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NEWSLETTER

Low level viremia in antiretrovirally treated HIV-infected patients – a virological perspective

Patrick Braun & Eva Wolf

Ultimate goal of any antiviral therapy is eradication. In HIV-infection, however, this is not possible with currently available medications and treatment strategies. Hence, the primary goal remains maximal viral suppression with longest possible duration to allow stabilization of the immune system.

Since in most cases the HI-virus remains detectable with at least a few HIV-1 RNA copies/ml despite highly active antiretroviral combination therapy, national and international professional associations have defined clinical cut-off values for determining therapy success. (Table 1)

Definition of low level viremia

The cut-off between virological success and failure is currently defined as 50 HIV-1 copies/ml. This value specifically is derived from clinical studies. The German-Austrian antiretroviral treatment guidelines define success as a lasting decline in viral load to below 50 HIV-1 RNA copies/ml. A value <50 copies/ml should be obtained within three to four, and in cases of very high initial viremia within six, months of therapy initiation.

The clinical cut-off value should not be mistaken with the specific lower level of detection of the testing system employed, which tends to be lower than the clinically relevant cut-off. To date, there is no evidence suggesting an increased risk for therapy failure or resistance development with viral loads below 50 HIV-1 RNA copies/ml.

The evaluation of viral loads between 50 and 200 or 500 HIV-1 RNA copies/ml in patients on antiretroviral therapy is problematic. A review published in 2014 states that 4 to 8% of successfully treated patients (HIV-1 RNA < 50 copies/ml) develop a persistent low viremia in the range of 50-200 copies/ml. (1) Low level viremia tends to be more frequently observed with boosted protease inhibitor based therapy than with NNRTIs, yet in most cases of PI-based treatments it is not linked to therapy failure due to resistance. [1] The risk for developing resistance appears to also vary among integrase inhibitors (RAL or EVG/cobi

Table 1: Definitions of therapy success and failure used by national and international professional associations

Professional association (year of guideline)	Therapy success	Low level viremia	Virological failure
DAIG, Deutsche AIDS-Gesellschaft (2015)	<50 copies/ml	50 – 200 copies/ml (e.g. blip)	<2 log ↑ after 4 weeks or >50 copies/ml after 6 months; 2 x >50 copies/ml after suppression
DHHS, Department of Health and Human Services (2016)	VL<LLOD (<20-75 copies/ml)	LLOD to 200 copies/ml	2x >200 copies/ml
IAS-USA, International AIDS Society (2016)	<50 copies/ml (week 24)	50 – 200 copies/ml	2x >200 copies/ml after suppression
E**ACS,** European AIDS Clinical Society (2015)	<50 copies/ml (week 24)	50 – 1000 copies/ml	2x >50 copies/ml (after 6 months)

LLOD= lower limit of detection

> DTG). Viral load values within this low range can have numerous causes and are not necessarily due to therapy failure secondary to resistance.

Causes for low level viremia and their clarification

Numerous factors can be associated with a short-term elevation of viral load, e.g. a vaccination against influenza or an infection with treponema pallidum. Concurrent interacting medications or biological substances, such as St. John's Wort, may decrease antiretroviral therapy plasma levels and efficacy, resulting in an increase in viral load. Also suboptimal adherence to therapy may have this effect. [2]

It is important to recheck an HIV-1 RNA level of more than 50 copies/ml in a patient with a previously undetectable viral load within four weeks. Often this detectable viral load is a single occurrence, called a "blip", and is not reproduced.

An explanation for detectable transient viremia may lie in measurement fluctuations of the assay used. It has been found that measurements at the lower end of the linear spectrum of a quantitative viral load assay become less precise as variance increases. In comparison to the RealTime system by Abbott or the Versant-kPCR by Siemens, viral load measurements performed with the TaqMan System by Roche have slightly higher values as well as a broader degree of variation. [3, 4] (Table 2) These and other studies however do not allow conclusive statements on which system may be superior. It is important to note, that a switch in assay may result in measurements that are not necessarily comparable. Currently, inter- and intra-assay comparisons with newer testing systems are being performed.

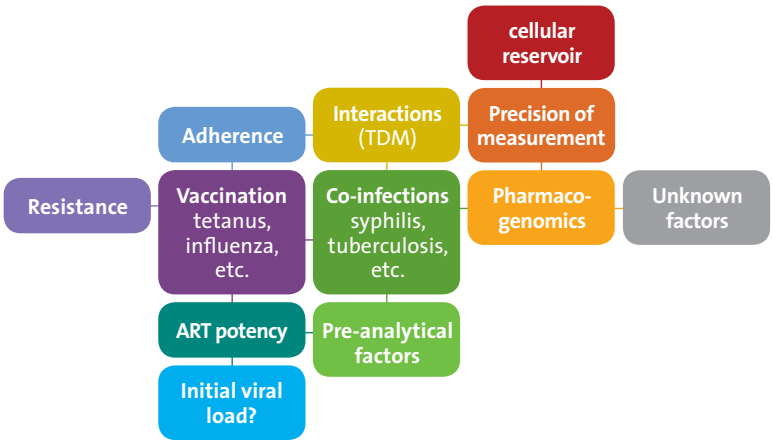
Another explanation for low viremia may lie in the preparation of the sample. The quantification of viruses detects RNA from HI-virions in EDTA plasma. If the EDTA blood sample is not centrifuged to obtain EDTA plasma within several hours or at the latest on the day of sampling, cell fractions with proviral DNA may be detected in plasma. Since some quantification systems isolate not only RNA but also DNA, such proviral DNA can serve as a matrix for PCR reactions and hence may provide false positive viral load results. False positive viral measurements can be avoided by processing the blood ideally within four hours and at least on the same day of sampling, and by performing sampling according to directions. [5]

Often one cannot explain low level viremia in antiretrovirally treated persons with potential causes including concurrent infection, vaccinations, suboptimal adherence or testing variations as listed in table 3. In such cases it is important to know more about the course of therapy. There is a difference, if a viral load never reaches undetectability and remains in the range of several hundred copies/ml after starting a new antiretroviral regimen, or if it becomes detectable after longer term viral suppression. The first scenario may indicate the use of a regimen with insufficient potency or duration to allow the current regimen to reach the maximal viral suppression. A decrease of viral load to below 50 HIV-1 RNA copies/ml may be then obtained through regimen change or intensification. Additionally, addressing adherence and drug monitoring are valuable to rule out other potential factors influencing potency, such as insufficient compliance, drug interactions

Table 2: Viral load in two clinical samples, measured in 10 independent runs with either HIV-1 RNA (m2000) RealTime (Abbott) or Cobas AmpliPrep/Cobas TaqMan HIV-1 v.2.0 (Roche) [3]

	Clinical sample <40 copies/ml (previous value measured with RealTime)		Clinical sample 80 copies/ml (previous value measured with RealTime)	
replicate	RealTime copies/ml	TaqMan v.2 copies/ml	RealTime copies/ml	TaqMan v.2 copies/ml
1	<40 (detected)	33	< 40 (detected)	58
2	<40 (detected)	50	66	110
3	<40 (detected)	57	75	112
4	<40 (detected)	59	78	125
5	<40 (detected)	63	80	127
6	<40 (detected)	70	85	177
7	50	101	91	209
8	57	103	102	214
9	62	110	113	241
10	83	155	137	295
Mean ± SD	–	**80.1**	**85.6 ± 28.8**	**166.8 ± 72.61**

Figure 1: Potential factors of low level viremia



as well as pharmacogenomic aspects including rapid metabolism.
In the second scenario, where viral load increases to > 50 HIV-1 RNA copies/ml after a phase of undetectability, one needs to investigate into potential therapy failure.
In both scenarios genotypic resistance testing is indicated. Resistance-associated failure in patients with persisting low level viremia is not uncommon. Persistent viral replication under the selective pressure of antiretroviral therapy usually leads to the evolution of more resistant HI-virus. The risk increases with increasing viral load and is hence higher in cases of 500 HIV-1 RNA copies/ml than it is in those with 100 copies/ml. Further, the risk of resistance is higher in regimes with a low barrier to resistance as seen for example with NNRTI-based combination therapy. Manufacturers' guidelines state that several hundred viruses per ml of plasma are needed to allow valid resistance testing. Accordingly, a larger sample volume needs to be drawn for an adequate result in patients with low level viremia. A comparison of nucleotide sequences from resistance analysis performed at different points in time may provide insight into viral evolution. If nucleotide sequences are identical, one can assume that the detected virus has not changed significantly with therapy. If nucleotide sequences differ reflecting differing viral populations, it cannot be determined whether this is due to evolving changes or if these viral populations originated from cellular reservoirs. [6] Detection of low level viremia in plasma does not necessarily indicate active viral replication. HIV-1 particles may be activated from latent reservoirs, for example through a (random) stimulation of antigens, and may not be able to replicate (only approximately 12% of infected cells house intact viral genome). [7]

A residual viremia with antiretroviral therapy appears to be associated with a higher immune activation, especially a higher CD8+ t-cell activation. It has been found that patients with viral loads between 50 and 200 copies/ml or viral blips had a larger proportion activated CD8+ cells (CD8+CD38+HLA-DR) than patients with viral suppression below 50 copies/ml. [8, 9] Certainly, it must be considered that in numerous patients with multi-resistant HI-viruses, viral suppression can no longer be reached despite novel therapy options. In these cases, the goal is to reduce viral load to the lowest level possible taking into consideration resistance patterns when making treatment choices.

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Table 3: Check-list and procedure in patients with low level

#	A) Characteristics and potential factors of low level viremia rocedure	Procedure
1	Blip	• repeat viral load measurement with a new blood sample drawn from the patient
2	possible mix-up of samples	• repeat viral load measurement with a new sample
3	pre-analytical factors, precision of measurement	• repeat viral load measurement following the specifications for pre-analysis (eg. EDTA-plasma separation) within 4 hours
4	suboptimal adherence	• check plasma drug concentrations
5	resistance	• perform genotypic resistance testing if low lever viremia is confirmed
6	concurrent infection	• repeat viral load measurement after the infection
7	vaccination	• repeat viral load measurement 4 to 8 weeks after vaccination
8	drug interaction(s)	• check plasma drug concentrations (trough concentrations)
9	pharmacogenomics (eg. rapid metabolizer)	• check plasma drug concentrations • if low and suboptimal adherence and drug interactions ruled out, check pharmacogenomics (eg. MDR-1 gene)
10	reduction in CD4 cell count	• underquantification of viral load? possibly repeat using a different assay • possibly rule-out HIV-2
11	increased cellular activation (CD8+CD38+ positive cells)	• rule-out other infections • monitor viral load closely
12	viruses derived from a cellular reservoir	• check viral evolution through comparison of nucleotide sequences from resistance tests performed at different points-in-time
	B) low viral load in various treatment scenarios	**Procedure**
I	No therapy	• if previously high viral loads, see #2 and #10 above
II	Initial therapy without viral suppression	• if therapy duration > 6 months, change or intensify treatment regimen • risk for developing resistance is higher in regimens with a low barrier to resistance • see #4, #5, #6, #8-#11
III	Viral load increase after viral suppression	• risk for developing resistance is higher in regimens with a low barrier to resistance • see #1-#13
IV	Salvage therapy	• evaluate low viral load in light of immune status • see #1-#13

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Herausgeber: InXFo GbR, Hirzstraße 17, 50937 Köln
Logistik-Team: Patrick Braun, Leonie Meemken, Eva Wolf
Technischer Support: Stefan Preis, Clinovate
Foto: Gunther Willinger