}$ ) than patients without low-level viraemia (appendix p 15). In multicentre analysis, male sex and lower baseline CD4 count remained positively associated with low-level viraemia whereas the association for age lost statistical significance (appendix p 156). In a separate multicentre analysis of predictors of nadir CD4-cell count, low-level viraemia of all ranges was associated with a lower nadir CD4-cell count than that in virologically suppressed patients (appendix p 17).



**Figure 3: Virological and treatment outcomes**

(A-C) Outcomes of patients on first-line ART with and without LLV. Extended Kaplan-Meier estimators of 40 580 patients on first-line ART without initial virological failure at the first viral load measurement, and with at least two viral load results and at least 1 year of follow-up without virological failure. (A) Virological failure (one or more viral load measurement of  $\geq 1000$  copies per mL). (B) Confirmed virological failure (two or more viral load measurements of  $\geq 1000$  copies per mL without subsequent resuppression of  $< 1000$  copies per mL on the same regimen). (C) Switch to second-line ART (defined as a switch from non-nucleoside reverse transcriptase inhibitor-based ART to protease inhibitor-based ART after at least one viral load measurement of 1000 copies per mL or more). (D) Virological failure (one or more viral load measurement of  $\geq 1000$  copies per mL) in patients on second-line ART with and without LLV. Extended Kaplan-Meier estimators of 1650 patients on second-line ART without initial virological failure at the first viral load measurement, and with at least two viral load results and at least 1 year of follow-up without virological failure. Shaded areas show 95% CI. Note: exposure group composition is updated at each timepoint to reflect the most recent viral load result for each patient. ART=antiretroviral therapy. LLV=low-level viraemia.



	Confirmed virological failure				Switch to second-line ART			
	Model 1*		Model 2†		Model 1*		Model 2†	
	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value	Adjusted HR (95% CI)	p value
Virological suppression <50 copies per mL	1 (ref)	..	1 (ref)	..	1 (ref)	..	1 (ref)	..
LLV 51–999 copies per mL	2.8 (2.6–3.1)	<0.0001	NA	NA	5.2 (4.4–6.1)	<0.0001	NA	NA
LLV 51–199 copies per mL	NA	NA	2.0 (1.8–2.2)	<0.0001	NA	NA	2.9 (2.4–3.7)	<0.0001
LLV 200–399 copies per mL	NA	NA	3.6 (3.2–4.1)	<0.0001	NA	NA	6.3 (4.9–8.1)	<0.0001
LLV 400–999 copies per mL	NA	NA	5.5 (4.8–6.4)	<0.0001	NA	NA	13.5 (10.8–16.8)	<0.0001
Sex: male	1.4 (1.3–1.5)	<0.0001	1.4 (1.3–1.5)	<0.0001	1.3 (1.1–1.6)	0.00028	1.3 (1.1–1.5)	0.00065
Age at start of ART	0.9 (0.8–0.9)	<0.0001	0.9 (0.8–0.9)	<0.0001	0.9 (0.9–0.9)	<0.0001	0.9 (0.9–0.9)	0.00031
CD4 count at start of ART ≥200 cells per µL	0.8 (0.8–0.9)	<0.0001	0.8 (0.8–0.9)	<0.0001	0.7 (0.5–0.8)	0.00067	0.7 (0.5–0.8)	0.00042
Calendar year of start of ART ≥2010	1.4 (1.3–1.5)	<0.0001	1.4 (1.3–1.6)	<0.0001	1.1 (0.9–1.3)	0.55	1.1 (0.9–1.4)	0.39

Analysis for patients on first-line ART excluded 9242 patients with initial virological failure, 17 855 patients with one viral load result, and 1777 patients with less than 1 year of follow-up on ART, resulting in 40 580 patients. Confirmed virological failure was defined as at least two viral load measurements of 1000 copies per mL or more without resuppression on first-line ART. Switch to second-line ART was defined as switch from non-nucleoside reverse transcriptase inhibitor-based ART to protease inhibitor-based ART after at least one viral load result of 1000 copies per mL or more. All models included a random effect for province. Virological suppression of less than 50 copies per mL was the reference value for low-level viraemia. The increment for age was set at 5 years. ART=antiretroviral therapy. LLV=low-level viraemia. \*Model 1 included LLV as a dichotomous variable. †Model 2 included LLV as a categorical variable.

**Table 3: Cox proportional hazards analysis of the association between low-level viraemia and confirmed virological failure or switch to second-line ART**

## Discussion

Our study assessed the effects of low-level viraemia in HIV-positive patients in low-income and middle-income countries receiving ART according to WHO guidelines. In this large-scale South African multicentre analysis, about a quarter of patients had low-level viraemia, which was found to be a strong predictor of subsequent virological failure and switch to second-line ART.

As in any analysis of real-world data, this study has some limitations that might have affected the results. Any selection of patient data for observational analysis can create selection bias. Changes in guidelines and local clinical practice might have created heterogeneity. Use of different viral load assays might have affected the comparability of low-level viraemia at lower ranges. Absence of pre-treatment viral load and drug resistance testing results precluded study of the mechanisms underlying low-level viraemia. However, the large sample size enabled extensive subset analyses, which demonstrated robustness of the findings and did not identify significant bias. Although missed visits and patient attrition might have resulted in incomplete follow-up data, data obtained from clinical programmes in a large number of urban and rural South African settings should accurately reflect current practice in low-income and middle-income countries.

The occurrence of low-level viraemia in this cohort was substantially higher than that in reports from high-income settings with contemporary ART. In these studies, the highest reported prevalence was 6.2% for low-level viraemia of 51–499 copies per mL during a median follow-up of 2.3 person-years.<sup>24</sup> In our analysis, the prevalence of low-level viraemia of 51–399 copies

per mL was 18% during a median of 2.4 person-years. However, the lower frequency of viral load monitoring used in clinical practice in low-income and middle-income countries might in part underlie these apparent differences, complicating head-to-head comparisons. The occurrence of virological failure in this cohort, which is similar to previously published data from treatment programmes in low-income and middle-income countries,<sup>3,8</sup> is also higher than that reported in high-income settings, although stark differences between individual countries exist.<sup>1</sup> The observed differences in prevalence of low-level viraemia and virological failure might therefore reflect lower overall rates of virological suppression in low-income and middle-income countries. Although not applicable to all clinics, a tendency towards higher rates of low-level viraemia were detected in rural and urban-rural settings compared with urban settings.

In this clinical cohort, virological failure during second-line ART occurred more frequently than in clinical trials done in low-income and middle-income countries.<sup>28</sup> Several factors could explain this difference, such as less stringent monitoring in clinical practice, allowing for accumulation of NRTI resistance, as well as persisting adherence problems present in patients who are switched from first-line to second-line ART. The role of these factors in regular clinical practice in low-income and middle-income countries requires further analysis.

Our findings show that patients with low-level viraemia are predisposed to subsequent virological failure. The risk was significantly increased even after a single measurement of low-range low-level viraemia of 51–199 copies per mL. Although previous studies from high-income countries

have shown an association between low-level viraemia and virological failure, these studies did not agree on the relevance of low-range low-level viraemia.<sup>13</sup> Less frequent virological monitoring and limited therapeutic options in low-income and middle-income countries might aggravate the effect of low-range low-level viraemia. However, we cannot exclude that high statistical power of this analysis might have enabled detection of this effect.

Our study showed a five-times increased risk of virological failure after high-range low-level viraemia of 400–999 copies per mL. Previous studies were mostly unable to study high-range low-level viraemia because of more stringent criteria for virological failure. The only exception was a single-centre Canadian cohort, in which 34 cases of repeated high-range low-level viraemia of 500–999 copies per mL were identified. Differences in study design notwithstanding, the Canadian study encountered a five-times increased risk of virological failure after high-range low-level viraemia, which is similar to our results.<sup>22</sup> This concordance strengthens our conclusion that high-range low-level viraemia confers a strongly increased risk of virological failure and therefore warrants clinical intervention.

Our results show that a quarter of patients with virological failure achieved virological resuppression (<50 copies per mL) without a change of regimen, suggesting intermittent non-adherence to ART. However, resuppression on the same regimen after virological failure was less likely if failure was preceded by low-level viraemia, which might indicate that these patients have more persistent adherence issues. Indeed, decreased ART drug concentrations have been encountered during low-level viraemia.<sup>14,15,29</sup> Alternatively, the longer duration of detectable viraemia in these patients might have increased selection of mutations conferring drug resistance. Loss of susceptibility to NNRTI-based regimens with a low genetic barrier to resistance has been observed during low-level viraemia in high-income settings.<sup>16,18</sup> The clinical significance of selection of drug resistance mutations during low-level viraemia in low-income and middle-income countries is poorly characterised. Patients reaching eligibility criteria for second-line ART remained on a failing regimen for a longer period (median 59.2 weeks after first detection of virological failure) than advised by current guidelines (directly after confirmation of virological failure, which is to occur within 12 weeks of first detection of virological failure), and were not switched in all cases. This delay might affect patient health, accumulation of drug resistance, and onward transmission of drug-resistant HIV, thus warranting intervention.

Viral load measurement is a powerful monitoring tool that is used worldwide to determine virological success and failure. The potential introduction of novel point-of-care viral load assays and dried-blood spot sampling could have infrastructural benefits in low-income and middle-income countries, but these methods currently do not have the ability to reliably

detect low-level viraemia. The current emphasis on the threshold of 1000 copies per mL for virological failure set by WHO, combined with the absence of provisions for management of low-level viraemia, implies that all values below this threshold denote virological success. Although some regional guidelines in low-income and middle-income countries contain limited interventions in case of low-level viraemia of 400–999 copies per mL, this has not been adopted by WHO.<sup>30</sup> Our results show that any detectable viral load between 51 and 999 copies per mL leads to poorer treatment outcomes than successful virological suppression of less than 50 copies per mL. Therefore, we recommend that WHO guidelines explicitly incorporate low-level viraemia as an early warning signal that deserves clinical action, including intensification of adherence counselling and repeat viral load testing.

The dynamics of virological failure and drug resistance might change upon adoption of higher genetic barrier first-line ART in low-income and middle-income countries. However, in our cohort the risk of virological failure after low-level viraemia in patients on high-genetic barrier second-line ART was similar to that of patients on first-line ART, suggesting that low-level viraemia will still be of importance in this context, and warranting re-evaluation of the threshold for virological failure.

In summary, this large-scale study shows that low-level viraemia occurs frequently and represents an important threat to virological success. Current WHO-guided clinical practice in low-income and middle-income countries is not geared towards early recognition and management of low-level viraemia. We urge policy makers and clinicians to incorporate management of low-level viraemia in their efforts to control the HIV epidemic.

#### Contributors

LEH and AMJW conceived and designed the study. LEH did data cleaning, pre-processing, and data analysis. LEH and AMJW wrote the first draft of the report. SC, HAT, and WDFV coordinated data collection. MM, LMH, DEG, DDR, HAT, WDFV, and AMJW advised during data analysis. All authors participated in writing of the final report.

#### Declaration of interests

SC reports grants from USAID-RTC during the conduct of the study; as well as grants from the US Centers for Disease Control and Prevention and WHO, outside the submitted work. LMH reports personal fees from Janssen, outside the submitted work. DDR reports personal fees from Merck, Gilead, Antiva, and Monogram, outside the submitted work. AMJW reports grants and consultancy fees from Janssen, Merck, ViiV Healthcare, and Gilead; grants and non-financial support from CLJI; and non-financial support and other from Virology Education, outside the submitted work, all paid to the University Medical Center Utrecht. All other authors declare no competing interests.

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