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Episodes of transient HIV viraemia (blips) in HIV-multi-experienced patients: the role of adherence

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ABSTRACT

Patients under combined antiretroviral therapy (cART) can experience unexplained episodes of transient HIV-RNA<1000 copies/ml preceded and followed by undetectable viraemia. The commonest hypothesis for blips is that are due to reduced adherence. It is not clear whether blips predict future virologic failure. Forty-five HIV-infected experienced patients with episodes of blips under optimal cART for at least 48 weeks were studied for level of adherence, CD4+, HIV-RNA, genotyping mutations and levels of adherence. All patients had been heavily pretreated. Good or optimal adherence was reported for all patients. The mean number of viral blips was 1.89. No statistical association was found between the number of blips and adherence scores. In this study patients with history of multiple blips presented a 28.8% risk of virologic failure despite good or optimal levels of adherence. Levels of adherence cannot always explain transient relapse of viraemia and other factors probably sustain blips. In addition, some patients with blips experienced a subsequent virologic failure despite good or optimal adherence. Changing therapy may be a prudent strategy for preserving future therapeutic options in experienced patients with blips

Introduction

If the goal of combined antiretroviral therapy (cART) is maximum suppression of HIV-RNA levels, virologic failure can be defined as the inability to achieve or maintain suppression of viral replication to levels below the limit of detection (<50 copies/mL). Suboptimal response to therapy is often associated with immunologic failure and/or clinical progression. The exact moment in which therapy should be changed when a patient became viraemic is not clear. There is no consensus latest Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents [DHHS, 2008] and debate is ongoing on the best therapeutic strategy. Some advise a change in therapy for any repeated detectable viraemia, whereas others advocate a less aggressive modality of action and think that there should be repeated values of viral load above 1000 copies/ml before switching therapy. In addition, there is a condition in which a patient under cARV can show intermittent isolated or multiple episodes of detectable low levels of HIV-RNA which are preceded and followed by undetectable viraemia. These intermittent episodes of viraemia usually are defined as viraemic "blips". Attainment of undetectable HIV-RNA after a change in therapy or episodes of high level or persistently detectable viraemia are not considered "blips". Isolated episodes of low level of viraemia may simply represent laboratory variations or, especially when more than 300 copies/ml, an actual rebound of HIV viral load. Many factors increase the likelihood of blips, first and foremost reduced adherence [Jones and Perelson, 2005; Podsadecki et al, 2007].

Other explanations given for blips include co-infections by opportunistic organisms, pharmacokinetic factors like absorption and metabolism of drugs, individual characteristics (enzymatic inter-individual variability), interactions with other medications and concomitant illness. Transient activation of the immune system by infectious agents may account for unexplained episodes of transient viraemia or viral blips. Random fluctuations around a mean viral load less than 50 copies/ml or statistical assay variations are other claims [Marcias et al., 2005; Jones and Perelson, 2005; Jones and Perelson, 2007]. Multiple reasons for transient relapse of viraemia may probably be present in the same patient.

Ongoing viral replication could increase the risk of drug resistance and permit the selection of resistant mutants but data on the real consequences of blips are contradictory. Generally speaking, Blips are not considered predictive of subsequent failure and should not be an indication for switching the cART regimen [Havlir et al., 2001; Mira et al., 2002; Sklar et al., 2002; Nettles et al., 2005; Di Mascio et al., 2005; Macias et al., 2005; Sungkanuparph et al., 2005; Lee et al., 2006].

This study examined the relation between transient rebound of HIV viraemia, treatment adherence,

resistance episodes and risk of treatment failure in HIV-multi-experienced patients showing blips under cART.

Patients and methods

Forty-five HIV-infected patients with at one or more episodes of transient viraemia or "blips" under optimal antiretroviral treatment for at least 48 weeks were studied. According to the literature "blips" were defined as levels of HIV-RNA 300-1000 copies/ml alternating to a value under 50 copies/ml. Patients with detectable viraemia <300 copies/ml were not enrolled in the study to exclude possible laboratory variations of HIV-RNA. All patients had been heavily pretreated and showed mutations in genotypic resistance tests performed before the introduction of the current antiviral therapy. All patients enrolled gave their informed consent to blood sampling and processing.

Laboratory monitoring: Patients were studied for antiretroviral drug resistance mutations in plasma, CD4+ cell count, HIV-RNA plasma levels and adherence to antiretroviral therapy.

The enumeration of CD4+ and CD8+ lymphocyte numbers was assayed in blood collected in EDTA-containing tubes; two-colour flow cytometric analysis was performed with the Becton Dickinson FACScan flow cytometer (Becton Dickinson, San Jose, CA). HIV-1-RNA levels were measured at -70°C stored plasma prepared from blood obtained in EDTA-containing tubes using a quantitative reverse polymerase chain reaction (Amplicor HIV Monitor; Roche Diagnostic Systems, Branchburg, NJ). The limit of detection was 50 copies/ml.

Adherence was assessed by self-reports. We reviewed adherence questionnaires submitted to each patient. The questionnaire covered medication use, particularly the name, dose and frequency of all antiretroviral drugs. We defined the percentage of prescribed doses reported taken over the previous seven day interval as the main measure of adherence.

Patients were classified as "highly adherent" if the questionnaires reported that they had taken 100% of prescribed doses. Patients were classified as "moderately adherent" if self-reports showed that they had missed no more than 15% of prescribed doses of therapy (85-99% adherence). Finally, patients who had taken less than 85% of their prescribed doses of therapy were defined as "non-adherent" (0-85% adherence).

Statistical analysis: Descriptive statistics were used to describe the sample. The chi square test was used to compare categorical variables, and Student's test for continuous variables. Univariate analysis was performed to examine the relation with adherence level by computing analysis of variance. All statistical analyses were performed using SPSS software programs (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics: Among the study population 32 patients were male (71.1%) and 13 were female (28.9%). The age (mean±SEM) of patients included in the analysis was 36.7±1.4 years. The main mode of HIV transmission was heterosexual exposure (44.4%); no patient was an active drug abuser.

Immuno-virological findings: Nadir level of CD4+ cells was 150.4±30 cells/mmc (mean±SEM). HIV-RNA zenith was 220900±29420 copies/ml (mean±SEM). CD4+ cell count at the start of current therapy was 236.9±37.9 (mean±SEM). The mean number of viral blips was 1.89±0.95 (mean±SEM) and the mean HIV-RNA at the blips was 649.5±29 copies/ml (mean±SEM). Differences between adherent and moderately adherent patients are shown in Table 1.

Antiretroviral regimens: All patients had been heavily pretreated: during a mean treatment period of 60.7 months patients received a mean of 7.2 antiretroviral drugs with a range of six to nine drugs. The pharmacologic history of this cohort showed that 66.6% of patients had received non nucleoside reverse transcriptase inhibitors (NNRTI) and 77.7% were exposed to protease inhibitors (PI). All patients had a history of virologic failures and showed mutations in genotypic resistance tests of performed before the introduction of latest guided antiretroviral therapy. The pharmacological history of two groups of patients with different levels of adherence did not show statistical differences. Months on current cART were 20±4.8 (mean±SEM): all patients took an antiretroviral regimen based on PI.

Table 1. Differences between adherent and moderately adherent patients with blips

	"Highly adherent" 29 patients (mean)	"moderately adherent" 16 patients (mean)	P
CD4+ nadir (cell/mmc)	165	129	0.562
HIV-RNA zenith (copies/ml)	222600	216600	0.929
Months on current cARV	21.2	18.3	0.218
Number of blips	1,8	2	0.579
HIV-RNA at blips (copies/ml)	614	714	0.097

Genotypic resistance mutations: After 12 months of follow-up after the last blip we observed virologic failure in only 13 patients. At the time of failure new PR plasma mutations were present in 53.8% of patients, whereas all patients presented new NRTI plasma mutations. There was no statistical association between the number of virologic blips and the number of new mutations at the time of failure. There was no evidence of significant differences between adherent and moderately adherent patients.

Adherence scores: Self-reported adherence data were available for all patients. At enrolment, 100% adher-

ence was reported in 29 (64.4%) of the 45 participants in the study ("highly adherent"); 80-99% adherence was calculated for 16 (35.6%) patients ("moderately adherent"). No statistical association was found between the number of virologic blips and adherence scores.

Discussion

Adherence to antiretroviral therapy is considered a key factor in maintaining therapeutic drug levels and virologic suppression, reducing the risk of drug resistance. Poor adherence to antiretroviral therapy has major consequences for patients with HIV infection, including failure to prevent viral replication, increased risk of developing viral resistance, development of clinical complications, and shortened survival. Although optimal or good adherence may be a key factor for virologic success, it may not be sufficient to obtain a continuous viral load under 50 copies/ml.

Between sustaining undetectable viral load and virologic failure of therapy there exists a poorly understood clinical situation described as viral blip. Despite continuous HIV-RNA lower than the level of detection, a number of patients experience unexplained episodes of transient viraemia. The commonest reason claimed for blips is a reduced compliance with the prescribed regimen [Jones and Perelson, 2007].

Adherence in this study on 45 patients with viraemic blips showed a 100% adherence in 29 and only 16 patients had a level of adherence of 85-99%. By convention, an acceptable level of adherence to therapy is estimated as more than 80% of prescribed doses [Miller and Hays, 2000; Braithwaite et al., 2006; Bangsberg, 2006]. In addition, undetectable viral loads were observed in patients with 80-95% range of adherence [Gross et al., 2000]. No statistical association was found in our study between adherence scores, number of virologic blips and number of new resistance mutations at failure. These data confirm that adherence levels do not always explain transient relapses of viraemia and that other factors are also likely to sustain viral blips.

Although adherence is crucial to obtain a complete immuno-virologic success during cARV and prevent the risk of new mutations, the relation between adherence and resistance to antiretroviral therapy is more complex than the claim that non-adherence increases the risk of drug resistance. Recent data indicate that every antiretroviral regimen has a unique adherence-resistance relationship. One study reported that 23% of drug resistance mutations occur in individuals in the top quintile of adherence (92-100%) and more than 50% of all drug resistance mutation are observed in 40% of patients with a range of adherence of 79-100% [Bangsberg et al., 2003].

Selection and accumulation of drug resistance during variable periods of low viraemia has been demonstrated [Parkin et al., 2000; Verhofstede et al., 2007] but there is an open debate on the real impact of blips on accumulation of new genotypic mutations that could contribute to future virologic failure. Some authors do not attribute clinical importance and prognostic significance to blips as transient episodes of detectable viraemia were not associated with virological failure in some cohort studies [Havlir et al., 2001; Mira et al., 2002; Sklar et al., 2002; Nettles et al., 2005; Di Mascio et al., 2005; Macias et al., 2005; Sungkanuparph et al., 2005; Lee et al., 2006; García-Gascó et al., 2008].

By contrast, the present study showed that patients with a history of multiple blips had a 28.8% risk of virologic failure despite good or optimal levels of adherence. It is important to emphasize that our cohort did not include patients with HIV-RNA <300 copies/ml unlike other samples, and it could be useful to exclude simple laboratory variations. A major difference between this population and other cohorts is that all subjects enrolled in this study were multi-experienced patients and presented resistance mutations at baseline. This may contribute towards a predisposition for subsequent virologic failure despite the small number of new mutations and to disclose a risk profile in patients with blips.

Our study did not confirm that new genotypic mutations observed at the time of failure were selected during blips, but intermittent episodes of low viraemia chronologically anticipated sustained viral rebound, indicating that blips are a risk factor for the emergence of drug resistance. This was confirmed by a recent paper demonstrating that drug resistance mutations can be selected during viral rebound not exceeding 1000 HIV-RNA copies/ml [Verhofstede et al., 2007].

One captivating explanation for the relation between transient rebounds of viraemia and virologic failure is that some blips might be due to the release of virus from cellular reservoirs where drugs do not penetrate and active replication could be taking place. On the other hand, transient rebounds of plasma viraemia are frequent among patients under cART and may be responsible for the continuous emergence of resistant viral strains with consequent replenishment of latent reservoirs. There is an inverse correlation between the decay of the reservoir and residual viral replication under cART.

The variable penetration of antiretroviral drugs into sanctuary sites may contribute to the differential evolution of human immunodeficiency virus and the emergence of drug resistance. The effects of HAART are usually assessed on blood samples, although several anatomical compartments have been reported as viral reservoirs. Viral evolution in these sanctuaries may differ from that in plasma, and resistance profiles discordant from those in plasma have been described. Inadequate drug penetration in reservoirs contributes to viral replication, resistance selection, and a subsequent risk of failure to control the virus in plasma.

Conclusions

Although it is well known that the goal of antiretroviral treatment is to maintain HIV-RNA at levels less than 50 cp/ml, data on the pathogenesis and role of intermittent viraemia in the development of drug resistance and treatment failure are scant and contradictory.

We can speculate that inadequate adherence is not the only key factor in the development of resistance mutations and that blips are a risk factor for virologic failure in multi-experienced patients. Moreover virologic failure was found in multi-experienced patients with viraemic blips and good adherence to therapy. Episodes of transient low viraemia probably increase the risk of failure in patients infected by HIV resistant strains despite the prescription of a guided ART. Changing therapy on the basis of resistance tests is a prudent strategy for preserving future therapeutic options in multi-experienced patients with blips.

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