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The role of HIV-DNA testing in clinical practice

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SUMMARY

HIV-1 RNA levels and CD4+T lymphocyte counts are currently the standard markers used in clinical practice for the management of HIV infection. Nowadays it is also possible to monitor the evolution of HIV infection by measuring HIV-DNA. This measurement is a useful new clinical marker mainly been used to date in experimental evaluations. HIV-DNA can be detected in lymphoid tissues and in PBMC even during powerful and prolonged antiretroviral therapy. Understanding the HIV-DNA marker, together with all the other standard markers used in clinical practice, is now essential in monitoring the progression of the infection. Furthermore, the measurement of the levels of HIV-DNA in different stages could indicate the spread of the infection reflecting the ability of antiretroviral therapy to purge reservoirs. This review highlights the importance of evaluating the HIV-DNA load which could provide an indirect estimate of the quantity of reservoirs. This is an important factor in establishing the progression of infection, sequencing therapy and predicting the failure of antiretroviral therapy at a early stage.

KEY WORDS: HIV-DNA, Reservoirs, Viral marker

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INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) has not only dramatically decreased morbidity and mortality in AIDS, but it has also transformed HIV infection into a manageable chronic condition (Burton *et al.* 2005; Mocroft *et al.* 2000; Palella *et al.,* 1998).

The study of HIV-DNA load could be useful to improve the life-long treatment of HIV infection. HIV-disease requires frequent virological and immunological monitoring to detect early therapeutic failures and to follow up the evolution of resistant viral variants (Mellors *et al.,* 2007). The CD4+ T lymphocyte count and the HIV-RNA load have been the standard markers used in clinical practice for the management and monitoring of HIV-infected patients (Paolucci *et al.,* 2001).

The CD4+ T-cell count provides knowledge on the patient's immunological status and HIV-RNA load gives information on the degree of viral replication at the time of the assay. The levels of viral load (VL) could vary because of the influence of several factors linked to both the virus and the host such as viral fitness and immunoactivation. Until recently, no markers could be used routinely to quantify the viral load in HIV reservoirs. Now, the evolution and dynamics of HIV infection can be evaluated by measuring HIV-DNA load, a more reliable virological marker providing information on the amount of virus in host immune tissues.

ABBREVIATIONS

cART: combined antiretroviral therapy
HAART: highly active antiretroviral therapy
HAD: HIV-associated dementia
LTR: long terminal repeat
PIC: pre-integration complex
PI: protease inhibitor
RR: relative risk
VL: viral load

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Moreover HIV-DNA load may give an estimate on the number of infected cells, including CD4+ T lymphocytes, and on the size of the viral reservoir from which infectious virions can potentially be released (Pomerantz 2003; Re *et al.,* 2006). Persistence of a large number of latently-infected cells poses a major obstacle to the eradication of HIV infection by antiretroviral treatment (Finzi *et al.,* 1997). In fact, it is evident now that, even in HAART-treated patients with sustained viral suppression, HIV cannot be eradicated because of the presence of long-lived, infected memory CD4+ T cells representing a viral reservoir, and HIV-1 latency (Chun *et al.,* 1998; Dornadula *et al.* 2001). Secondly, it can be useful in clinical management in predicting very early failures and in sequencing the combined antiretroviral therapy (Dornadula *et al.,* 1999; Bell *et al.,* 2001).
While measuring the HIV-DNA load is now easier than in the past through the use of real-time PCR, there is no commercial test available and the assay has to be established in house and is therefore not easy to implement in a routine diagnostic setting.
This review highlights the potential role of HIV-DNA load in clinical practice taking into consideration the need to implement more standardized tests in routine diagnostic settings.

Different forms of HIV-DNA

Different types of HIV-DNA have been found in infected cells: linear integrated, linear and circular non-integrated forms of HIV-DNA are identified during a productive infection.
Following entry into host cells, the reverse transcriptase (RT) enzyme converts the viral genome from RNA into a linear double-stranded DNA (dsDNA) (Gomez and Hope 2005; Swiggard *et al.,* 2005).
Through the pre-integration complex (PIC), the newly-formed HIV-DNA will enter the nucleus but not all of it will become integrated into the host genome (Pauza *et al.,* 1994). In the nucleus the final product of the integration process will be the linear integrated HIV-DNA, also called HIV-provirus. HIV-provirus is fundamental to the transcription process and virus production, but it also gives stability to the retroviral genome integrated in the host cells. Depending on the state of infected CD4+ T cells, HIV-provirus could take two different pathways. It could be actively transcribed in them if they are activated or it could remain in a latent state if they revert back to a quiescent state and become memory CD4+ T cells (Robinson and Zinkus 1990). The stable, integrated and transcriptionally silent provirus is not affected by antiretroviral therapy.
The other forms of HIV-DNA can be considered side products and they are represented by linear and circular non-integrated forms distinguished by the presence of a long terminal repeat (LTR) sequence in a 1-LTR circle or 2-LTR circle. A huge quantity of non-integrated HIV-DNA in the nuclei of infected cells is generally believed to be the result of multiple infections (Bukrinsky *et al.,* 1991; Teo *et al.,* 1997).
In both resting and activated CD4+ T cells, non-integrated HIV-DNA represents the most prevalent form of HIV-DNA and a large amount of it can be detected in vivo. Moreover, most of HIV-DNA appears to be in non-integrated forms particularly in those patients with detectable VL in the plasma (Blankson *et al.* 2000; Sharkey *et al.,* 2000; Butler *et al.,* 2001).
Although these forms of HIV-DNA are labile intermediates, their detection could be indicative of a productive infection. Sharkey *et al.* suggested that the episomal 2-LTR form of HIV-DNA can be used as the marker of recent infection or ongoing replication in those patients with undetectable viraemia under HAART (O'Doherty *et al.,* 2002).
Although it is possible to distinguish between the integrated and non-integrated forms of HIV-DNA (Brussel *et al.,* 2005; Vandergraaff *et al.,* 2001; Alcamí *et al.,* 1995), so far there has been no standardized methods to measure the number of cells with integrated HIV-DNA.
Due to difficulties in different techniques, it still seems feasible only in a few major laboratories.
The recombination forms of non-integrated HIV-DNA cannot generate a productive infection but they can express the viral genes by being actively transcribed.
Their role in the pathogenesis of HIV infection is still unclear (Chen *et al.,* 1997). Transcription is promoted by the presence in LTR sequences of promoter and enhancer elements which present a high affinity for several cellular transcription factors, such as NF- B (Nabel and Baltimore 1987; Stevenson *et al.,* 1990). The role of these viral transcripts also remains unclear even though

their presence has been documented in vitro early after HIV infection (Stevenson and Haggerty *et al.,* 1990). However, Wu *et al.* suggested that they could play an important role in generating a productive HIV infection (Wu *et al.,* 2004).

HIV-DNA: reservoir establishment and persistence

Persistence of the virus depends on the long-term survival of a pool of infected cells called resting CD4+ T lymphocytes which constitute the HIV reservoir.

The frequency of latently infected cells is ~1 in 10^6 resting CD4 T cells. This reservoir is generated early during an acute infection by reversion of the infected CD4+ T cells into quiescent memory lymphocytes. These resting CD4+ T cells are transcriptionally silent but they can produce new infectious virions when activated again (Haggerty *et al.,* 2001; Brooks *et al.,* 2001; Chun *et al.* 1997). To date, the major obstacle to eradication of HIV infection is HIV-DNA archived in the life-long memory of CD4+ T lymphocytes (Wong *et al.* 1997; Zhang *et al.* 1999; Finzi *et al.* 1999). This reservoir could persist despite several years of undetectable viraemia under HAART (Siliciano *et al.* 2003; Strain *et al.* 2003).

Although the mechanisms of persistence remain unclear, the stability of this set of cells can be explained in the following ways (Sedaghat *et al.* 2008). First of all, both the long half-life of resting CD4+ T cells and the possibility of their self-renewal by proliferation may contribute to persistence of this pool of infected cells (Pierson *et al.* 2000). Secondly, the latently-infected cells with harboured transcriptionally-silent HIV-DNA seem to be protected from host immune responses and the cytopathic effects of the virus, thereby further increasing their chances of survival (Ramratnam *et al.* 2000).

Moreover, persistent low level replication or sporadic bursts of replication during HAART (e.g., due to occasional lapses in adherence) can also replenish the reservoirs in people who appear to be treated successfully (Chun *et al.* 2005). The residual viral replication could be responsible for continuous cross-infection between the resting and activated T-cell compartments and therefore for resetting the size of the HIV reservoir (Havlir *et al.* 2001). Finally, we could hypothesize that intermittent phases of viraemia called "blips" could also continuously refill the reservoir (Mira *et al.* 2002; Jones *et al.* 2007; Ramratnam *et al.* 2004).

Although eradication of HIV infection seems remotely possible with currently available drugs, new therapeutic strategies such as intensification (Persaud *et al.* 2000) and addition of new antiretroviral drugs to target the reservoirs could further reduce the levels of ongoing replication and the size of the HIV reservoir (Shen and Siliciano 2008).

One more good reason to study stably-integrated HIV-DNA in the infected cells is that we may consider it a large viral library containing all quasi-species, including the original wild-type and the resistant variant strains generated during suboptimal regimens (Ruff *et al.* 2002). Boni *et al.* analyzed HIV-1 drug-resistant variants in plasma and peripheral blood mononuclear cells in 56 therapy-naive patients with recent and chronic infection to assess the prevalence of mutations associated with drug resistance and compare cell-free and cell-associated strains. Their results showed a different resistance profile between plasma and PBMC compartments and the authors concluded suggesting the PBMC compartment be explored before of the first line of treatment (Bon *et al.* 2007).

There is a direct correlation between the number of integrated sequences of mutant viral strains and the exposure time to HAART in those patients with a history of suboptimal therapy or with cycles of discontinued therapy (Silva *et al.* 2006). Interestingly, in the absence of drug influence, resurgence of the wild-type is observed in patients with drug-resistant strains in resting CD4+ T cells and also in those with undetectable viraemia who have started structured interruption of antiretroviral therapy (Deeks *et al.* 2001). In fact, the wild-type presents better fitness than the mutant strains and therefore it replicates faster and more effectively. The importance of evaluating archived resistant strains was also confirmed by the French PRIMO cohort study in which virological failure was observed in three patients who, despite empirical HAART, presented resistant strains in the reservoir following primary HIV infection (Ghosn *et al.* 2006).

The archived drug-resistant strains represent a major restriction on treatment options. Further, in the absence of a universally-accepted method of determining different genotypes of the virus in

the reservoir, those drug-resistant strains may cause future failures, not only in patients with no stable control over viral replication in spite of the treatment, but also in new patients infected directly by mutant strains.

HIV-DNA: role in the monitoring of the therapeutic strategies

The study of HIV-1 DNA could be considered another important marker in clinical practice, especially in evaluating the sequencing of antiretroviral therapy in every stage of the HIV infection.

There are several treatment strategies that could benefit from an assessment of HIV-DNA including: primary infection, chronic infection with simplification or structured interruption strategies. In addition, HIV-DNA testing could also consider that similar considerations apply to patients with a complex treatment history, in whom the assessment of previously archived resistance may be helpful. In order to avoid the subsequent appearance of resistant viral strains and treatment failures, the exact time to begin each strategy must be established with the utmost care. Currently, based on the US Department of Health and Human Services guidelines, CD4+ T-cell count and HIV-RNA load are the two markers used to decide when to start treatment and how to monitor the effectiveness of HAART (DHHS Panel on Antiretroviral Guidelines for Adult and Adolescents 2009; Rosenberg *et al.* 2000). The persistence of HIV-DNA in resting CD4+ T cells emphasizes the usefulness of a new viral marker besides HIV-RNA load in the follow-up of HIV-infected patients.

Primary infection

HIV-DNA load could provide more information on the state of HIV-1 infection and the benefits of an early HAART since it has been demonstrated that low CD4+ T-cell count and high HIV-DNA load during primary infection lead to rapid progression to AIDS and increase in mortality (Goujard *et al.* 2006).

An important focus on HAART is the benefits of an early treatment during primary infection. The current guidelines still remain vague on the importance of prompt treatment in those patients with seroconversion <6 months earlier. Although early treatment appears to be effective in controlling viral replication, gain in CD4+ T-cell count and delay in disease progression to AIDS (Cossarizza *et al.* 2004; Strain *et al.* 2005), more data are needed to better address the issue on how truly tangible the advantages are. In this connection, HIV-DNA load could provide more information on the state of HIV-1 infection and the benefits of an early HAART. In their study, Garrigue *et al.* reported the different pathways that HIV-RNA and the HIV-DNA take in plasma and latently-infected cells in patients with primary infection under HAART.

The authors observed, in general, a gradual decrease in the levels of HIV-DNA and HIV-RNA in PBMC in patients with primary infection after 18-month follow-up with undetectable HIV-RNA in the plasma. In particular, HIV-DNA load decreased by 1 log and was detectable in 92.3% of patients whereas HIV-RNA load decreased by 3.6 log with a sharp decline in PBMC in the first 6 months followed by a plateau (Garrigue *et al.* 2000).

In our opinion, knowledge of the exact amount of HIV-DNA and monitoring the decay of HIV-DNA load after first line therapy could provide better data for selection of subsequent therapeutic strategies, such as simplification or interruption, than the values of CD4+ T-cell count and HIV-RNA load.

Chronic infection

The introduction of HAART in clinical practice has brought great benefits to patients with chronic HIV infection as shown by a drastic decline in opportunistic infections, hospitalizations, etc. (Mocroft *et al.* 2003).

However, HAART has also presented new challenges with regard to drug toxicity in the management of HIV-infected patients. As the immunological and virological benefits have to be weighed against the risks of prolonged exposure to potentially toxic drugs, it is never easy to establish when to initiate HAART. From HIV-DNA load new data could be extrapolated to clinical progression and it could help clinicians in making decisions on the right time to initiate HAART or to change therapeutic strategies. It was suggested before that HIV-DNA load be used as an independent marker of disease progression. In fact, Kostrikis *et al.* demonstrated that five years after infection patients who had started HAART with

CD4 + T-cell count <350 μL and HIV-DNA load <3 log_{10} copies/10^6 PBMCs presented a lower risk of clinical progression to AIDS than those with CD4+ T-cell count >350 μL and HIV-DNA load >3 log10 copies/10^6 PBMCs (Kostrikis *et al.* 2002). Rouzioux *et al.* showed in an elegant study that the risk of clinical progression to AIDS could be better described by HIV-DNA load rather than by other markers (Rouzioux *et al.* 2005). In fact, they reported that for 383 patients at the time of seroconversion the crude RR of clinical progression to AIDS was 5.80 for each 1-log_{10} increase in HIV-DNA load whereas it was 2.97 for each 1-log_{10} increase in HIV-RNA load and 1.25 for each 100-cell/μL decrease in CD4+ T-cell count. Perhaps an extended evaluation of CD4+ T-cell count, HIV-RNA load and HIV-DNA load in each patient could give the best estimate on both the state of viral infection and the risk of disease progression.

VL is not always useful in evaluating the risk of developing diseases linked to HIV infection. In a condition of undetectable VL, recent data in the literature show that approximately 10.1/100 person year developed HIV-associated dementia (HAD) (Shiramizu *et al.* 2007; Langford *et al.* 2006). This illustrates the virological and immunological status change in different compartments during HIV infection.

The possibility that monocytes/macrophages could harbour different forms of HIV-DNA increases speculation over the usefulness of this marker in evaluating the evolution of infection, including AIDS-related neurological conditions. The latest paper by Shiramizu *et al.* shows that the amount of HIV-DNA in peripheral blood mononuclear cells is correlated to the neurocognitive disorders.

In particular they conducted a longitudinal study to examine HIV-associated neurocognitive disorders in the Hawaii Aging with HIV Cohort that includes older >50 years old and younger 20-39 years old patients. In addition, the participants of the study were divided into: normal cognition, minor cognitive motor disorder and HIV-1 associated dementia. The levels of HIV-RNA and the CD4 cell count were similar in the three groups, whereas HIV-DNA levels differed significantly among all three groups. In particular, a correlation was found between circulating levels of HIV-DNA and HIV-1 associated neurocognitive disorders. To better understand this result and to exclude a possible correlation between HIV-DNA levels and HIV-RNA the measurement of HIV-DNA was repeated in patients with undetectable levels of HIV-RNA. The results confirmed that the levels of HIV-DNA are correlated to the neurocognitive status.

The authors emphasized that all patients took therapy with no differences in CNS penetration. These findings suggest that HIV-DNA has an important role in the pathogenesis of neurologic disorders and that it is a key marker in the follow up of HIV infection (Shiramizu *et al.* 2009).

Moreover, HIV-DNA load could give clinicians an opportunity to initiate HAART in those patients whose immune status falls in the so-called “grey zone”. There is a slower clinical progression to AIDS in those with CD4+ T-cell count <350 μL and HIV-DNA load <3.2 log than in the ones with CD4+ T-cell count >350 μL and HIV-DNA load >3.2 log. (Kostrikis *et al.* 2002). This result suggests that even in the presence of high CD4+ T-cell counts, HIV-DNA load of 3.2 log could represent the threshold at which HAART could be started. Knowledge of HIV-DNA load helps in evaluating the virological and immunological aspects of each patient necessary to establish the right moment to initiate HAART and also to better assess the future therapeutic scenario.

HIV-DNA load could also become a tool to predict the long-term success of HAART in naïve patients under the initial therapy, perhaps as a marker of virological failure in those with initial viral response to therapy. Several studies indicated that the quantification of different forms of HIV-DNA in PBMC could provide information on the prolonged effectiveness of HAART. In particular, a small follow-up study on 51 patients indicated that HIV-DNA load was the only predictive parameter of virological rebound in the group of non-responders.

In fact, baseline HIV-DNA load was higher in the patients with virological failure to HAART than in those with sustained undetectable viraemia under HAART. The total decay of integrated HIV-DNA and non-integrated 2-LTR HIV-DNA forms could be considered complete success of the antiretroviral therapy in those patients with persistent undetectable HIV-RNA load under HAART (Hatzakis *et al.* 2004; Gillim-Ross *et al.* 2005; McDermott *et al.* 2005; Havlir *et al.* 2005). In ad-

dition, HIV-DNA load was found to be the only predictive marker of residual viraemia in HAART. We may hypothesize that HIV-DNA load gives indirect information on the capacity of the virus to infect new cells which contributes to persistence of the HIV latent reservoir (Pellegrin *et al.* 2003). Decrease in HIV-DNA load ought to be considered the cornerstone of therapeutic sequencing. In fact, lower HIV-DNA loads are predictive of sustained undetectability of plasma viraemia in those patients switching from a protease inhibitor (PI)-based regimen to simplified therapy (d'Ettorre *et al.* 2007; Viard *et al.* 2004; Burton *et al.* 2005).

This therapeutic strategy could be a safe and attractive option to certain groups of patients under certain circumstances because it not only improves adherence but also reduces the toxicity of antiretroviral drugs.

It was also observed in the structured treatment interruption studies that higher HIV-DNA loads at the beginning of interruption of drug intake was later reflected in faster increases in HIV-RNA load as well as HIV-DNA load with a loss of previous virological gains obtained with HAART (Lewin *et al.* 2008). HIV-DNA load can be useful as a prognostic factor of long-term CD4+count-guided interruption of antiretroviral treatment. Sarmati *et al.* found a virological and immunological score associated with treatment interruption of more than 180 days that include four parameters: nadir CD4 cell count >269 cells/mmc, baseline CD4+ cell count >675 cells/mmc, baseline proviral HIV-DNA levels of <323 cp/10^6 PBMC and CD4+cells count at 14 days of interruption >564cells/mmc. They conducted a pilot prospective, open label, multicenter trial comprising 62 HIV-seropositive patients who decided to interrupt therapy after two or more years of successful HAART and observed at multivariate regression analysis that HIV-DNA level of 323 cp/10^6 PBMC and a CD4 count at day 14 after interruption more than 564 cells/mmc were independently associated to a reduced risk of restarting therapy. In addition they found that patients with a nadir of CD4 cell count >269 cell/mmc at baseline, proviral HIV-DNA levels >323 cp/10^6 PBMC and CD4 at 14 days after interruption, >564 cell/mmc were more likely to remain drug-free for a long period of time (Sarmati *et al.* 2009).

In addition, monitoring the HIV-DNA load could also be useful to obtain information on the consequence of the HAART interruption on viral reservoirs. The study conducted by Palmisano *et al.* on 37 HIV positive subjects enrolled in the Istituto Superiore di Sanità-Pulsed antiretroviral Therapy (ISS-PART) clinical trial compared 24 months of intermittent (arm B) versus continuous (arm A) HAART in chronic HIV infection. The authors found that fixed-time treatment interrup-

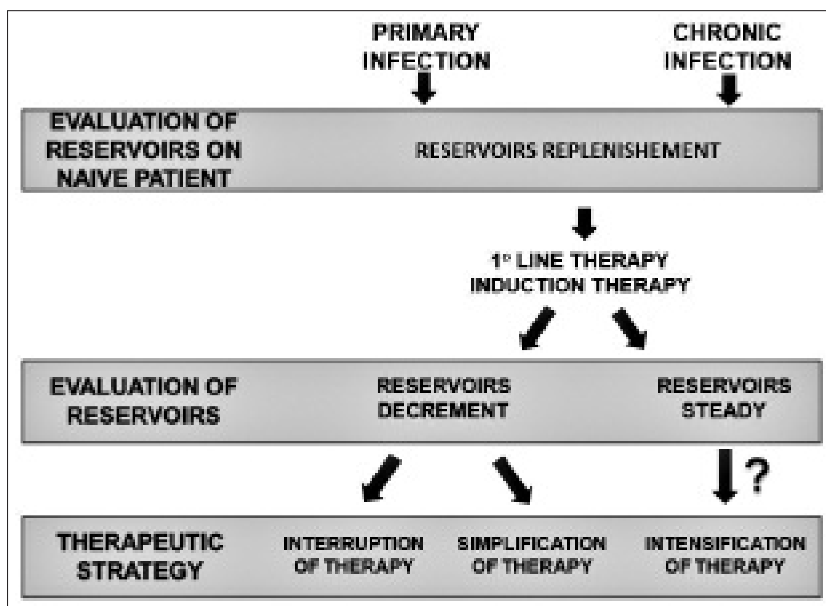

FIGURE 1 - *Model of potential uses of HIV-DNA in the clinical management of HIV infection.*

tion in patients who had reached a steady-state level of HIV-DNA has a negligible effect on total HIV-1 DNA and on the number of provirus infected cells. These considerations suggest that the number of HIV-1 DNA copies corresponds to the number of both resting both activated infected cells and thus the value of HIV-DNA can be consider a marker of viral burden (Palmisano *et al.* 2008).

It is not uncommon to witness under HAART some important instances in which patients have several blips of viraemia and show no sign of gain in CD4+ T-cell count.

Under these circumstances, a crucial decision on either changing or intensifying the therapy needs to be made to avoid accumulation of resistant viral strains and useless administration of new drugs. However, this kind of patient is not defined as a complete failure to the therapy. In such cases, HIV-DNA load can provide an indication on whether to change or intensify the current antiretroviral therapy.

Intensification of the therapy in patients with several viral blips helps us to reach a decay of latent cells in the HIV reservoir, thereby reducing the state of transient viraemia (Jones and Perelson 2007). Altogether these data clearly substantiate the importance of observing progressive levels of HIV-DNA during the management of different phases of HIV infection.

Therefore, HIV-DNA load provides clinicians with a broader panorama of data previously not available with CD4+ T-cell count and HIV-RNA load which only give information on rapid and partial changes. The values of this new viral marker at different times in the clinical history of an HIV-infected patient should be integrated with the previous clinical data for better evaluation of the disease and for better control of the therapeutic efficacy.

HIV-DNA load is an important marker on which the management of antiretroviral treatment for each HIV-infected patient should be based. Evaluation of this marker at different stages in the history of infection, and consequently information on the size of HIV reservoir, can help clinicians to choose the best strategies and to control the progression of HIV infection (Figure 1). In addition, the decrease in HIV-DNA load would facilitate patients in optimizing various therapeutic strategies such as intensification, simplification and structured interruption of therapy. In future, this research tool should become a part of clinical practice in refining therapeutic strategies.

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