Plasma HIV-1 copy number and in vitro infectivity of plasma prior to and during combination antiretroviral treatment

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Abstract

Some studies on untreated patients have shown a general correlation between plasma HIV copy number and plasma infectivity in in vitro models. Recent observations also indicate that HIV-RNA level is an important predictor of perinatal transmission and may also have a role in heterosexual transmission. To further analyse the correlation between HIV viral load and plasma infectivity, we studied the relationship between HIV-1 plasma copy number and plasma infectivity prior to and during treatment with antiretroviral combination regimens in HIV-1 infected adults. Plasma infectivity was assessed in vitro by coculture of plasma from HIV-positive patients with PHA-stimulated fresh PBMC from uninfected donors. A positive plasma isolation, in almost all cases (43/45) and irrespective of treatment status, was associated with an HIV viral load higher than 100 000 copies per ml, with higher plasma HIV-1 RNA values in isolation-positive samples compared with isolation-negative samples (median values, 710 000 vs. 37 500 copies per ml, respectively). SI and NSI strains had similarly high viral load values (470 000 vs. 790 000 copies per ml), but CD4 counts were lower in the SI phenotype group. Our data indicate that low levels of viral load are only exceptionally associated with isolation from plasma in the in vitro model we used. This observation confirms indirectly the presence of an association between viral load and infectivity. The requisite of a high plasma viral load in order to obtain infectivity (i.e. positivity of HIV isolation from plasma) also seems maintained under antiretroviral treatment, adding confidence in the conclusion that reductions in viral load translate into reduction of plasma infectivity. Due to the extreme complexity of factors determining transmission, a very prudent interpretation of the results is essential when information from experimental studies has to be transferred to clinical situations requiring assessment of risks or clinical decisions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: HIV; Plasma infectivity; Plasma viral load; Antiretroviral treatment

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1. Introduction

Plasma HIV copy number is an important factor in predicting progression of HIV disease and survival. HIV viral load is also generally accepted as an indirect marker of ongoing or residual HIV replication and represents the main parameter used to manage antiretroviral therapy. HIV copy number is also considered a marker of infectivity for plasma and other biological samples, with higher values linked to higher probability of HIV transmission, as suggested by studies on HIV perinatal and sexual transmission (Garcia et al., 1999; Mofenson et al., 1999; Pedraza et al., 1999). In discordant couples at high-risk of transmission, both viral load and viral isolation are risk factors for heterosexual transmission of HIV-1 (Pedraza et al., 1999).

An in vivo assessment of the risk of HIV transmission is difficult and assessment and quantitation of HIV infectivity is usually performed through in vitro experiments based on coculture models, which use biological samples from HIV-infected patients and cells from uninfected donors. In this setting, we had already shown a good correlation between high RNA copy number and HIV plasma isolation in asymptomatic and early symptomatic HIV-1 infected patients with low CD4 (Ercoli et al., 1995). This correlation indicates that plasma HIV-1 copy number reflects viral infectivity of plasma in patients not pretreated with antiretroviral therapy. Little is known about this relation in patients undergoing antiretroviral treatment. In general, the reduction in plasma viral load obtained through antiretroviral combination regimens is expected to translate into an overall reduction of plasma infectivity. At the start of the antiretroviral therapy, however, a strong evolutionary selection is introduced into a steady state in which total HIV-1 copy number in plasma is probably composed of a mixture of variants with variable characteristics. In a group of predominantly untreated patients (80% untreated, 20% on zidovudine monotherapy) Piatak et al. have shown an average ratio of 1/60 000 between titers of infectious HIV-1 and circulating levels of plasma virus determined by QC-PCR, with several factors possibly responsible for this phenomenon, including production of genetically defective virus (Piatak et al., 1993). The ratio between infectious particles and total viral load can be considered a measure of the degree of infectivity of plasma. During antiretroviral treatment, this ratio could be higher than in pretreatment status because of the additional selection pressure introduced by antiretroviral treatment. We decided to investigate this issue using samples collected prior to and during antiretroviral combination treatment.

Plasma infectivity was assessed by coculture of plasma from HIV-positive patients with PHA-stimulated fresh peripheral blood mononuclear cells (PBMC) from uninfected donors, and coculture results (positive or negative) were related to HIV-1 RNA copy number, with the aim to define, (1) an HIV-RNA threshold value associated with isolation positivity; (2) possible differences in the number of HIV-1 RNA copies associated with a positive isolation prior to and during antiretroviral treatment. In our model, an increased ratio between infectious and non-infectious particles would translate into a lower viral load associated with plasma isolation. We were also able to analyse biological phenotypes of positive isolates with respect to syncytium-inducing (SI) profile (SI vs. NSI, non syncytium-inducing). SI phenotype is an independent marker of clinical and immunological progression (Koot et al., 1993; Richman and Bozzette, 1994) and is indicative of HIV tropism, growth and replication characteristics (Fenyo, 1995; Alkhatib et al., 1996) and was also reported to play a role in HIV materno-fetal transmission, lower response to antiviral treatment and development of decreased zidovudine susceptibility (Fenyo, 1995; Katzenstein et al., 1996; Rusconi et al., 1996). In this study, we compared frequency of isolation of SI virus prior to and during antiretroviral treatment and examined the relationship between SI/NSI phenotype, HIV-1 RNA copy number and CD4 count. In...
defining the above correlations, HIV isolation from plasma was considered an overall measure of plasma infectivity.

2. Patients and methods

2.1. Patient population

Samples were obtained from patients enrolled in a double blind, randomised multicentre trial with inclusion criteria of AIDS (defined as a previous history of at least one clinical AIDS-defining event) or CD4+ below 200/mm³ and no previous antiretroviral treatment (ISS 047) (Floridia et al., 1999). Treatments during the study were zidovudine (AZT, ZDV, 200 mg tid) plus didanosine (ddI, 200 mg bid), plus nevirapine, 200 mg bid or zidovudine plus didanosine plus nevirapine-matching placebo.

2.2. CD4 count determination

The lymphocyte phenotyping of PBMCs for CD4 count was performed in the same laboratory at each centre, using licensed test kits according to existing quality assurance programs.

2.3. Preparation of plasma samples

All plasma samples were processed within 4 h of sampling. Heparinized (for plasma isolation) and EDTA-treated (for RNA quantitation) blood samples obtained from each patient were centrifuged at 200 × g for 15 min to separate plasma and cellular fractions. Samples were processed at each centre site, preparing aliquots of 1.5 ml which were locally stored at −70°C and subsequently analysed at a single laboratory (Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy) at the end of the study.

2.4. HIV-1 RNA quantitation

HIV-1 RNA plasma copy quantification was performed centrally using the NASBA technique (cut-off, 4000 copies per ml) (Van Gemen et al., 1993), with samples below the cut-off reanalysed with a more sensitive assay (detection threshold 400 copies per ml). All samples below the detection threshold with the more sensitive assay were assigned the value of 399 copies per ml in quantitative analyses.

2.5. Plasma isolation

Heparinized plasma (1 ml) from the patient was added to 2 ml of culture medium containing 10 × 10⁶ PHA stimulated HIV-seronegative donor PBMCs and 20 U/ml IL-2. This suspension was incubated in a T25 flask for 2 h at 37°C in 5% CO₂ atmosphere. After incubation, 8 ml of medium containing 20 U/ml of IL-2 were added; cultures were incubated at 37°C in a 5% CO₂ atmosphere for 28 days. Feeding was performed twice a week: half of the supernatant was removed and replaced once with fresh culture medium and once with fresh culture medium including 10 × 10⁶ PHA-stimulated donor PBMCs. The supernatant was tested weekly for HIV-1 p24 antigen using a commercial ELISA kit (Abbott HIVAG-1 monoclonal). Positivity was defined by two consecutive HIV p24 antigen ‘out of range’ (O.D. > 2) values.

2.6. SI/NSI phenotype

Supernatants (50 μl) from HIV-1 positive cultures were added to duplicate wells containing 50 000 MT-2 cells in 150 μl medium in 96 well plates. HTLV-IIIB/H9 was used as positive control in each assay, and uninoculated wells served as negative controls. Cells were examined microscopically for cytopathology at 3–4-day intervals for a total of 14 days. After each examination, half of the culture volume was removed and replaced with fresh medium. To confirm the infectious potential of each inoculum, duplicate cultures containing 200 000 PHA-stimulated HIV-negative donor PBMCs were set up and fed along with the MT-2 cultures. At day 14, supernatants from the PBMC cultures were assayed for p24 production; isolates were scored as NSI only, if the PBMC cultures were positive for p24 and MT-2 cultures were negative for cytopathology.
2.7. Statistical analysis

Plasma isolation data (positive/negative, SI/NSI phenotype) were tabled by plasma HIV-1 RNA copy number (median and geometric mean), CD4+ count and treatment status (on treatment vs. no treatment) at the time of sampling. Comparison of continuous variables was performed using Wilcoxon’s two-sample test. Probabilities to be isolation-negative according to treatment status were expressed by relative risk plus 95% confidence interval. In order to evaluate the effect of treatment on plasma isolation controlling for its effect on RNA, we used a logistic regression model which considered as outcome variable a positive plasma isolation and as explicative variables RNA (dichotomised, $<100\,000$ or $\geq100\,000$ copies per ml), CD4 count and treatment status. Statistical analyses were made using the statistical software EPI-INFo, version 6.04 (Centers For Disease Control & Prevention, USA; World Health Organization, Geneva, Switzerland, 1997) and the statistical package SAS (SAS Inc., Cary, NC, USA), version 6.12.

3. Results

3.1. Plasma isolation and HIV-1 RNA copy number

Plasma isolation (qualitative coculture of plasma from HIV-positive patients with uninfected PHA-stimulated PBMC from uninfected donor) was performed on 124 samples from 57 patients. Forty-eight samples (39%) tested positive and 76 negative (61%). Thirty-six patients (63%) had at least one isolation-positive sample.

Data on both HIV-1 RNA copy number and isolation results were available for 119 samples: a positive plasma isolation, in almost all cases (43/45) and irrespective of treatment status, was associated with an HIV viral load higher than 100 000 copies per ml (Fig. 1). A negative isolation result, however, did not exclude high RNA levels (Fig. 2). Overall, isolation-positive samples had higher plasma HIV-1 RNA values compared with isolation-negative samples (copies per ml: mean, 546 175; median 710 000 versus: mean, 13 643; median 37 500; $P < 0.000001$, Wilcoxon’s two-sample test).

![Isolation-positive samples](image-url)
Fig. 2. HIV plasma copy number and CD4 cell count associated to isolation-negative samples.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>HIV-1 RNA (copies per ml)</th>
<th>CD4 (counts per mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>All samples</td>
<td>119</td>
<td>55 068</td>
</tr>
<tr>
<td>Isolation-positive</td>
<td>45 (37.8%)</td>
<td>546 175</td>
</tr>
<tr>
<td>Isolation-negative</td>
<td>74 (62.2%)</td>
<td>13 643</td>
</tr>
</tbody>
</table>

3.3. Isolation-positive samples: HIV-1 RNA copy number and CD4 cell count according to SI/NSI phenotype

Data on SI/NSI status were available for 45 of the 48 isolation-positive samples. SI and NSI strains were not different in terms of concomitant HIV-1 RNA copy number (mean, 475 480; median, 470 000 for SI vs. mean 585 372; median, 790 000 for NSI; P = 0.33). SI strains, compared with NSI strains, tended to be associated with lower CD4 counts at sampling (mean, 69/mm³; median 27/mm³ for SI vs. mean 104/mm³; median 84/mm³ for NSI; P = 0.11, Table 2).
3.4. Effect of treatment

3.4.1. RNA

On-treatment samples were collected between week 24 and 48 of treatment. Pre-treatment samples had a lower probability to be isolation-negative with respect to on-treatment samples (23/57 vs. 53/67, relative risk = 0.51, 95% CI 0.36–0.72). As expected, on-treatment samples had overall lower RNA values (mean, 8954; median, 9400) compared with pretreatment samples (mean, 424 912; median, 575 000; \( P < 0.000001 \)). Isolation-positive samples were characterised by high RNA values in both on-treatment and pre-treatment samples, although values were lower for the samples collected on-treatment (mean and median, 583 166 and 180 000, respectively), compared with pretreatment samples, 738 593 and 810 000, respectively; \( P = 0.026 \). The results of the logistic regression analysis, performed to estimate the strength of the association between a positive plasma isolation and high RNA values and to assess the role of treatment controlling for its effect on RNA, showed that only HIV-1 RNA was associated significantly with an increased risk of a positive isolation result (OR for HIV copy number \( > 100 \) 000 compared with a value below 100 000: 41.3, CI 95% 9.1–186.8, \( P < 0.0001 \)), with treatment status and CD4 count not associated with an increased risk of a positive plasma isolation (odds ratios 1.32 and 1.00; \( P = 0.63 \) and 0.65, respectively). RNA and CD4 data by treatment status and isolation outcome are summarised in Table 3.

3.4.2. CD4

On-treatment samples (n = 63) had higher CD4 counts compared with pre-treatment (n = 59) samples (mean, 189.7/mm\(^3\); median 194/mm\(^3\) vs. 88.6 and 62.5/mm\(^3\), respectively). In contrast with the RNA results, however, the difference between isolation-positive samples and isolation-negative results observed in the whole group (i.e. not considering treatment status) was evident only among pre-treatment samples (mean, 63.9/mm\(^3\); median 33/mm\(^3\) vs. 124 and 139/mm\(^3\), respectively), with on-treatment samples showing only a minor difference between isolation-positive and isolation-negative groups (mean, 164.2/mm\(^3\); median 162/mm\(^3\) vs. 196.4 and 196/mm\(^3\), respectively) (Table 3).

3.4.3. HIV-isolation results

At baseline, 34 patients were isolation-positive and 23 isolation-negative. Follow-up isolation results were available for 28 out of 34 isolation-positive patients and for 19 of 23 isolation-negative patients. During treatment, most of isolation-positive patients became isolation-negative, with a trend for a higher negativisation rate among patients receiving the triple combination (total, 20; triple combination 13 vs. double combination 7). Most of the patients who were isolation-negative at baseline remained isolation-negative during follow-up (17 out of 19 with available data). Only two patients who were isolation-negative at baseline became isolation-positive during treatment. Both had very high baseline viral load (640 000 and 1 370 000 copies per ml, respectively).

3.4.4. SI/NSI phenotype

At baseline, among the 34 isolation-positive patients, 11 had an SI phenotype and 23 an NSI phenotype. Follow-up data were available for nine SI and 19 NSI patients. During follow-up, 15 patients with NSI baseline phenotype and five

### Table 2

HIV-1 RNA copy number and CD4 count by SI/NSI phenotype

<table>
<thead>
<tr>
<th></th>
<th>HIV-1 RNA (copies per ml)</th>
<th>CD4 (counts per mm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N )</td>
<td>Mean</td>
</tr>
<tr>
<td>Isolation-positive (SI + NSI)</td>
<td>45</td>
<td>546 175</td>
</tr>
<tr>
<td>SI phenotype</td>
<td>15 (33.3%)</td>
<td>475 480</td>
</tr>
<tr>
<td>NSI phenotype</td>
<td>30 (66.7%)</td>
<td>585 372</td>
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</table>
Table 3
HIV-1 RNA copy number and CD4 count by isolation results and treatment status

<table>
<thead>
<tr>
<th></th>
<th>HIV-1 RNA, (copies per ml)</th>
<th>CD4 (counts per mm$^3$)</th>
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<tr>
<td></td>
<td>$N$</td>
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<td>Isolation-positive (total)</td>
<td>45 (37.8%)</td>
<td>546 175</td>
</tr>
<tr>
<td>Isolation-negative (total)</td>
<td>74 (62.2%)</td>
<td>13 643</td>
</tr>
<tr>
<td>Before treatment (total)</td>
<td>56 (47.1%)</td>
<td>424 912</td>
</tr>
<tr>
<td>On treatment (total)</td>
<td>63 (52.9%)</td>
<td>8954</td>
</tr>
<tr>
<td>Before treatment (isolation-negative)</td>
<td>23 (41.1%)</td>
<td>192 209</td>
</tr>
<tr>
<td>Before treatment (isolation-positive)</td>
<td>33 (58.9%)</td>
<td>738 593</td>
</tr>
<tr>
<td>On treatment (isolation-negative)</td>
<td>51 (81.0%)</td>
<td>4138</td>
</tr>
<tr>
<td>On treatment (isolation-positive)</td>
<td>12 (19.0%)</td>
<td>238 166</td>
</tr>
</tbody>
</table>
patients with SI baseline phenotype became isolation-negative. Five patients remained isolation-positive maintaining their baseline phenotype (NSI, 3; SI, 2) and three patients remained isolation-positive switching to the opposite phenotype (1 switch NSI-SI, 2 SI-NSI).

4. Discussion

Infectivity of plasma and other biological samples from HIV-positive patients is a relevant issue in several situations in which an assessment of the risk of HIV transmission may be necessary. Methods to assess infectivity must rely on in vitro models and are not easily applicable to most of the situations occurring in clinical practice. Therefore, the risk of HIV transmission is commonly assessed estimating the amplitude of the exposure using data relating to quantity and type of sample, and amount of virus contained in the sample. Plasma samples of equal volume may differ significantly in terms of viral load. Based on previous data from others and us a general direct relationship between plasma viral load and plasma infectivity can be assumed in untreated patients. The main goals of our study were to define this relationship during antiretroviral treatment and to evaluate whether the same level of viral load was associated with characteristics of infectivity (i.e. positivity of HIV isolation from plasma) irrespective of status of antiretroviral treatment.

The main finding of our study was that isolation positivity was almost invariably associated with a viral load of at least 100 000 copies per ml, in both pre-treatment and on-treatment samples. These results indicate that in the in vitro model we used, low levels of viral load are only exceptionally associated with isolation from plasma, confirming the association already found by others between HIV-1 viral load and plasma infectivity.

The maintained presence of this association also in samples collected during treatment and the existence among isolation-positive samples of only a limited viral load difference (about 0.5 log) between before-treatment and on-treatment samples suggests that therapy does not induce major changes in the proportion of infectious/non-infectious HIV particles which compose global HIV viral load and that on-treatment viral load levels can be considered roughly equivalent to pre-treatment viral load levels in terms of infectivity. This conclusion also is supported by the results of a logistic regression analysis, which showed no significant effect of treatment after controlling for RNA values.

Our data confirm clearly the positive effect of combination regimens on plasma viral load and CD4 cell count. The concomitant negativisation of plasma isolation observed during treatment in most of the patients who were isolation-positive at baseline indicates that combination treatment reduces infectivity through a reduction in viral load. This assumption is also supported by the trend for a higher negativisation rate that we observed among patients receiving the more potent antiretroviral regimen.

Two studies on perinatal transmission (Garcia et al., 1999; Mofenson et al., 1999) showed no HIV transmission from mother to child with viral load levels below 500 and 1000 copies per ml, respectively. Similarly, our in vitro results showed no positive isolation among samples with a viral load below 1000 copies, supporting the concept of a low or absent risk of infectivity in the presence of a low or undetectable viral load.

Although HIV plasma isolation is a very infrequent event in the presence of low viral load, negative plasma isolation does not automatically indicate low viral load, particularly in previously untreated patients. This observation is in agreement with the findings in perinatal transmission from Garcia et al. (1999), in which it was not possible to define a threshold for a 100% rate of transmission and in which about 60% of women with HIV-RNA above 100 000 copies per ml did not transmit HIV infection to their newborns. Similar results in the setting of heterosexual transmission were provided by Pedraza et al. (1999), who showed a broad distribution of viral load among non-transmitters subjects, with three out of the four patients with viral load at or above 100 000 copies per ml remaining non-transmitters.
We therefore support the hypothesis that high viral load, although representing a major factor influencing transmission and infectivity, is not per se sufficient to determine infectivity and that other factors, particularly in vivo, may be involved and need to be studied, with particular reference to host response factors, HIV biological characteristics and replication dynamics. Among HIV biological characteristics, we could not estimate a possible influence of HIV drug resistance on plasma infectivity because samples were not tested for resistance status.

CD4 counts in on-treatment samples were similar in isolation-positive and isolation-negative samples. This is not unexpected, being a consequence of the CD4 increase induced by treatment. It also confirms that, as expected, plasma infectivity is mainly related to HIV viral load and not to CD4 levels.

SI/NSI phenotype was also studied. Given the fast-growth kinetics reported for SI isolates, higher viral load values could have been expected for SI compared with NSI isolates. We did not find major viral load differences between SI and NSI isolates, but SI phenotype resulted associated to lower CD4 counts, as already reported by others (Fenyo, 1995). This observation is compatible with the assumption that SI strains can determine CD4 decline not only by direct destruction but also by formation of syncytia in which both infected and uninfected cells are involved.

Assuming that an SI phenotype is associated with an increased risk of progression, it may also be important to define to what extent treatment may induce reversal of SI phenotype. In our sample, almost half of the isolation-positive, SI-positive patients became isolation-negative during treatment, and two patients switched from an SI to an NSI phenotype. These changes may be considered as positive, and indicate that treatment can revert, at least transiently, negative prognostic markers. Although favourable changes (such as negativisation of plasma isolation results or switch from SI to NSI phenotype) involved the majority of patients, some patients remained isolation-positive during follow-up and a limited number switched from negative to positive or from an NSI to an SI phenotype. These observations confirm that combination regimens can induce positive changes in most but not all patients treated, and that a proportion of non-responders can be expected for CD4, viral load and other virological markers. The identification of baseline characteristics predicting treatment failure would represent an important tool to individualise treatment and improve its effectiveness. In the whole trial population studied, we found that high baseline viral load was a factor linked to lower response to treatment (Floridia et al., 1999). Although this might lead to the use of more potent regimens in patients with high baseline viral load, this strategy has not yet been confirmed by specifically designed controlled trials.

From a methodological point of view, plasma isolation has clearly some limits as a measure of infectivity. Use of plasma may determine a carryover of some amounts of drugs present in the plasma, with a possible interference in cell infection. This effect, which is difficult to remove in all plasma isolation procedures, is, however, tempered by dilution procedures occurring prior to challenging cells. We did not perform experiments to assess the magnitude of this effect in our study. However, similar experiments to exclude this effect were conducted by Zhang et al. (1996) in a study assessing HIV virion kinetics at different time points following initiation of nevirapine. The results showed that, on treatment, the virus-inactivated plasma had only very modest effects on slowing viral load in culture, with the infectivity titers of pretreatment plasma samples not altered by adding in a 1:1 ratio HIV-inactivated plasma from patients receiving nevirapine (Zhang et al., 1996).

As for all laboratory models, the assessment of infectivity may be dependent on the target cells used. In this work, we used fresh PBMC from uninfected donors, which should sufficiently guarantee a relatively wide target cell profile. However, we recognise that the model used only provides a very indirect measure of infectivity and that in clinical situations, there are many other variables interfering with HIV transmission that we were not able to analyse. Although the results we showed provide potentially useful information to the clinician, due to the extreme complexity of
factors determining transmission, a very prudent interpretation is essential when in vitro data have to be transferred to clinical situations requiring assessment of risk or clinical decisions.

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References


