Despite being highly diverse, immunovirological status strongly correlates with clinical symptoms during primary HIV-1 infection: a cross-sectional study based on 674 patients enrolled in the ANRS CO 06 PRIMO cohort

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Objectives: To analyse immunovirological status during primary HIV-1 infection (PHI) according to contemporary clinical status and time since infection.

Methods: Plasma HIV-RNA and peripheral blood mononuclear cell (PBMC) HIV-DNA levels and CD4 cell counts were determined at enrolment in the ANRS PRIMO cohort. Time since infection was estimated based on both the number of antibodies on western blot at enrolment (0–1, 2–4 or ≥5 specific antibodies) and the estimated interval between infection and enrolment based on clinical and epidemiological features. Patients were classified according to the presence or absence of clinical symptoms at enrolment.

Results: Between 1996 and 2006, 674 patients were enrolled an estimated median of 47 days after infection. Median marker values were as follows: HIV-RNA 5.10 log10 copies/mL (range 1.70–8.33); HIV-DNA 3.30 log10 copies/10^6 PBMCs (1.84–4.93); and 506 CD4 cells/mm^3 (40–1542). Median HIV-RNA and PBMC HIV-DNA levels were significantly higher in patients with 0 or 1 specific antibody (n=71) than in patients with 2–4 (n=228) or ≥5 antibodies (n=375). Symptomatic patients had significantly higher HIV-RNA and PBMC HIV-DNA levels and lower CD4 cell counts. However, 10% of symptomatic patients recruited shortly after infection had favourable immunovirological status.

Conclusions: Plasma HIV-RNA, PBMC HIV-DNA and CD4 cell count values were highly diverse and correlated strongly with clinical status during PHI. Early diagnosis was not always associated with severe PHI. Combining PBMC HIV-DNA with HIV-RNA, CD4 cell count and clinical symptoms would have allowed identification of 179 patients (26.5%) at high risk of rapid disease progression who did not meet current guidelines for early treatment initiation.

Keywords: HIV, primary infection, diversity, HIV-DNA, HIV-RNA, CD4 cell count, clinical symptoms

Introduction

Primary HIV-1 infection (PHI) is defined as the period from initial infection to complete seroconversion. PHI appears to be symptomatic in >50% of cases,1 yet >90% of cases of PHI go undiagnosed,2 mainly because clinical manifestations are highly variable,3 and because no particular symptom or combination of symptoms has sufficient diagnostic sensitivity and
specification. The severity, number and, in some studies, duration of symptoms during PHI correlate with the rate of subsequent disease progression, especially in the case of neurological PHI. High levels of HIV-RNA and HIV-DNA and low CD4 cell count at PHI diagnosis are also predictive of rapid disease progression.

Few data are available on the precise range of virological marker values during PHI. One previous study showed that HIV-RNA levels ranged widely from one patient to another during the 120 days after infection. Another study also suggested that most patients with a very early diagnosis of HIV infection have severe PHI.

ANRS PRIMO is one of the largest prospective cohorts of patients enrolled shortly after primary HIV infection. Here we examined the range of plasma HIV-RNA, cellular HIV-DNA and CD4 cell count values during PHI, according to clinical status and the time since infection based on the western blot profile. We also assessed whether HIV-1 drug resistance and HIV-1 subtype had an impact on clinical presentation. Finally, we examined whether early diagnosis during PHI is associated with more severe clinical and immunovirological status.

Patients and methods

Study population

The study involved patients with PHI enrolled in the French multicentre ANRS PRIMO CO 06 cohort created in November 1996. The Ethics Committee of Cochin Hospital approved the study, and all the patients gave their written informed consent. PHI is defined by a negative or indeterminate HIV ELISA associated with positive plasma HIV-RNA or p24 antigenemia, or with an evolving western blot profile (no anti-Pol antibodies), or with HIV seropositivity after a negative antibody test <6 months previously. At enrolment the patients had a physical examination and blood samples were collected for immunological and virological studies. All the patients were antiretroviral-naive at enrolment in the cohort. The present study is a cross-sectional analysis of data collected at enrolment.

Classification of clinical status

The patients had a physical examination at the inclusion visit then at months 1, 3 and 6, and every 6 months thereafter. They were asked whether they had experienced any of the following symptoms: fever, rash, mouth ulcers, arthralgia, pharyngitis, loss of appetite, weight loss, malaise, myalgia, tiredness or fatigue, nausea, headache, photophobia, night sweats, diarrhoea, genital sores, vomiting, anal sores or stiff neck. For this study, symptomatic PHI was classified as moderate (fewer than three symptoms and illness lasting <15 days) or severe (three or more symptoms involving at least two organ systems (i.e. skin and gastrointestinal tract) and/or biological abnormalities grade ≥2 according to the ANRS scale (www.anrs.fr) (excluding total and CD4 lymphocyte counts) and/or illness lasting >15 days, and/or hospitalization, and/or neurological PHI (meningitis confirmed by cerebrospinal fluid analysis and/or encephalitis and/or facial palsy)).

Estimation of the time since HIV infection

The date of infection was estimated as the date of symptom onset minus 15 days, the date of an incomplete western blot minus 1 month or the midpoint between a negative and a positive ELISA test. Time since infection was also estimated according to the number of emerging antibodies (0–1, 2–4 or ≥5) on the HIV-1 western blot profile at the time of enrolment into the cohort.

Laboratory methods

HIV-RNA was quantified with the Cobas Amplicor HIV-1 Monitor 1.5 assay (Roche Diagnostics, Meylan, France) or the Versant HIV-1-RNA 3.0 assay (Bayer Diagnostics, Emeryville, CA, USA), as recommended by the manufacturers. Both methods have a detection limit of 50 HIV-RNA copies/mL.

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood by centrifugation on a one-layer Ficoll Hypaque gradient. PBMC HIV-DNA was extracted and quantified by real-time PCR as previously described. This method detects all forms of intracellular HIV-DNA, i.e. unintegrated and integrated linear DNA, as well as 1-long terminal repeat (LTR) and 2-LTR circles.

Samples with undetectable HIV-RNA were systematically restested in the Necker Hospital virology laboratory with the ANRS generic test kit (Biocentric) targeting the LTR gene, in order to determine the possible role of HIV-1 diversity. Individual patients’ results were verified if the HIV-RNA and PBMC HIV-DNA values were, respectively, <3 log10 copies/mL and ≥2 log10 copies/10⁶ PBMCs, or ≥3 log10 copies/mL and ≤2 log10 copies/10⁶ PBMCs. These thresholds corresponded to the lowest interquartile for patients diagnosed most rapidly after infection (0 or 1 antibody on the western blot). All samples containing ≤3 log10 copies/mL HIV-1-RNA were restested with the ANRS HIV-RNA LTR generic kit. As no commercial kit was available for HIV-DNA quantification, we compared HIV-RNA levels in the corresponding plasma sample tested with a commercial kit and with the ANRS generic kit when the sample contained ≤2 log10 copies/10⁶ PBMCs. As the primers located in the LTR region are identical in the ANRS HIV-RNA kit and the HIV-DNA assay, similar HIV-RNA values obtained with a commercial kit and with the ANRS HIV-RNA kit ruled out underestimation of the HIV-DNA level.

Drug resistance was evaluated by amplifying the HIV-1 reverse transcriptase (RT) and protease genes in plasma HIV-RNA samples obtained at enrolment, as described elsewhere. Resistance to nucleoside RT inhibitors, non-nucleoside RT inhibitors and protease inhibitors was defined according to the 2008 HIV-1 genotypic resistance interpretation algorithm of the French National Agency for Research on AIDS (ANRS) (www.hivfrenchresistance.org).

The HIV-1 subtype was determined by sequencing the RT gene (600 bp) and aligning it with 37 HIV-1 reference strains using the Clustal V program (hiv-web.lanl.gov). Pairwise evolutionary distances were estimated with Kimura’s two-parameter method. A phylogenetic tree was constructed by neighbour joining (Neighbor program implemented in the Phylip package). The reliability of the tree topology was estimated from 1000 bootstrap replicates (data not shown).

Statistical analysis

Medians, ranges and interquartile ranges were computed for continuous variables and compared using the non-parametric Kruskal–Wallis test. We used a locally weighted regression of each marker value on the time since infection, using a bandwidth of 0.80 and tricube weighting. Figures were manipulated using Stata/SE 9.0 software (Stata Press, College Station, TX, USA).

Results

Demographic and clinical characteristics

Between November 1996 and October 2006, 674 patients were enrolled in the ANRS PRIMO cohort (Tables 1 and 2). Median age was 34 years (range 15–79) and 560 patients (83%) were male. Seventy-one patients (10.5%) had 0 or 1 antibody, 228

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patients (34%) had 2–4 antibodies and 375 patients (55.5%) had ≥5 antibodies on the HIV-1 western blot at enrolment. The estimated median time between infection and enrolment was 47 days (range 16–241) and correlated with the number of antibodies detected on the enrolment western blot, increasing from 28 days (range, 18–49) in patients with 0–1 antibody to 39 days (range 16–118) in patients with 2–4 antibodies and 63 days (range 18–241) in patients with ≥5 antibodies (P<0.0001). PHI was asymptomatic in 13% of patients (n=87), moderately symptomatic in 69% (n=468) and severe in 18% (n=119, including 21 patients with neurological PHI). The median time between infection and enrolment differed significantly according to the presence and severity of symptoms (54 days in asymptomatic PHI, 46 days in moderate PHI and 41 days in severe PHI; P<0.0001).

### HIV-RNA and PBMC HIV-DNA levels

At enrolment the HIV-RNA level ranged from <1.7 to 8.33 log10 copies/mL, with a median of 5.06 log10 copies/mL (two patients had confirmed HIV-RNA <1.7 log10 copies/mL). The median HIV-RNA level correlated negatively with the number of antibodies and the time since infection. The highest levels were observed in patients with 0 or 1 antibody (5.70 log10 copies/mL, range 2.78–8.33) than in patients with 2–4 (5.23 log, range 2.45–7.43) or ≥5 antibodies (4.85 log, range <1.70–6.90). HIV-RNA values were 1.5 log10 copies/mL lower in patients enrolled 120 days after infection than in patients enrolled 15 days after infection (Figure 1a). The median HIV-RNA level was lower in women (4.6 log10 copies/mL) than in men (5.1 log10 copies/mL) (Table 2). This difference remained significant in a multivariate analysis adjusted for clinical status, time since infection, CD4 cell count at enrolment, HIV-1 subtype and the presence of drug resistance mutations. Among the 299 patients who had <5 antibodies on the western blot at enrolment, 39 (13%) had HIV-RNA <4.5 log10 copies/mL (lower quartile of the overall distribution), even though they all had symptomatic PHI.

HIV-DNA levels at enrolment ranged from <1.84 to 4.93 log10 copies/10^6 PBMCs, with a median of 3.32 log10 copies/10^6 PBMCs; 14 patients had confirmed values of <1.84 log10 copies/10^6 PBMCs, and one of these 14 patients had a concomitant HIV-RNA level of <1.7 log10 copies/mL. PBMC HIV-DNA levels correlated negatively with the number of antibodies and the time since infection. The highest levels were observed in patients with 0 or 1 antibody (3.49 log, P<0.0001) (Figure 1b). Among the 299 patients who had <5 antibodies on the inclusion western blot, 63 (21%) had PBMC HIV-DNA levels <3 log10 copies/10^6 PBMCs, and 14 patients had high HIV-RNA (<1.7 log10 copies/mL) and low PBMC HIV-DNA (<3 log10 copies/10^6 PBMCs).

### Table 1. Demographic and clinical characteristics of 674 patients at the time of diagnosis of primary HIV infection

<table>
<thead>
<tr>
<th>Sex, n (%)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission, n (%)</td>
<td>homosexual</td>
<td>446 (66.2)</td>
</tr>
<tr>
<td>HIV-RNA level (log10 copies/mL)</td>
<td>&lt;1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Primary infection, n (%)</td>
<td>asymptomatic</td>
<td>446 (66.2)</td>
</tr>
<tr>
<td>Moderate</td>
<td>488 (72.2)</td>
<td>75 (11.1)</td>
</tr>
<tr>
<td>Severe</td>
<td>119 (17.7)</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>Time from infection to inclusion (days), median (range)</td>
<td>47 (16–241)</td>
<td>47 (16–241)</td>
</tr>
</tbody>
</table>

### Table 2. Immunovirological parameters of 674 patients at the time of primary HIV infection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=674)</th>
<th>Males (n=560)</th>
<th>Females (n=114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cell count (cells/mm³)</td>
<td>506 (40–1542)</td>
<td>498 (40–1542)</td>
<td>554 (171–1393)</td>
</tr>
<tr>
<td>HIV-RNA (log10 copies/mL)</td>
<td>5.1 (&lt;1.7–8.3)</td>
<td>5.1 (&lt;1.7–7.4)</td>
<td>4.6 (2.3–8.3)</td>
</tr>
<tr>
<td>HIV-DNA (log10 copies/10^6 PBMCs)</td>
<td>3.3 (&lt;1.8–4.9)</td>
<td>3.3 (&lt;1.8–4.9)</td>
<td>3.3 (&lt;1.8–4.3)</td>
</tr>
</tbody>
</table>

Data are presented as median (range).
The median HIV-RNA level was lower in asymptomatic patients (4.5 log₁₀ copies/mL, range 1.70–5.93) than in patients with moderate symptoms (5.1 log₁₀, 1.70–7.43) and in patients with severe symptoms (5.3 log₁₀, 3.25–8.33) (P<0.0001) (Figure 3a). The correlation between the HIV-RNA level and clinical status persisted when the number of specific antibodies at enrolment was taken into account. The highest HIV-RNA levels were observed in patients with severe clinical PHI and a short interval between infection and enrolment, while the lowest levels were observed in asymptomatic patients with a long interval between infection and enrolment.

Asymptomatic patients also had lower HIV-DNA levels than symptomatic patients, even when the number of specific antibodies at enrolment was taken into account. The median values were 3.16 log₁₀ copies/10⁶ PBMCs (range 1.84–4.14) in asymptomatic PHI, 3.34 log₁₀ (range <1.84–4.93) in moderately symptomatic PHI and 3.4 log₁₀ (range 2.0–4.6) in severe PHI (P=0.0004) (Figure 3b).

The median CD4 cell count was significantly higher in asymptomatic PHI (624 cells/mm³, range 91–1508) than in moderately symptomatic PHI (506 cells/mm³, range 40–1542) and severe PHI (411 cells/mm³, range 78–1393) (P<0.0001) (Figure 3c), and this correlation persisted after taking into account the number of specific antibodies.

Overall, among the 274 patients with symptomatic PHI who were enrolled shortly after infection (i.e. with <5 antibodies), 27 patients had favourable immunovirological status (i.e. CD4 cell count >500 cells/mm³, HIV-RNA <4.5 log₁₀ copies/mL and PBMC HIV-DNA <3 log₁₀ copies/10⁶ PBMCs). Three of these 27 patients were diagnosed very early after infection (0–1 antibody) (Figure 3a).

**HIV-1 subtypes and genotypic resistance**

The virus was subtype B in 514 patients and subtype non-B in 160 patients (CRF-02 in 87 cases). Sixty-one (38%) of the 160 patients harbouring non-B viruses were immigrants, of whom 40 originated from sub-Saharan Africa.

Seventy-one viruses harboured mutations conferring resistance to at least one antiretroviral drug: 53 viruses were resistant to one class, 12 to two classes and 6 to at least one member of each of the three main classes.

Clinical status and the estimated time between infection and enrolment did not differ between patients with subtype B and non-B infection or between patients with resistant and those with wild-type strains. The correlation between immunovirological status and clinical status remained significant after adjustment for genotypic resistance and HIV-1 subtype. Patients with...
subtype B infection had a significantly higher median CD4 cell count (521 cells/mm$^3$, range 40–1542) and a significantly lower median PBMC HIV-DNA level (3.29 log$_{10}$ copies/10$^6$ PBMCs, range 1.8–4.93) than patients with subtype non-B infection (471 cells/mm$^3$, range 128–1493, $P = 0.01$; 3.40 log$_{10}$ copies/10$^6$ PBMCs, range 1.8–4.55, $P = 0.02$). Patients with resistant strains had significantly lower HIV-RNA levels (median 4.79 log$_{10}$ copies/mL, range 2.45–7.15) than patients with wild-type strains (median 5.10 log$_{10}$ copies/mL, range 1.70–8.33, $P = 0.02$).

**Discussion**

We report the largest study of clinical, virological and immunological characteristics recorded shortly after HIV-1 infection. To the best of our knowledge, this is the first study correlating plasma HIV-RNA, PBMC HIV-DNA, HIV subtype, HIV resistance, CD4 cell count and clinical presentation in recently infected individuals. The HIV-RNA level, the CD4 cell count and the PBMC HIV-DNA level all varied widely from one patient to another, regardless of the precise time since infection (based on both the number of antibodies on the western blot at enrolment and the estimated interval between infection and enrolment).

The HIV-RNA lower quartile was 3 log$_{10}$ copies/mL in the 71 patients diagnosed the shortest time after infection (0 or 1 antibody on western blot) (Figure 3). We also identified 27 patients who had favourable immunovirological status despite having symptomatic PHI and a short interval between infection and enrolment. This challenges the view that HIV-RNA levels <50,000 copies/mL (4.7 log$_{10}$ copies/mL) are infrequent during the acute infection.$^3$ Other authors have suggested that, as HIV-RNA levels in a given patient are highly variable during the 120 days after infection, peak levels during this early period might not be predictive of the rate of subsequent disease progression.$^{11}$ Our model showed a difference in HIV-RNA levels of only 1.5 log$_{10}$ copies/mL between patients enrolled 15 and 120 days after infection, possibly explaining why this marker is predictive of disease progression even when measured early after infection.$^{23}$

PBMC HIV-DNA levels were highest in the 71 patients with a very early diagnosis (median 28 days after infection), suggesting that the cellular viral reservoir is established very rapidly.$^{27,28}$ PBMC HIV-DNA values were less widely dispersed than HIV-RNA values. Hubert et al.$^{29}$ also found that the variability in CD4 cell decline was explained more by PBMC HIV-DNA levels than by HIV-RNA levels during the first 6 months after infection.
We have previously found that PBMC HIV-DNA levels during PHI correlate with the subsequent risk of disease progression. Indeed, we previously performed longitudinal analysis to examine potential predictors of the occurrence of an acquired immunodeficiency syndrome (AIDS)-related clinical event or a CD4 cell count, 350 cells/mm$^3$ in a subset of 163 patients enrolled in the same PRIMO cohort. We found that a CD4 cell count, 500 cells/mm$^3$ was associated with a 77% risk of progression at 2 years, compared with only 5% when CD4 cell count was 750 cells/mm$^3$. An HIV-RNA count of $5 \log_{10}$ copies/mL was associated with a 55% risk of progression at 2 years, compared with 8% among patients with levels $4 \log_{10}$ copies/mL. In addition, PBMC HIV-DNA $3.4 \log_{10}$ copies/10$^6$ PBMCs was associated with a 62% risk of progression at 2 years, compared with 13% in patients with values $2.9 \log_{10}$ copies/10$^6$ PBMCs. In multivariate analysis, only CD4 cell count and PBMC HIV-DNA level were independently predictive of disease progression.

PBMC HIV-DNA levels were significantly higher in symptomatic patients than in asymptomatic patients in the present study, and symptomatic PHI has been linked to a higher risk of disease progression. Together, our data suggest that PBMC HIV-DNA is a useful additional prognostic marker in HIV infection.

HIV-RNA levels were significantly lower in patients harbouring resistant strains than in patients harbouring wild-type strains, probably because resistant viruses are less fit. Resistance did not appear to influence the early CD4 cell count or clinical status during PHI. Early identification of such patients is important, however, as drug resistance mutations can undermine the response to treatment initiated during PHI.

Patients harbouring subtype non-B viruses had significantly lower CD4 cell counts and significantly higher PBMC HIV-DNA levels than other patients, despite a similar time since infection, suggesting a risk of more rapid clinical and/or immunological progression.

French and American guidelines recommend early antiretroviral treatment for patients with severe PHI and/or a CD4 cell count of $350$ cells/mm$^3$. One-third of our patients (228/674) met at least one of these criteria. A further 179 patients (26.6%), with none of the former criteria, had a PBMC HIV-DNA level of $3.4 \log_{10}$ copies/10$^6$ PBMCs, which is predictive of more rapid disease progression.

In conclusion, in this large series of patients studied early during primary HIV infection, plasma HIV-RNA and PBMC HIV-DNA levels and the CD4 count correlated strongly with clinical status, regardless of the precise moment during PHI at which they were
Immunovirological status correlates with PHI symptoms

diagnosed. Virological and immunological status varied widely from one patient to another, regardless of clinical status during PHI. These findings show that early diagnosis is not always associated with severe PHI. Together, HIV-RNA and PBMC HIV-DNA levels and the CD4 cell count, even when determined very soon after HIV infection, can help to predict spontaneous clinical outcome and to identify patients most likely to benefit from early treatment. The recent advent of new potent and well-tolerated drug classes might warrant a reconsideration of the potential benefits of treatment initiation during PHI.

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Transparency declarations
None to declare.

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