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ORIGINAL RESEARCH

How reliable is an undetectable viral load?

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Objectives

An article by the Swiss AIDS Commission states that patients with stably suppressed viraemia [i.e. several successive HIV-1 RNA plasma concentrations (viral loads, VL) below the limits of detection during 6 months or more of highly active antiretroviral therapy (HAART)] are unlikely to be infectious. Questions then arise: how reliable is the undetectability of the VL, given the history of measures? What factors determine reliability?

Methods

We assessed the probability (henceforth termed reliability) that the $n + 1$ VL would exceed 50 or 1000 HIV-1 RNA copies/mL when the nth one had been <50 copies/mL in 6168 patients of the Swiss HIV Cohort Study who were continuing to take HAART between 2003 and 2007. General estimating equations were used to analyse potential factors of reliability.

Results

With a cut-off at 50 copies/mL, reliability was 84.5% ($n = 1$), increasing to 94.5% ($n = 5$). Compliance, the current type of HAART and the first antiretroviral therapy (ART) received (HAART or not) were predictive factors of reliability. With a cut-off at 1000 copies/mL, reliability was 97.5% ($n = 1$), increasing to 99.1% ($n = 4$). Chart review revealed that patients had stopped their treatment, admitted to major problems with compliance or were taking non-HAART ART in 72.2% of these cases. Viral escape caused by resistance was found in 5.6%. No explanation was found in the charts of 22.2% of cases.

Conclusions

After several successive VLs at <50 copies/mL, reliability reaches approximately 94% with a cut-off of 50 copies/mL and approximately 99% with a cut-off at 1000 copies/mL. Compliance is the most important factor predicting reliability.

Keywords: adherence, compliance, contagiousness, HAART, reliability, viral load

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Introduction

The Swiss Federal AIDS Commission's review of data concerning the contagiousness of patients treated with highly active antiretroviral therapy (HAART) concluded that patients with stably suppressed viral load (VL) on treatment were extremely unlikely to pass on their infection [1]. The Commission posited that viraemia be considered 'stably suppressed' if successive measures, spanning at least 6 months, had revealed VLs below limits of detection and if compliance with HAART was assured. This 'Swiss statement' has generated considerable controversy. One point of contention is the reliability of an undetectable VL. If an individual has an HIV-1 RNA level below the detection limits at point *x*, how about timepoint *y*, when the individual engages in sexual intercourse?

The Swiss HIV Cohort (SHCS), with its serial measures of HIV-1 RNA concentrations, provides a basis for testing the following theory: if a patient has an undetectable VL,

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what is the likelihood that the next VL will also be undetectable? By analogy, this likelihood also applies to sexual contact some time after a measured undetectable VL, a situation where real-time measurements are not practicable.

Patients and methods

Study population

We selected participants of the SHCS, a prospective, clinic-based, observational study of HIV-1 infected adults initiated in 1988 (www.shcs.ch) [2]. After written informed consent was obtained, detailed demographic, lifestyle and clinical information were collected. Follow-up visits took place approximately every 6 months. The project was based upon a sub-set of patients with several VL measurements on HAART, with at least one below 50 HIV-1 RNA copies/mL, between May 2003 and December 2007 ($n = 6168$). Patients with no VL measurement subsequent to the one with < 50 copies, and those VLs where SHCS records indicated that the patients had no treatment at the time of measurement, were not included (Fig. 1).

Assessments

We defined reliability as the probability that the $n + 1$ VL would exceed 50 or 1000 copies/mL when the n previous VL was < 50 copies/mL. The cut-off of 1000 copies/mL was chosen because no transmission has been shown below that value in patients who are not on treatment in discordant heterosexual couples and from mother to child [3,4].

The factors we considered as potentially predictive for detectable VLs were time-independent variables (sex, age at joining the cohort, mode of acquisition of HIV, sexual preference, ethnic origin, type of the first treatment received) and time-dependent variables (number of visits, treatment, CD4 cell counts and compliance). The type of treatment was determined at the time of the measurement of every VL. It was classified as nonnucleoside reverse transcriptase inhibitors (NNRTIs) + nucleoside reverse transcriptase inhibitors (NRTIs), NRTIs only, unboosted protease inhibitors (PIs) + NRTI, boosted PIs + NRTIs and other regimens. Adherence data were collected at the time of measurement of the VL with a window of 3 months. Adherence was categorized based on the 4 weeks previous to the patient interview using the following three classes: those who forgot more than one dose of antiretroviral therapy; those who forgot one dose; and those who never forgot any dose.

Patients who present high VLs (≥ 1000 copies/mL) after several successive undetectable values may be particularly relevant regarding contagiousness. We reviewed the patient files in Zurich, Bern and Geneva, representing 49% of the episodes of VLs ≥ 1000 copies/mL. Reasons for these high VLs were sought in the charts and categorized as: (1) stopping HAART; (2) lack of adherence; (3) no explanation found; and (4) no information available.

Statistical analysis

The observed probabilities for the VL to be < 50 or < 1000 copies/mL at time of the $n + 1$ test were assessed with the exact 95% confidence interval (CI) (Clopper–Pearson's method).

The probability for the VL to be > 50 copies/mL at time of the $n + 1$ test was also estimated using a logistic generalized estimating equations (GEE) model (with a non-structured correlation) to analyse the impact of factors such as the compliance and use of HAART as first treatment on the five first VLs. Firstly, a univariate analysis was performed. The main suspected factors introduced in the multivariate model were compliance, the type of the current HAART and the type of the first HAART. The confounding factors were the time interval between two successive VLs, the centres, the CD4 cell count and the age at first undetectable VL after 1 May 2003. Ethnicity, sex and mode of acquisition of HIV were not introduced in the model because the number of confounding factors was limited (convergence of the GEE model) and because these factors were not significant in univariate analysis. The odds ratio (OR) was assessed. Statistical significance was considered established if a P-value was < 0.05. The analyses were performed with SPPS version 15.0.1 (SPSS Inc., Chicago, IL, USA) and S-Plus 8.0 (TIBCO Software Inc., Palo Alto, CA, USA).

Results

Patient characteristics

A total of 6168 patients had at least one VL below 50 copies/mL after 1 May 2003 and an additional subsequent VL measurement while presumed receiving HAART. The selection of patients is detailed in Figure 1. Patients' median age was 41 years [interquartile range (IQR) = 36–48] and 69.2% were men. The mode of acquisition of HIV was heterosexual for 38.6%, homosexual for 36.6% and intravenous (IV) drug use for 20.6%. Most of the patients were White (82.4%); 65.3% had HAART as their first treatment. At the time of measurement of VLs, compliance information was available for 86.2%, of whom 79.8% claimed to have omitted no dose during the 4 weeks preceding the visit. A total of 2691 patients (43.6%) had a VL ≥ 50 copies/mL and 423 (6.9%) a

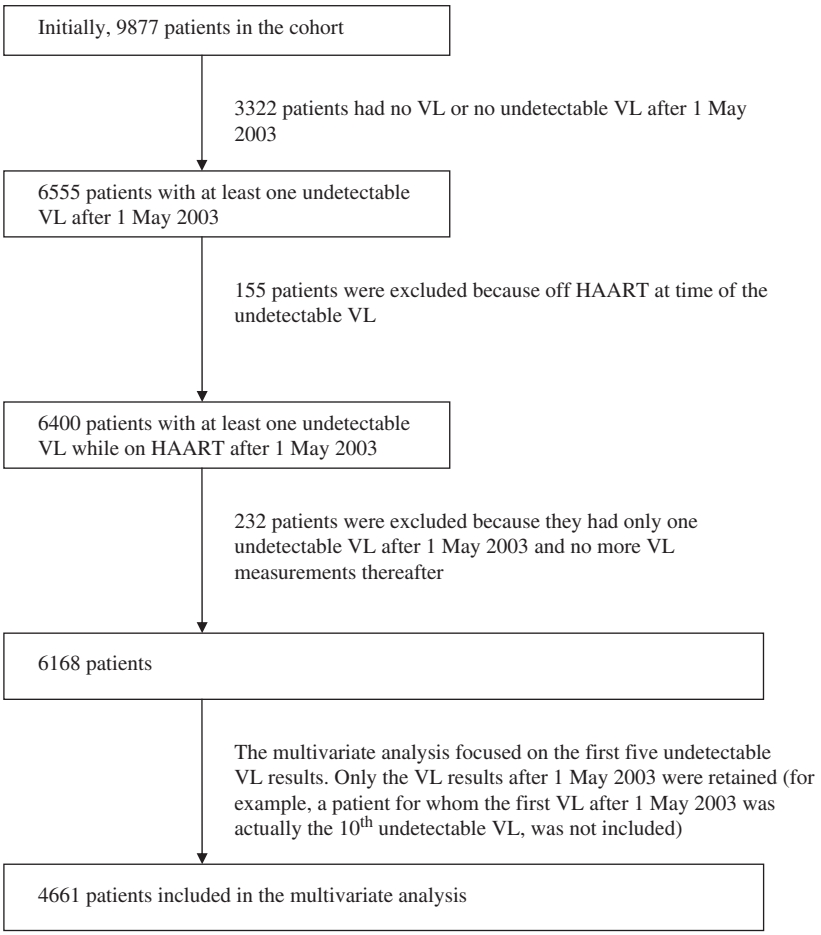

Fig. 1 Selection of patients from the Swiss HIV Cohort Study.

VL $\geq$ 1000 copies/mL (counting only the first detectable VL after 1 May 2003). The median time between two successive VLs was 93 days (range 1–1351 days, IQR 84–120). Among the 6168 patients, 4661 had at least one of the five first undetectable VLs after 1 May 2003. Their characteristics are shown in Table 1.

Cut-off at 50 copies/mL

The observed probability for VL to be undetectable at the $n+1$ test given that the n previous ones were undetectable increased until $n=5$ and appeared to be stable for $n>5$ (Fig. 2). It ranged from 84.5% (95% CI = 83.4–85.7) for $n=1$ to 94.5% (95% CI = 93.6–95.4) for $n=5$.

When more than one dose was missed in the previous 4 weeks, the observed probability was 69.8% (95% CI = 63.9–75.3) for $n=1$ (Fig. 3, top left panel). When the compliance was better, the probability was higher: 84.7% (95% CI = 81.1–87.8) if one dose was missed and 86.2% (95% CI = 84.7–87.5) if no dose was missed, for $n=1$. Whatever the compliance, reliability increased regularly until $n=5$. Values remained stable for $n>5$.

For $n=1$, patients with HAART as first treatment had better reliability: 86.4% (95% CI = 85.1–87.7) *vs.* 80.3% (95% CI = 78.0–82.3). The reliability increased until $n=5$ and reached 95.4% (95% CI = 94.4–96.) for patients with HAART as first treatment and 92.6% (95% CI = 90.6–94.2) for the other patients (Fig. 3, top right panel).

In the univariate analysis for risk of detectability, the following factors had a P-value greater than 0.05: sex ($P=0.32$), CD4 cell count ($P=0.14$), mode of acquisition of HIV ($P=0.08$) and ethnicity ($P=0.46$). The significant variables were the number of previous visits with an undetectable VL ($P<0.001$), the type of HAART ($P<0.01$), compliance ($P<0.001$), first treatment ($P<0.001$), time interval between two VLs ($P<0.001$), centre ($P<0.001$) and age at first undetectable VL after 1 May 2003 ($P<0.001$). In the multivariate analysis, the CD4 cell count ($P=0.11$) and

Table 1 Characteristics of the 6168 patients

Male	4271 (69.2%)
Age, years (median and IQR)	41 (36–47)
Weight, kg (median and IQR)	67 (60–75)
Mode of acquisition of HIV (%)	
Heterosexual	2379 (38.6%)
MSM	2255 (36.6%)
Intravenous drug use	1273 (20.6%)
Other	261 (4.2%)
Ethnic group	
White	5084 (82.4%)
Black	724 (11.7%)
Other	360 (5.8%)
First HAART	
Yes	4028 (65.3%)
No	2140 (34.7%)
First treatment	
NNRTI + NRTI	1233 (20.0%)
NRTI only	1965 (31.9%)
Unboosted PI + NRTI	2310 (37.5%)
Boosted PI + NRTI	508 (8.2%)
Other	152 (2.5%)

IQR, interquartile range; MSM, men who have sex with men; HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

age ($P = 0.18$) were no longer significant. Reliability increased with the number of previous undetectable VLs and was higher for patients with HAART as first treatment than for other patients. Good compliance (no dose or one dose missing in last 4 weeks) also increased reliability, as did the type of HAART: NRTIs showed better reliability than other types. As expected, the $n + 1$ VL was more correlated to the previous one when the time interval was shorter (see Table 2).

Of the first detectable VLs after VL < 50 copies/mL, 62.6% were 50–199 copies/mL, 15.8% 200–499 copies/mL, 5.9% 500–999 copies/mL and 15.7% above 1000 copies/mL. When the VL was detectable at 50–999 copies/mL, the following one was again undetectable in 69.4% of cases (which would define the detectable VL as a 'blip') [5–11]. When the VL was more than 1000 copies/mL, the following was undetectable in only 30.8% of cases.

Cut-off at 1000 copies/mL

The observed probability for VL to be > 1000 copies/mL at the time of the $n + 1$ test increased slowly until $n = 4$ and seemed to be stable for $n > 4$ (Fig. 2). It ranged from 97.5% (95% CI = 97.0–98.0) for $n = 1$ to 99.1% (95% CI = 98.6–99.4) for $n = 4$.

When more than one dose was missed in the last 4 weeks, reliability was 91.7% (95% CI = 87.7–94.7) for $n = 1$ (Fig. 3, bottom left panel). When the compliance was better, reliability was higher: 97.4% (95% CI = 95.5–98.7) if one dose was missed, and 98.2% (95% CI = 97.7–98.7) if no dose was missed.

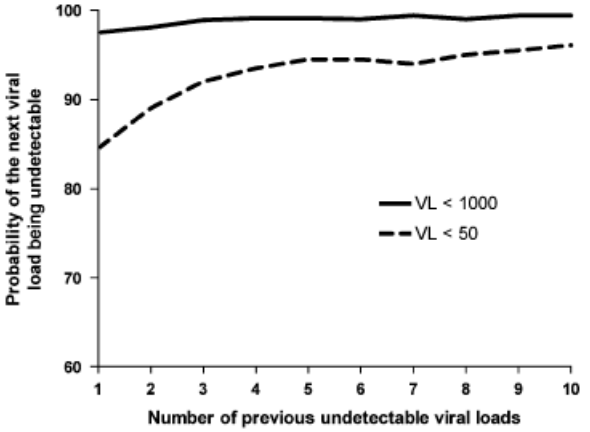

Fig. 2 Probability for the next viral load to be undetectable (test results with cut-offs at 50 and 1000 HIV-1 RNA copies/mL).

For $n = 1$, patients with HAART as first treatment had better reliability: 98.1% (95% CI = 97.5–98.6) *vs.* 96.3% (95% CI = 95.0–97.3). At $n = 4$, reliability reached 99.2% (95% CI = 98.7–99.6) for patients with first HAART and 98.7% (95% CI = 97.7–99.3) for other patients (Fig. 3, bottom right panel).

A VL ≥ 1000 copies/mL after ≥ 2 undetectable VLs is particularly puzzling, and potentially relevant regarding infectiousness. There were 325 such results in 311 patients. All relevant patient files (160 episodes, 49%) were reviewed in Geneva, Bern and Zurich. All these patients were recorded as still taking HAART in the SHCS database at the time of the $n + 1$ VL measurement. In four cases, the information regarding high VL was in error. Information on causes of high VL was completely lacking in 12 charts; in many of these cases, patients had missed appointments around the time of the VL measurements. This left 144 episodes with potentially useful information. In 55 of these (38.2%), patients had stopped their treatment for > 4 days before VL measurement; an additional 44 (30.5%) admitted to gross problems with compliance prior to measurement. Five patients (3.5%) were not on HAART. In eight patients (5.6%), the high VL was ascribed to viral escape with multi-resistant HIV. For the remaining 32 episodes (22.2%), no plausible explanation could be found.

Discussion

After several successive VLs at < 50 copies/mL, reliability reaches approximately 94% with a cut-off of 50 copies/mL, and approximately 99% with a cut-off at 1000 copies/mL. These conclusions are based on a large patient sample of

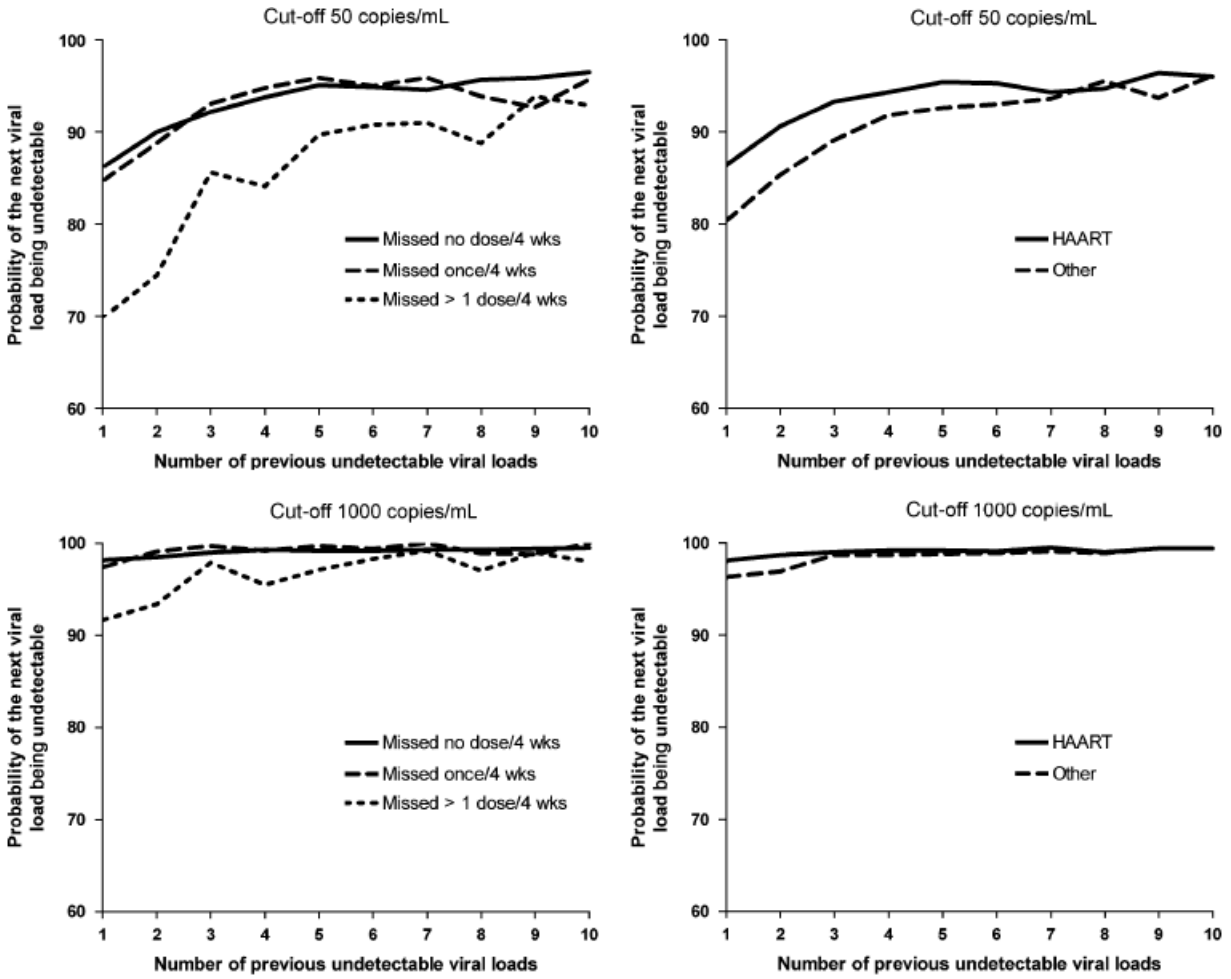

Fig. 3 Probability for the next viral load to be undetectable according to compliance and first treatment (test results with cut-offs at 50 and 1000 HIV-1 RNA copies/mL).

more than 6000 (representative of general medical practice) and the provision of more than 50 000 VL measurements. With a cut-off of 50 copies/mL at the $n + 1$ visit, there were 4661 patients with at least one detectable VL to analyse and to correlate with potential prognostic factors, yielding robust models and conclusions. Apart from the number of previous undetectable VLs, compliance is an important factor predicting reliability. The type of first antiretroviral treatment administered is also significant: reliability was lower in patients who started on NRTI mono- or bi-therapy in the 1990s, than in those who started with HAART. Our findings are in agreement with numerous other studies reporting that loss of virological control is more frequent in such patients [12–16].

At the cut-off of 50 copies/mL, we also found that reliability was significantly different depending upon the treatment centre. This centre effect was still apparent in multivariate analysis. However, it has little biological plausibility. On the contrary, we assume that it is caused by a lack of homogeneity in laboratory procedures, based on the following: (1) the centre effect disappears when the higher cut-off (1000 copies/mL) is used; (2) in centres with low reliability, a much higher proportion of detectable VLs reflects low values (< 200 copies/mL) than in centres with higher reliability; and (3) the centre effect disappears between 2003 and 2007, a time when more uniform procedures for extracting nucleic acids and measuring VL were introduced in Switzerland.

To interpret these results correctly, it is important to understand the procedures of the SHCS, including its limits. Compliance data depend on patient interviews. The question asked is: 'How many doses have you missed during the last 4 weeks?'. Reported compliance rates are high; it is possible that many patients tell their interviewer what they think he or she wants to hear, and overestimate their compliance. Cohort visits occur approximately every

Table 2 Univariate and multivariate analysis of the probability of the next viral load being undetectable (cut-off at 50 HIV-1 RNA copies/mL)

	Univariate analysis		Multivariate analysis	
Factor	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
Number of previous undetectable viral loads				
1	1.00	–	1.00	–
2	1.04 (1.02–1.06)	<0.001	1.03 (1.01–1.05)	0.001
3	1.08 (1.06–1.09)	<0.001	1.06 (1.04–1.07)	<0.001
4	1.09 (1.07–1.10)	<0.001	1.06 (1.04–1.07)	<0.001
First treatment				
Other	1.00	–	1.00	–
HAART	1.05 (1.04–1.07)	<0.001	1.03 (1.02–1.04)	<0.001
Current treatment				
NNRTI	1.00	–	1.00	–
Boosted PI	0.94 (0.92–0.96)	<0.001	0.95 (0.93–0.96)	<0.001
NRTI	0.95 (0.93–0.98)	<0.001	0.98 (0.96–1.00)	0.03
Unboosted PI	0.97 (0.96–0.99)	<0.001	0.98 (0.96–0.99)	<0.001
Compliance				
Missed >1 dose/4 weeks	1.00	–	1.00	–
Missed 0 or 1 dose/4 weeks	1.14 (1.10–1.17)	<0.001	1.11 (1.08–1.14)	<0.001
Interval between two successive VLs				
≤120 days	1.00	–	1.00	–
>120 days	0.96 (0.94–0.97)	<0.001	0.97 (0.96–0.98)	<0.001
Centre[†]				
Zurich, Lausanne, Bern	1.19 (1.16–1.22)	<0.001	1.18 (1.15–1.21)	<0.001
Geneva, Lugano, St Gallen	1.12 (1.09–1.16)	<0.001	1.13 (1.10–1.16)	<0.001
Basel	1.00		1.00	
Age at the first undetectable VL of the sequence (per 10 years of age)	0.99 (0.98–0.99)	<0.001	1.00 (0.99–1.00)	0.22
CD4 cell count (cells/μL)				
≤200	1.00	–	1.00	
>200 and ≤350	0.99 (0.97–1.01)	0.41	0.99 (0.97–1.01)	0.13
>350	1.01 (0.99–1.03)	0.49	1.00 (0.99–1.02)	0.91
Sex[‡]				
Women	1.00			
Men	0.99 (0.98–1.01)	0.31		
Mode of acquisition of HIV[‡]				
Heterosexual	1.00			
MSM	1.00 (0.99–1.02)	0.77		
Intravenous drug use	0.98 (0.96–1.00)	0.03		
Other	0.99 (0.96–1.02)	0.57		
Ethnic origin[‡]				
White	1.00			
Black	1.01 (0.99–1.03)	0.32		
Other	1.01 (0.99–1.04)	0.30		

[†]Centres were grouped according to their OR in an analysis introducing the centres and the number of previous undetectable VLs (Zurich 1.19, Lausanne 1.21, Bern 1.16, St Gallen 1.13, Geneva 1.13, Lugano 1.10, Bern 1.00).
[‡]Variables not introduced in the multivariate analysis.
MSM, men who have sex with men; HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; OR, odds ratio; CI, confidence interval.

6 months, when patients undergo a standardized interview and examinations. However, VL measurements typically accrue every 3 months. Therefore, the database contains many such measurements that cannot be correlated exactly with data on adherence. For instance, a patient may have an undetectable VL and cohort visit on 1 March and indicate truthfully that he was perfectly compliant with treatment during the 4 weeks preceding his visit. He may interrupt treatment on 15 May, and have a VL of 5000 copies/mL on 1 June. He may then start treatment again on 15 June, with undetectable VL and self-report of perfect compliance on 1 September. In such a case, the SHCS database would yield no explanation for the elevated VL on 1 June.

Therefore, we conducted a limited hospital chart review of patients with unexplained high VL (≥1000 copies/mL after ≥2 undetectable VLs). In 72.1% non-compliance, treatment interruption or insufficient treatment was indicated as the cause; in 5.6% resistance and viral escape was cited. This left 22.2% of episodes unexplained. This may be

an overestimate, because chart reviews many years after the event are not very informative. However, our data leave open the possibility that unexplained high VLs in seemingly well-controlled patients, although rare, can occur.

This possibility is well accepted for lower VLs (<1000 copies/mL), which accounted for 84.3% of the detectable VLs we analysed. When both preceded and followed by undetectable measurements (69.4% in our study), such low VLs are regarded as blips. Most studies have found no association between blips and virological or clinical failure [5,9–11,17–19]. Problems with compliance have been implicated as causative factors [5,17,18], corresponding to our own findings of a strong and highly significant association between adherence and a detectable VL above 50 copies/mL.

In conclusion, the reliability of undetectable VL depends on the number of previous measurements, continued compliance with antiretroviral treatment and having started such treatment with HAART. The first two factors were identified in the article by the Swiss AIDS Commission [1]. Our data leave open the possibility that unexplained rises in VL to ≥ 1000 copies/mL, although rare, may occur.

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