Original article

Virological outcome and management of persistent low-level viraemia in HIV-1-infected patients: 11 years of the Swiss HIV Cohort Study

Noémie Boillat-Blanco[1†], Katharine EA Darling[1†], Franziska Schoni-Affolter[2], Danielle Vuichard[3], Mathieu Rougemont[4], Rosamaria Fulchini[5], Enos Bernasconi[6], Manel Aouri[7], Olivier Clerc[1], Hansjakob Furrer[8], Huldrych F Günthard[9], Matthias Cavassini[1], the Swiss HIV Cohort Study*

[1]Infectious Diseases Service, Department of Medicine, University Hospital and University of Lausanne, Lausanne, Switzerland
[2]Data Coordination Centre for the Swiss HIV Cohort, Lausanne, Switzerland
[3]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
[4]Division of Infectious Diseases, Geneva University Hospital, Geneva, Switzerland
[5]Division of Infectious Diseases, Department of Medicine, Cantonal Hospital, St Gallen, Switzerland
[6]Division of Infectious Diseases, Hospital of Lugano, Lugano, Switzerland
[7]Division of Clinical Pharmacology and Toxicology, University Hospital and University of Lausanne, Lausanne, Switzerland
[8]Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland
[9]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

*Corresponding author e-mail: matthias.cavassini@chuv.ch
†These authors made an equal contribution to the manuscript

Background: Management of persistent low-level viraemia (pLLV) in patients on combined antiretroviral therapy (cART) with previously undetectable HIV viral loads (VLs) is challenging. We examined virological outcome and management among patients enrolled in the Swiss HIV Cohort Study (SHCS).

Methods: In this retrospective study (2000–2011), pLLV was defined as a VL of 21–400 copies/ml on ≥ three consecutive plasma samples with ≥8 weeks between first and last analyses, in patients undetectable for ≥24 weeks on cART. Control patients had ≥ three consecutive undetectable VLs over ≥32 weeks. Virological failure (VF), analysed in the pLLV patient group, was defined as a VL>400 copies/ml.

Results: Among 9,972 patients, 179 had pLLV and 5,389 were controls. Compared to controls, pLLV patients were more often on unboosted protease inhibitor (PI)-based (adjusted odds ratio [aOR; 95% CI] 3.2 [1.8, 5.9]) and nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)-only combinations (aOR 2.1 [1.1, 4.2]) than on non-nucleoside reverse transcriptase inhibitor and boosted PI-based regimens. At 48 weeks, 102/155 pLLV patients (66%) still had pLLV, 19/155 (12%) developed VF and 34/155 (22%) had undetectable VLs. Predictors of VF were previous VF (aOR 35 [3.8, 315]), unboosted PI-based (aOR 12.8 [1.7, 96]) or NRTI-only combinations (aOR 115 [6.8, 1,952]), and VLs>200 during pLLV (aOR 3.7 [1.1, 12]). No VF occurred in patients with persistent very LLV (21–49 copies/ml; *n*=26). At 48 weeks, 29/39 patients (74%) who changed cART had undetectable VLs, compared with 19/74 (26%) without change (*P*<0.001).

Conclusions: Among patients with pLLV, VF was predicted by previous VF, cART regimen and VL≥200. Most patients who changed cART had undetectable VLs 48 weeks later. These findings support cART modification for pLLV>200 copies/ml.

Introduction

HIV viral load (VL) in treated patients is a marker for treatment response [1] and is a strong predictor of disease progression [2]. The aim of combined antiretroviral therapy (cART) is to render the VL undetectable. In clinical practice, we may observe a transiently detectable VL in as many as 25–50% of patients previously well-controlled on cART [3]. Less common is persistent low-level viraemia (pLLV), when HIV RNA is detectable at low levels upon sequential analyses, occurring in 4–8% of patients [3–5].

To what extent pLLV is a marker of residual replication, and a promoter of immune activation, virological failure (VF) and clinical progression, is unclear [4,6]. Some studies support cART modification when pLLV occurs, given the potential for accumulating resistance mutations and virological progression [3,4,7–26]. Others do not, arguing that VF occurs in a minority of cases and rarely when viraemia is <50 HIV RNA copies/ml [4,10,13–27].

One difficulty in drawing conclusions from published data is the wide inter-study variability in LLV and VF definitions. Definitions which fit study data are not always applicable to clinical practice. This is reflected in current HIV treatment recommendations: the 2012 International AIDS Society (IAS)-USA Panel recommendations describe a lack of consensus regarding the management of patients with VLs of 50–200 copies/ml [28]; the 2013 US Department of Health and Human Services (DHHS) guidelines describe the clinical implications of VLs of 48–200 copies/ml as controversial [29]. Both recommendations mention the AIDS Clinical Trials Group definition of VF as a confirmed detectable VL>200 copies/ml following VL suppression [28,29]. However, the decision to alter cART in patients with pLLV>200 copies/ml is complicated by the reduced reliability of resistance assays when HIV RNA levels are <500 copies/ml [30].

To shed light on the clinical implications of pLLV in a large cohort, we examined the virological outcome and management of this phenomenon in HIV-1 infection within the Swiss HIV Cohort Study (SHCS).

Methods

Study design

We conducted a retrospective analysis from 1 January 2000 to 30 November 2011 of patients on cART enrolled in the SHCS. The SHCS is an ongoing, nationwide, prospective observational cohort of HIV-infected patients, with continuous enrolment at seven centres in Switzerland and by affiliated private physicians [31]. Cohort patients undergo data collection (socio-demographic characteristics, comorbidities, cART regimen, treatment adherence and clinical course) and blood sampling ($CD4^+$ T-cell count, VL and renal function) at inclusion and twice-yearly thereafter. VL values measured between two consecutive cohort visits are also recorded. Approval for this study was obtained from the local ethical committees of all participating centres.

Inclusion criteria

Patients with an undetectable VL (VL below the assay detection threshold) during ≥24 weeks on cART and who subsequently presented a pLLV, defined as a VL between 21 and 400 copies/ml, detectable on ≥ three consecutive plasma samples for ≥8 weeks between the first and final analyses, made up the pLLV group. Control patients had ≥ three consecutive VL values ≤20 copies/ml for ≥32 weeks (that is, the same length of follow-up as the patients with pLLV: 24 plus 8 weeks) without cART-change. Patients could not be both pLLV patients and controls.

Rationale for pLLV definitions

We chose 400 copies/ml as the upper pLLV limit as this is the threshold for reliable resistance assays at SHCS centres. VL assays and thresholds for 'detectable' viraemia have varied in the past decade in Switzerland. The predominant detection threshold was 20 copies/ml between 2000 and 2003, 50 copies/ml between 2004 and 2005, 40 copies/ml between 2006 and 2008, and 20 copies/ml since 2009, with the Cobas AmpliPrep/Cobas Taqman HIV-1 assay, version 2 (Roche Diagnostics, Basel, Switzerland). We chose 21 copies/ml as the lower pLLV limit to include every detectable viraemia. VLs of 21–49 copies/ml were defined as persistent very low level viraemia (pVLLV).

Definitions and recorded parameters

pLLV was classified according to the predominant VL detection threshold at the time: 20 copies/ml (2000 to 2003 and from 2009 onwards) and 40–50 copies/ml (2004 to 2008). To reflect the different thresholds over time, we included 'year at inclusion' as a variable in our analysis. Undetectable VL was defined as a VL≤20 copies/ml or as a VL under the detection threshold when the limit of detection was >20 copies/ml. VF was defined as a single VL>400 copies/ml. Previous VF was defined as a single VL>400 copies/ml, having been undetectable on cART, or inability to achieve a VL<400 after 24 weeks on a cART regimen. VF analysis was restricted to pLLV patients.

When available, therapeutic drug monitoring (TDM) results were recorded, as performed by liquid chromatography-tandem mass spectrometry (drug levels classified as very low [<10th centile], low [<25th centile], in the expected range [25th–75th centile] and high [>75th centile], as previously described [32]). Genotyping resistance testing was recorded, when available, and analysed using the Stanford HIV drug resistance database [33]. Adherence to cART was the lowest adherence throughout the period with an undetectable VL prior to pLLV for pLLV patients, and throughout control patient follow-up. 'Suboptimal adherence' was defined as ≥ one dose omission per month [34]. Other definitions of suboptimal adherence were analysed but did not correlate with any outcomes even in univariate analysis.

cART regimens were described according to their composition: non-nucleoside reverse transcriptase inhibitor

(NNRTI)-based regimens containing one NNRTI and two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); ritonavir-boosted or unboosted protease inhibitor (PI)-based regimens containing one PI and two NRTIs; NRTI combinations containing three NRTIs; and other drug class combinations containing a PI with an NNRTI and/or new drugs such as raltegravir (RAL), maraviroc (MVC) or enfuvirtide.

Treatment modifications and the choice of alternative cART were recorded up to 24 and 48 weeks (±4 weeks) after the last VL value in pLLV patients. Treatment modification was defined as addition of a new antiretroviral drug class to the regimen, change from a PI-based to an NNRTI-based regimen or vice versa, change from an unboosted to a boosted PI, change from an old to a new generation PI (darunavir [DRV]) and modification of the NRTI combination plus documented reason for change as 'treatment failure' in the medical records (see below). Treatment discontinuation was defined as stopping cART for ≥4 weeks as previously described [35]. Switch of cART to mono- or dual-NRTI therapy was defined as treatment for 'viral fitness' [36]. cART discontinuation for <4 weeks and treatment changes not described above were considered as minor and not recorded as treatment modifications. The reasons for cART modification are defined within the SHCS as: physician's decision, patient's decision, abnormal fat distribution, toxicity, treatment failure (virological, immunological or clinical) and unknown.

Laboratory values

Baseline CD4+ T-cell count values were taken as those just prior to pLLV onset for pLLV patients and those at the time of inclusion, or the nearest value thereafter, for control patients. CD4+ T-cell counts obtained at 24 and 48 weeks (±4 weeks) after the last pLLV value were analysed, respectively, as 24- and 48-week outcomes after pLLV. Baseline VL values were taken as the peak values observed during the pLLV period. Peak VL values observed during the 24 and 48 weeks (±4 weeks) after the last pLLV value or prior to cART modification (when cART modification occurred) were analysed, respectively, as 24- and 48-week outcomes after pLLV. In the case of treatment modification in the pLLV group, the nearest CD4+ T-cell counts and VLs obtained at 24 and 48 weeks (±4 weeks) after treatment modification were analysed. Virological outcome was also analysed separately according to the level of viraemia during pLLV: 21–49, 50–200 and 201–400 copies/ml.

Statistical analyses

Factors associated with pLLV were evaluated by comparing baseline characteristics of pLLV patients to those of controls using multivariable logistic regression with backward selection. Year at study inclusion, corresponding to the VL detection threshold at each time point, was included in the multivariable analysis.

Virological outcome of pLLV was evaluated by comparing baseline pLLV values to those at 24 and 48 weeks after the last pLLV value, or at the time of cART modification, using the Wilcoxon–Mann–Whitney test for continuous variables and χ^2 test for categorical variables.

Predictors of VF were evaluated by comparing pLLV patients who developed VF to those who did not develop VF, using multivariable logistic regression as described above. Patients lacking VL values after the last pLLV value and/or before any cART modification were excluded from the analysis.

Factors associated with cART modification were evaluated by comparing characteristics of pLLV patients in whom cART was modified to those of pLLV patients whose treatment was unchanged, again using multivariable logistic regression. Virological and immunological outcomes at the time of cART modification were compared to these parameters 24 and 48 weeks after treatment change, using the Wilcoxon–Mann–Whitney test. Patients whose treatment was discontinued for ≥4 weeks were excluded from the analysis. Patients with a second cART modification or cART stop during the follow-up period without previous virological and immunological results were also excluded from the analysis.

Statistical analyses were performed using Stata software, version 11.2 (StataCorp, College Station, TX, USA).

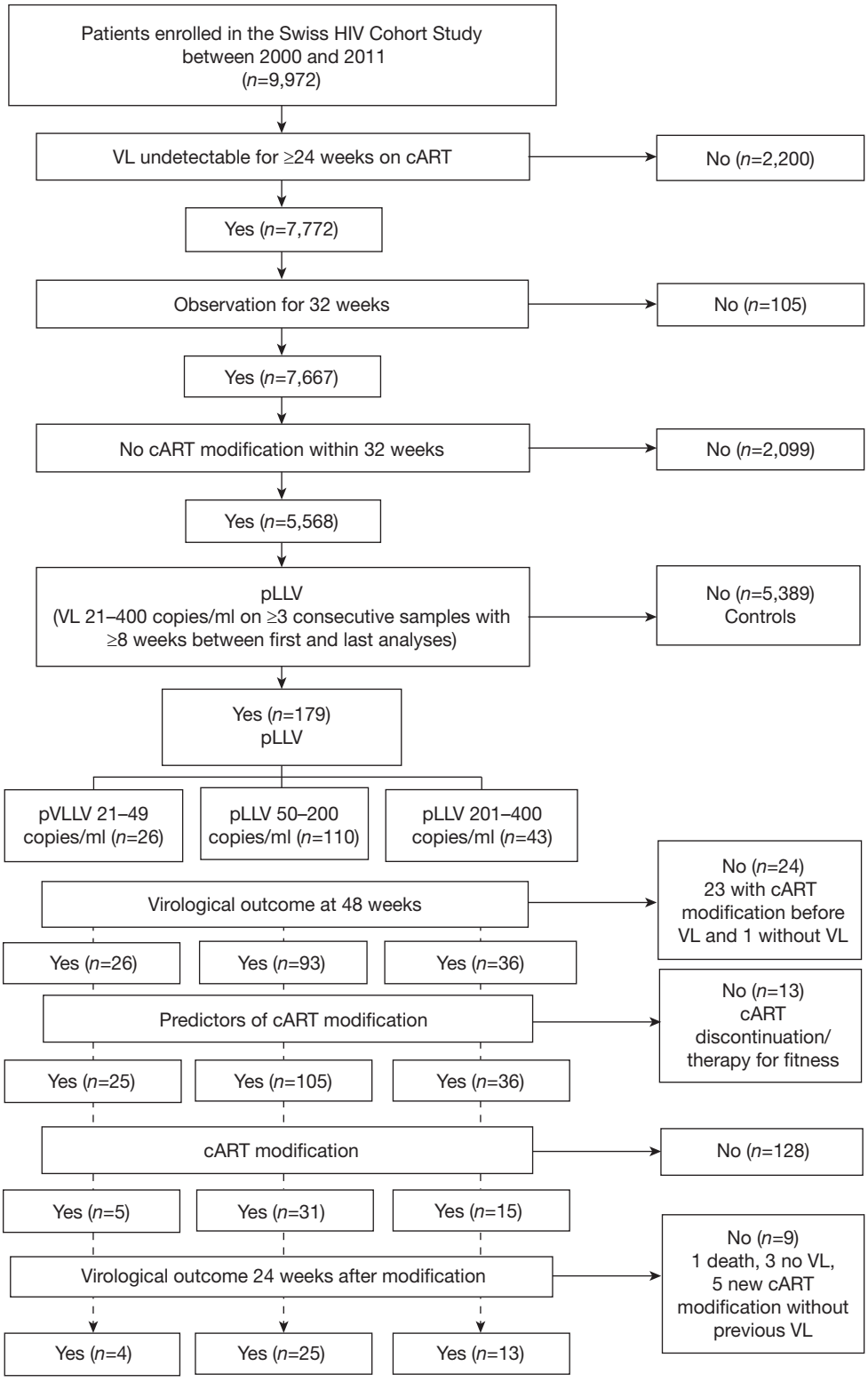
Results

Patient characteristics and factors associated with pLLV

Between 1 January 2000 and 30 November 2011, 179 patients (of 9,972) presented pLLV while 5,389 patients were included as controls (Figure 1). Median pLLV duration was 25 weeks (IQR 18–30) after a median undetectable duration of 43 weeks (IQR 39–52). The total duration (time from first undetectable VL to last pLLV value) was 67 weeks (IQR 61–81) and a median of 3 undetectable VL values (IQR 2-4) were recorded. Control patients had ≥3 consecutive undetectable VLs during a median of 76 weeks (IQR 67–92) and a median of 6 undetectable VL values (IQR 5–7) were recorded.

The majority of pLLV patients were included before 2004 and after 2008 when VL detection threshold was 20 copies/ml. Compared to controls, pLLV patients had been on their current cART for shorter durations, were more often on unboosted PI-based regimens and NRTI combinations, and were more often attending Centre B (Table 1). During the period of pLLV, TDM was performed more frequently in pLLV patients. However, prior to pLLV onset, there was no difference in TDM practices between pLLV patients and controls (data not shown).

Figure 1. Flow chart of study patient selection



cART, combined antiretroviral therapy; pLLV, persistent low-level viraemia; pVLLV, persistent very low-level viraemia; VL, viral load.

Table 1. Factors associated with persistent low-level viraemia

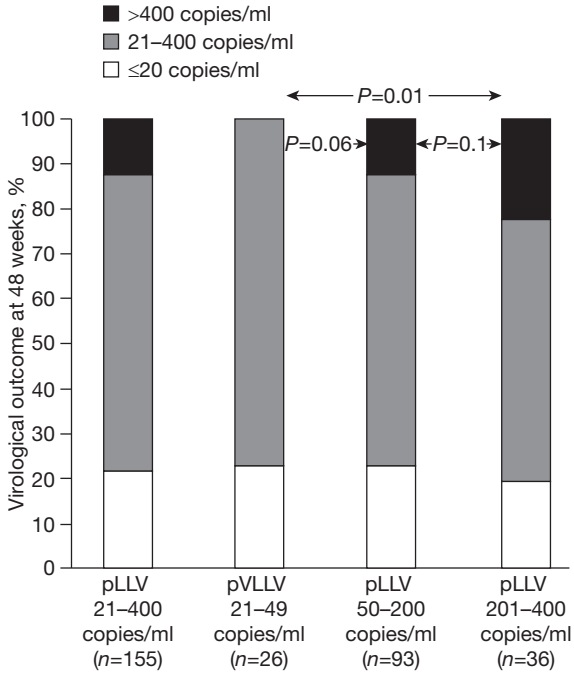
Patient characteristics	pLLV (*n*=179)	Control group (*n*=5,389)	Bivariate analysis[a]		Multivariable analysis[a]	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Mean age, years (SD)	46 (10)	44 (10)	1.02 (1, 1.03)	0.01	1.01 (1, 1.03)	0.12
Male sex	127 (71)	3,764 (70)	1.05 (0.76, 1.46)	0.75	–	–
Caucasian	150 (84)	4,430 (82)	1.12 (0.75, 1.68)	0.58	–	–
Mode of HIV acquisition				0.83		
Heterosexual	73 (41)	2,306 (43)	Ref	Ref	–	–
MSM	70 (39)	2,079 (39)	1.06 (0.76, 1.48)	0.72	–	–
IDU	36 (20)	1,004 (19)	1.133 (0.75, 1.7)	0.55	–	–
SHCS centre of follow-up				0.003		–
A	66 (37)	2,181 (40)	Ref	Ref	Ref	Ref
B	30 (17)	429 (8)	2.31 (1.48, 3.6)	<0.001	3.02 (1.78, 5.13)	<0.0001
C	21 (12)	726 (13)	0.96 (0.58, 1.57)	0.86	1.06 (0.6, 1.88)	0.83
D	24 (13)	725 (13)	1.09 (0.68, 1.76)	0.71	1.02 (0.54, 1.92)	0.95
E	24 (13)	772 (14)	1.03 (0.64, 1.65)	0.91	1.15 (0.62, 2.11)	0.66
F	8 (4)	224 (4)	1.18 (0.56, 2.49)	0.66	1 (0.39, 2.59)	0.99
G	6 (3)	332 (6)	0.6 (0.26, 1.39)	0.23	0.26 (0.06, 1.1)	0.07
Year at inclusion				<0.0001		–
2000–2003	44 (25)	1,104 (20)	Ref	Ref	Ref	Ref
2004–2008	63 (35)	3,124 (58)	0.51 (0.34, 0.75)	0.001	0.52 (0.3, 0.88)	0.02
≥2009	72 (40)	1,161 (22)	1.56 (1.06, 2.28)	0.02	2.25 (1.32, 3.85)	0.003
Mean $CD4^+$ T-cell count, cells/mm^3 (SD)	530 (265)	528 (276)	1 (1, 1)	0.92	–	–
>5 log HIV VL before cART	59 (45)	1,546 (38)	1.34 (0.94, 1.91)	0.1	1.32 (0.92, 1.9)	0.13
AIDS-defining illness	67 (37)	1,466 (27)	1.6 (1.18, 2.18)	0.003	–	–
Nadir $CD4^+$ T-cell count ≤200 cells/mm^3	125 (70)	3,348 (62)	1.41 (1.02, 1.95)	0.04	1.42 (0.96, 2.12)	0.08
Previous mono or dual therapy	75 (42)	1,828 (34)	1.41 (1.04, 1.9)	0.03	–	–
Diabetes mellitus	7 (4)	201 (4)	1.05 (0.49, 2.27)	0.9	–	–
Psychiatric comorbidity	25 (25)	705 (22)	1.19 (0.75, 1.89)	0.46	–	–
Regular alcohol consumption[b]	52 (46)	1,968 (49)	0.88 (0.61, 1.28)	0.5	–	–
Mean number of past regimens (SD)	5 (3)	4 (3)	1.04 (0.99, 1.08)	0.1	–	–
cART regimen				0.009		–
NNRTI-based	51 (28)	2,148 (40)	Ref	Ref	Ref	Ref
Boosted PI-based	63 (35)	1,763 (33)	1.51 (1.03, 2.19)	0.03	1.45 (0.93, 2.29)	0.1
Unboosted PI-based	27 (15)	490 (9)	2.32 (1.44, 3.74)	0.001	3.58 (1.93, 6.64)	<0.0001
NRTI combinations[c]	19 (11)	484 (9)	1.65 (0.97, 2.83)	0.07	2.39 (1.2, 4.77)	0.01
Other drug class combinations[d]	19 (11)	504 (9)	1.59 (0.93, 2.71)	0.09	2.11 (1.04, 4.26)	0.04
Mean duration of treatment, years (SD)	2.7 (1.8)	3.1 (1.6)	0.81 (0.73, 0.92)	0.001	0.8 (0.68, 0.91)	0.001
HCV coinfection	9 (7)	369 (10)	1.37 (0.92, 2.06)	0.1	–	–
HBV coinfection	45 (46)	1,254 (38)	0.74 (0.37, 1.47)	0.4	–	–
Mean renal clearance, ml/min[e] (SD)	98 (31)	104 (31)	0.99 (0.99, 1)	0.03	–	–
Mean BMI, kg/m^2 (SD)	23 (4)	24 (4)	0.98 (0.94, 1.03)	0.42	–	–
TDM performed until last LLV value	32 (18)	617 (11)	1.68 (1.14, 2.49)	0.01	1.78 (1.081, 2.94)	0.02
Suboptimal adherence[f]	34 (25)	1,149 (24)	1.06 (0.71, 1.57)	0.78	–	–

Values are *n* (%) unless otherwise indicated. [a]Factors associated with persistent low-level viraemia (pLLV). [b]> Once per week. [c]15 lamivudine-zidovudine-abacavir; 1 lamivudine-zidovudine-tenofovir; 1 lamivudine-stavudine-abacavir; 1 zidovudine-didanosine-abacavir; 1 lamivudine-zidovudine. [d]Protease inhibitor (PI) with non-nucleoside reverse transcriptase inhibitor (NNRTI) and/or raltegravir, maraviroc, enfuvirtide. [e]Cockroft-Gault formula for estimating glomerular filtration rate. [f]≥ One dose omission per month. BMI, body mass index; cART, combined antiretroviral therapy; HIV VL, HIV viral load; IDU, injecting drug users; MSM, men who have sex with men; Ref, reference; SHCS, Swiss HIV Cohort Study; TDM, therapeutic drug monitoring.

To minimize the impact of VL detection threshold variations with time, we also examined pLLV patients with at least one VL value over the highest detection threshold (>50 copies/ml, *n*=153). The main difference was that this pLLV population had a higher rate of suboptimal adherence and more advanced HIV disease (see *Factors associated with pLLV among patients with at least one VL > 50 copies/mL* [Additional file 1]). Parameters potentially influencing cART pharmacokinetics, such as body mass index and renal function, were not associated with pLLV.

Genotypic resistance testing was performed during or after pLLV in 31/179 (39%) patients. Most patients (*n*=23) had at least a major PI-, NNRTI- or

Figure 2. Virological outcome of the patients with pLLV at 48 weeks



Virological outcome of the 155 patients for whom 48-week data were available, either following the last viral load (VL) measurement during the persistent low-level viraemia (pLLV) period or at the time of combined antiretroviral therapy modification. Patients are grouped according to VL value at the onset of pLLV. pVLLV, persistent very low-level viraemia.

NRTI-resistance mutation, four patients had minor PI-resistance and four patients had no resistance (see *Genotypic resistance data for the 31 patients undergoing resistance testing* [Additional file 1]).

Virological and immunological consequences of pLLV

In the pLLV group, 107/179 (60%) and 155/179 (87%) patients had virological follow-up data up to 24 and 48 weeks, respectively, after the last pLLV value and before any cART modification. At 48 weeks, 102/155 patients (66%) still had pLLV, while 19/155 (12%) presented VF and 34/155 (22%) had become undetectable (Figure 2). Predictors of VF were previous VF, unboosted PI-based or NRTI-only combinations and VL>200 copies/ml during pLLV (Table 2 and Figure 2). No patient with pVLLV (n=26) experienced VF up to 48 weeks after the last pVLLV value (Figure 2). Among patients with pLLV values of 50–200 copies/ml (n=93) and those with values of 201–400 copies/ml (n=36), 12% and 22%, respectively, experienced VF up to 48 weeks after the last pLLV value. In the bivariate analysis, diabetes was a predictor of VF. However, diabetic patients were more often on unboosted PI-based or NRTI-only combinations than non-diabetic patients, and this factor was not predictive in the multivariable model.

Regarding immunological outcome, the mean baseline CD4$^+$ T-cell count in pLLV patients was 532 (±SD 277). This increased at 48 weeks after the last pLLV value to 563 cells/mm^3 (±SD 133; P=0.01).

Treatment modification after last pLLV value

By 48 weeks after pLLV, 51/179 patients (29%) underwent cART modification (see *Timing of cART modification among patients with pLLV* [Additional file 1]). In the multivariable analysis, predictors of cART modification after pLLV were unboosted PI-based and NRTI-only cART, onset of pLLV after 2003, and particularly after 2008, and VL values >50 copies/ml since the first pLLV value (Table 3). Of pLLV patients in whom TDM was performed during the period of pLLV, those with lower drug levels (<25th centile) more often changed cART. However, patient numbers were low and this observation was not statistically significant (data not shown). The most common reason for cART modification, as documented in medical records by treating clinicians, was 'treatment failure' (29/51, 57%; see *Reason for cART modification as derived from patients' medical records and choice of new regimen* [Additional file 1]). Finally, regarding the choice of alternative cART, 22/51 patients (43%) had treatment intensification by the addition of one or more drugs from another antiretroviral class (see *Reason for cART modification as derived from patients' medical records and choice of new regimen* [Additional file 1]).

Virological outcome after treatment modification

Of the 51/179 patients who underwent cART modification by 48 weeks post-pLLV, virological follow-up was available at 24 weeks and at 48 weeks in 42/51 (82%) and 39/51 (76%) patients, respectively (see *Virological and immunological outcome in pLLV patients after cART modification* [Additional file 1]). By 24 weeks, 30/42 patients (71%) had VLs<20 copies/ml compared to 28/86 patients (33%) on unchanged cART (P<0.001). VF occurred in 2/42 (5%) post-change and in 10/86 (12%) on unchanged cART (P=0.3). By 48 weeks, 29/39 patients (74%) with modified cART had VLs<20 copies/ml compared to 19/74 patients (26%) without change (P<0.001). VF occurred in 0/39 patients (0%) post-change and in 7/74 patients (9%) on unchanged cART (P=0.09).

Discussion

We observed that patients with pLLV of 21–400 HIV RNA copies/ml were more often on unboosted PI-based and NRTI-only combinations than control patients. Progression to VF occurred in 12% and 22%

Table 2. Predictors of virological failure within 48 weeks after last LLV value

	Virological failure (*n*=19; 12%)	No virological failure (*n*=136; 88%)	Bivariate analysis[a]		Multivariable analysis[a]	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
SHCS centre of follow-up				0.01		–
A	7 (37)	51 (38)	Ref	Ref	–	–
B	1 (5)	27 (20)	0.27 (0.03, 2.31)	0.23	–	–
C	2 (11)	17 (13)	0.86 (0.16, 4.53)	0.86	–	–
D	7 (37)	10 (7)	5.1 (1.46, 17.76)	0.01	–	–
E	1 (5)	19 (14)	0.38 (0.04, 3.33)	0.39	–	–
F	1 (5)	7 (5)	1.04 (0.11, 9.77)	0.97	–	–
G	0 (0)	5 (4)	–	–	–	–
Diabetes mellitus	3 (16)	2 (1)	12.56 (1.95, 80.92)	0.01	–	–
HBV coinfection	6 (12)	2 (4)	3.47 (0.67, 18.04)	0.14	–	–
HCV coinfection	16 (41)	23 (59)	0.53 (0.22, 1.27)	0.15	–	–
Mean number of regimen changes (SD)	6 (4)	4 (3)	1.14 (1, 1.3)	0.06	–	–
Previous mono or dual therapy	9 (47)	56 (41)	1.29 (0.49, 3.37)	0.61	–	–
Virological failure in the past	15 (79)	52 (38)	6.05 (1.9, 19.2)	0.002	34.61 (3.79, 316)	0.002
cART regimen				0.04		–
NNRTI-based	2 (11)	45 (33)	Ref	Ref	Ref	Ref
Boosted PI-based	5 (26)	50 (37)	2.25 (0.42, 12.18)	0.35	1.93 (0.32, 11.62)	0.5
Unboosted PI-based	5 (26)	17 (13)	6.62 (1.17, 37.41)	0.03	12.79 (1.7, 96)	0.01
NRTI combinations	4 (21)	9 (7)	10 (1.58, 63.1)	0.01	115 (6.77, 1,952)	0.001
Other drug class combinations	3 (16)	15 (11)	4.5 (0.69, 29.56)	0.12	3 (0.4, 22.39)	0.3
Suboptimal adherence	8 (67)	32 (27)	5.31 (1.5, 18.86)	0.01	–	–
pLLV				0.03		–
21–49 copies/ml	0 (0)	26 (100)	Ref	Ref	–	–
50–200 copies/ml	11 (58)	82 (60)	Ref	Ref	–	–
201–400 copies/ml	8 (42)	28 (21)	2.81 (1.03, 7.63)	0.04	3.69 (1.12, 12.12)	0.03

Values are *n* (%) unless otherwise indicated. 179 patients with persistent low-level viraemia (pLLV): exclusion of 23 patients with combination antiretroviral therapy (cART) modification before any virological follow-up and exclusion of 1 patient without virological follow-up. [a]Factors associated with virological failure. LLV, low-level viraemia; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; Ref, reference; SHCS, Swiss HIV Cohort Study.

of patients with pLLV values of 50–200 copies/ml and 201–400 copies/ml, respectively, by 48 weeks after the last pLLV value, but in no patient with pVLLV. Among patients with pLLV, predictors of VF were previous VF, unboosted PI-based or NRTI-only combinations and VL≥50 copies/ml and particularly >200 copies/ml during pLLV. Treatment was modified in 28% of pLLV patients by 48 weeks and was influenced by cART regimen at pLLV onset and availability of new drug classes. Treatment modification led to significantly more pLLV patients becoming undetectable.

The association between unboosted PI-based and NRTI-only combinations, and pLLV and VF is consistent with previous studies showing lower potency of such regimens [28,37]. Our 48-week VF data are in the lower range compared to previous studies among pLLV patients, which report VF rates of 13–37%, depending on pLLV and VF definitions [10,12,23]. However, we observed that VF rate increased with pLLV value, notably in patients with VLs>200 copies/ml. The association between pLLV value and VF has been described in other studies, as has the association between VF and emergence of new resistance mutations. Laprise *et al.* [12] recently reported that, among 165 patients with pLLV of 50–999 copies/ml, all degrees of pLLV were associated with increased risk of VF (defined as a VL>1,000 copies/ml) and that cumulative VF incidence increased with VL. Taiwo *et al.* [26] observed among 31 patients followed over 24 weeks that new resistance mutations increased with VL once VL>100 copies/ml. Conversely, Delaugerre *et al.* [16] did not identify VL value as being predictive of resistance acquisition among 48 patients with VLs of 40–500 copies/ml during a 6-month period. The results of other studies are also controversial. Some conclude that pLLV predicts resistance mutation emergence and VF [10,16,38], while others describe no such association. Furthermore, cART intensification among pVLLV patients does not always reduce pLLV [17,18,25]. These contradictory results may be explained by the different pLLV definitions used and the different causative mechanisms, such as viral release from sanctuary sites or residual replication during suboptimal therapy [39].

Treatment modification was related to unboosted PI-based and NRTI-only combinations and to pLLV onset after 2003, particularly after 2008, and was

Table 3. Predictors of cART modification within 48 weeks after pLLV

Patient characteristics	Within 48 weeks after last LLV value: cART modification (*n*=51; 31%)	cART continuation (*n*=115; 69%)	Bivariate analysis[a]: OR (95% CI)	*P*-value	Multivariable analysis[a]: OR (95% CI)	*P*-value
Mean age, years (SD)	48 (12)	46 (9)	1.01 (0.99, 1.05)	0.24	–	–
Male sex	36 (71)	81 (70)	1.01 (0.49, 2.08)	0.98	–	–
Caucasian	42 (82)	96 (83)	0.92 (0.39, 2.21)	0.86	–	–
Mode of HIV acquisition				0.45		–
Heterosexual	25 (49)	45 (39)	Ref	Ref	–	–
MSM	18 (35)	45 (39)	0.72 (0.35, 1.5)	0.38	–	–
IDU	8 (16)	25 (22)	0.58 (0.23, 1.47)	0.25	–	–
SHCS centre of follow-up				0.02		–
A	23 (45)	39 (34)	Ref	Ref	–	–
B	3 (6)	23 (20)	0.22 (0.06, 0.82)	0.02	–	–
C	9 (18)	11 (10)	1.39 (0.5, 3.85)	0.53	–	–
D	10 (20)	12 (10)	1.41 (0.53, 3.78)	0.49	–	–
E	5 (10)	17 (15)	0.5 (0.16, 1.53)	0.22	–	–
F	0 (0)	8 (7)	–	–	–	–
G	1 (2)	5 (4)	0.34 (0.04, 3.09)	0.04	–	–
Year at inclusion				0.1		–
2000–2003	7 (14)	32 (28)	Ref	Ref	Ref	Ref
2004–2008	18 (35)	40 (35)	2.06 (0.77, 5.53)	0.15	5.1 (1.32, 19.66)	0.02
≥2009	26 (51)	43 (37)	2.76 (1.07, 7.16)	0.04	10.38 (2.63, 41)	0.001
Mean CD4+ T-cell count, cells/mm^3 (SD)[b]	516 (253)	561 (268)	1 (1, 1)	0.31	–	–
>5 log HIV VL before ART	17 (44)	38 (48)	0.85 (0.4, 1.84)	0.69	–	–
Nadir CD4+ T-cell count ≤200 cells/mm^3	40 (78)	79 (69)	1.66 (0.76, 3.6)	0.2	1.91 (0.8, 4.6)	0.15
Previous mono or dual therapy	21 (41)	49 (43)	0.94 (0.48, 1.84)	0.86	–	–
Diabetes mellitus	4 (8)	2 (2)	4.81 (0.85, 27.15)	0.08	–	–
HBV coinfection	2 (33)	4 (67)	1.13 (0.2, 6.46)	0.9	–	–
HCV coinfection	13 (31)	29 (69)	0.84 (0.34, 2.05)	0.7	–	–
Psychiatric comorbidity	4 (13)	19 (29)	0.35 (0.11, 1.21)	0.08	–	–
Regular alcohol consumption[c]	16 (46)	32 (44)	1.08 (0.48, 2.43)	0.85	–	–
Mean number of past regimens (SD)	4.4 (3.2)	4.6 (3.1)	0.98 (0.88, 1.09)	0.67	–	–
cART regimen				0.002		–
NNRTI-based	7 (16)	36 (31)	Ref	Ref	Ref	Ref
Boosted PI-based	17 (33)	44 (38)	1.99 (0.74, 5.32)	0.17	2.05 (0.73, 5.75)	0.18
Unboosted PI-based	12 (24)	13 (11)	4.75 (1.54, 14.66)	0.007	16.44 (3.64, 74.31)	<0.0001
NRTI combinations	12 (24)[d]	7 (6)[e]	8.82 (2.56, 30.3)	0.001	11.13 (2.85, 43.5)	0.001
Other drug class combinations[f]	3 (6)	15 (13)	1.03 (0.23, 4.52)	0.97	1.02 (0.22, 4.78)	0.98
Mean duration of treatment, years (SD)	3.4 (2.4)	2.5 (1.5)	1.3 (1.07, 1.54)	0.01	–	–
TDM performed during pLLV	8 (16)	21 (18)	0.83 (0.34, 2.03)	0.69	–	–
Low TDM, <25th centile	5 (63)	8 (38)	2.71 (0.5, 14.54)	0.25	–	–
Suboptimal adherence[g]	15 (34)	29 (31)	1.14 (0.53, 2.45)	0.73	–	–
HIV VL since first LLV				0.54		–
21–49 copies/ml	3 (6)	12 (10)	Ref	Ref	Ref	Ref
50–200 copies/ml	29 (57)	71 (62)	1.63 (0.43, 6.22)	0.47	1.51 (0.33, 6.89)	0.6
201–400 copies/ml	12 (24)	22 (19)	2.18 (0.51, 9.28)	0.29	2.95 (0.53, 16.33)	0.21
>400 copies/ml	7 (14)	10 (9)	2.8 (0.57, 13.75)	0.21	2.67 (0.42, 16.8)	0.3

Values are *n* (%) unless otherwise indicated. Exclusion of 13 patients who discontinued combined antiretroviral therapy (cART) or who were started on therapy for fitness. [a]Factors associated with cART modification. [b]At 48 weeks or at cART change. [c]> Once per week. [d]Non-nucleoside reverse transcriptase inhibitor (NNRTI) combination: 9 lamivudine-zidovudine-abacavir; 1 lamivudine-stavudine-abacavir; 1 zidovudine-didanosine-abacavir; 1 lamivudine-zidovudine. [e]NNRTI combination: 6 lamivudine-zidovudine-abacavir; 1 lamivudine-zidovudine-tenofovir. [f]Protease inhibitor (PI) with NNRTI and/or raltegravir, maraviroc, enfuvirtide. [g]≥ One dose omission per month. BMI, body mass index; HIV VL, HIV viral load; IDU, injecting drug users; LLV, low-level viraemia; MSM, men who have sex with men; pLLV, persistent low-level viraemia; Ref, reference; SHCS, Swiss HIV Cohort Study; TDM, therapeutic drug monitoring.

driven mainly by clinician perception of treatment failure. Continuing suboptimal therapy could be explained by the therapeutic options available at the time of the study. Continuing NNRTI-based regimens is contrary to recommendations proposing prompt NNRTI discontinuation when pLLV occurs,

to reduce drug-resistant mutant selection in this low genetic barrier treatment [29]. However, NNRTI-based cART was not associated with increased VF risk compared to boosted PI-based regimens. That patients on unboosted PI-based and NRTI-only combinations were more likely to change treatment was expected, given recommendations advising against these combinations because of inferior potency [2,28,37,40,41]. The recommendations were supported by the higher VF risk among patients on unboosted PI-based and NRTI-only combinations. The association between year of pLLV and cART modification probably reflects the availability of new antiretroviral drug classes.

It is noteworthy that, whilst the majority of patients who changed cART achieved undetectable VLs, cART modification did not affect VF rate at 24 weeks. By 48 weeks, however, VF rate was higher among patients on unchanged cART, lending support to the 2013 DHHS guidelines [29]. This underlines the importance of adequate follow-up when performing longitudinal studies.

Our study has limitations. We may have underestimated VF rate in pLLV patients as some underwent cART modification during the follow-up period and this decreased the length of pLLV follow-up. Second, the HIV RNA assay detection threshold changed over the study period, possibly leading to different pLLV populations over time. Third, there were few pVLLV patients so it is difficult to obtain statistically meaningful results for this sub-group. However, this highlights the difficulty in studying pLLV and pVLLV, as these events are uncommon: whilst our sample was small, it came from 9,972 potentially eligible patients. Against these limitations, our analysis spans over a decade of pLLV experience in a clinical setting representative of an entire country [42]. In addition, our study includes extended follow-up of patients with regular and frequent VL measurements, and reliable documentation of cART history, reported treatment adherence and other variables. In this way, our study adds to that of Laprise *et al.* [12] which was not sufficiently powered to analyse the effects of cART and treatment adherence on pLLV occurrence and its management.

In conclusion, we observe that clinicians were more likely to change patients on unboosted PI-based and NRTI-only regimens, and when new drug classes were available. We observed a strong correlation between pLLV levels of 201–400 copies/ml and VF. Treatment modification when pLLV was >200 copies/ml was associated with increased virological suppression and reduced VF by 48 weeks. Whilst a randomized prospective study to examine the impact of cART modification in pLLV patients could strengthen our findings, in practice, as pLLV is a rare event, a sufficiently-powered trial would require the collaboration of multiple national cohorts.

Acknowledgements

NB-B, KEAD, FS-A and MC contributed to study conception, study design, study performance, data analysis and manuscript writing. DV, MR, RF, EB, MA, OC, HF and HFG contributed to study conception, study design, study performance and manuscript review.

We thank all the patients of the SHCS as well as the study nurses, physicians and laboratories at each SHCS centre for making this study possible. We also thank Cristina Cellerai, Service of Immunology and Allergy, Lausanne University Hospital, Lausanne, Switzerland, for providing information on the viral load assays employed at the Lausanne centre during the study period, and Martin Rickenbach, SHCS, Lausanne centre, for providing information on the assays employed, and the limits of detection, at all other SHCS centres for the same time period.

This study has been financed within the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant number 148522, project number 677), and by the SHCS research foundation. The data are gathered by 7 outpatient clinics, 15 regional hospitals and 36 practitioners.

Members of the Swiss HIV Cohort Study: Aubert V, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of 'Positive Council'), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Nicca D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Centre), Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F, Schmid P, Schüpbach J, Speck R, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

Disclosure statement

The authors declare no competing interests.

Additional file

Additional file 1: Supplementary data can be found at http://www.intmedpress.com/uploads/documents/3275_Boillat-Blanco_additional_file_1.pdf

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Accepted 6 May 2014; published online 25 June 2014