Prevalence of drug resistance genotypes causing broad cross-resistance to nucleos(t)ide analogues

Oscar Gallego, Carmen de Mendoza, Angélica Corral and Vincent Soriano

A total of 786 patients failing antiretroviral therapy were examined for the presence of HIV genotypes causing broad cross-resistance to nucleos(t)ide analogues. They were found to be present in 40% of patients. The Q151M complex and 67/69 inserts were also recognized in 3% of patients. Although thymidine-associated mutations, which favour drug removal (pyrophosphorylation), were involved in the majority of cases, mutations reducing drug binding were relatively infrequent, probably because of their effect on viral fitness.

All plasma samples referred to our laboratory for drug resistance testing between January 2000 and February 2003 were examined. A total of 786 specimens were analysed. The following changes at the RT gene were investigated: E44D, K65R, T69D/N/G/S, L74V, V75A/M/T/S/I, Y115F, V118I, and M184V. TAM (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/R/N) were considered separately, as well as the two classic MNR genotypes (67/69 inserts–deletions and the Q151M complex) and the new Q145M mutation.

At the time of the analysis, the median length on antiviral therapy in the study population was 58 months [interquartile range (IQR) 39–84]. Overall, drug resistance mutations appeared in 86.7% of patients, with a median number of five (IQR 3–8) at the RT gene. The most prevalent single changes were T215Y/F, 49.1% (n = 386); M41L, 42.1% (n = 331); M184V, 41% (n = 322); and E44D/A with or without V118I, 26.2% (n = 206). If the definition of broad cross-resistance to NRTI is applied to genotypes yielding to reduced susceptibility to at least three drugs, their rate in the study population was as follows. The presence of three or more TAM (including T215Y/F) was recognized in 291 patients (37%). This genotype has been associated with resistance to zidovudine, stavudine and abacavir [7]. If changes at codon M41L or L210W were present, resistance to tenofovir should also be expected [3,9]. The presence of M184V plus three or more TAM (including T215Y/F) has been associated with a significant loss of susceptibility to didanosine, abacavir, and

Fig. 1. Prevalence of single drug-resistance mutations (above) and major drug-resistant genotypes causing broad cross-resistance to nucleos(t)ide analogues in HIV-infected patients failing antiretroviral therapy. MNR, Classic multinucleos(t)ide-resistant genotypes Q151M complex and 67/69 inserts; TAM, thymidine-associated mutations. *Including T215Y/F in all instances.
lamivudine, besides zidovudine and stavudine. This genotype was recorded in 114 patients (39%). The genotypes E44D/A with or without V118I plus three or more TAM (including T215Y/F), which produce resistance to zidovudine, stavudine, lamivudine and abacavir [5] were present in 165 individuals (21%). The genotype L74V plus M184V, which causes resistance to abacavir, didanosine, lamivudine and zalcitabine, was recorded in 40 patients (5.1%). The genotype K65R plus M184V, which causes resistance to abacavir, didanosine, lamivudine, zalcitabine and tenofovir [3], was found in only four (0.5%). Finally, the combination of three or more TAM (including T215Y/F) plus L74V was observed in 40 patients (5.7%) (Fig. 1).

None of the seven individuals who carried viruses with K65R had three or more TAM (including T215Y/F), a genotype that could be particularly awful. Overall, classic MNR genotypes were recognized in 24 patients (3%), half of the cases being the Q151M complex, and the remaining 12 being different rearrangements between codons 67 and 69. Ten of the latest carried inserts of two amino acids, whereas two had a codon 67 deletion. Of note is the fact that no cases of the newly multi-RT inhibitor resistance mutation Q145M were seen.

Drug-resistant genotypes have been associated with lower viral load values and higher CD4 cell counts in HIV-infected patients on antiretroviral therapy, presumably because of their impact on virus replication capacity [10,11]. Viruses harbouring mutations at codons 65, 74 or 184 within the RT gene show a significant impairment in their inherent ability to replicate [11,12]. Other RT mutations, such as TAM, may affect virus replication capacity less [11]. We assessed whether some influence on viral load values could be recognized in individuals carrying viruses with broad cross-resistance to nucleos(t)ide analogues. We did not find a significant difference between mean viral load values in patients with viruses harbouring M184V plus three or more TAM (including T215Y/F) (4.1 logs), K65R plus M184V (4.3 logs), and L74V plus M184V (4.2 logs). However, many of these individuals carried viruses with other drug-resistance mutations at the RT gene (mean number of 8.7, 10.5 and 10, respectively), besides those genotypes, which could have compensated for their effect on virus replication capacity.

In conclusion, more than 40% of treatment-experienced HIV-infected patients currently failing antiretroviral therapy carry viruses with genotypes associated with broad cross-resistance to nucleos(t)ide analogues. Changes at the RT favouring drug removal (pyrophosphorylase) seem to account for the majority of cases, whereas RT mutations causing reduced drug binding are rare (approximately 10%), hypothetically because of their effect on viral fitness. Therefore, new RT inhibitors with preserved antiviral activity mainly against viruses with multiple TAM are urgently needed.

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### References

Intra-hepatic messenger RNA levels for interferons and related genes in hepatitis C virus/HIV co-infected patients

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Intra-hepatic levels of mRNA for IFN-α, IFN-γ, IFN type 1 receptor (IFNAR-1) and PKR were determined in hepatitis C virus (HCV)/HIV co-infected and HCV mono-infected patients. In co-infected patients, IFN-α mRNA was upregulated and correlated with HIV-1 viraemia. IFN-γ, IFNAR-1 and PKR mRNA were detected in mono-infected, but not in co-infected patients. These findings suggest that in HCV/HIV co-infected individuals the ability to respond to IFN-α is impaired, probably because of the absence of its receptor.

Hepatitis C virus (HCV) infection runs a more rapid and severe course of liver disease in HIV-1-co-infected patients [1]. Although it is likely that an imbalanced immune response could contribute to this phenomenon, the underlying pathogenetic events have not been fully elucidated [1,2]. Since new therapeutic antiretroviral regimens have prolonged the survival time of HIV-infected patients, treatment of HCV has become a major challenge in these individuals. In this respect, a better understanding of the pathogenetic events, and identification of predictive parameters able to design a more appropriate therapeutic regimen are highly desirable.

It was recently shown that in HCV-infected patients intra-hepatic messenger RNA levels for IFN and IFN-related genes are altered compared with those found in patients with non-infectious liver disease. In particular, IFN-α and IFN-β mRNA levels were lower, and IFN-γ mRNA levels were higher in liver biopsies from HCV-infected, compared with non-alcoholic steato-hepatitis patients. Moreover, in HCV-infected patients IFN-γ mRNA levels were correlated with those of IFN type I receptor (IFNAR-1) and IFN regulatory factor 1, probably as a result of a coordinated induction. It is noteworthy that high IFN-α and IFN-β mRNA levels were associated with low staging, suggesting a possible protective role of IFN-α and IFN-β against fibrosis [3].

In this study we analysed mRNA levels for IFN-α, IFN-γ, IFNAR-1 and PKR in liver biopsies of HCV/HIV-co-infected patients to compare their levels with those found in singly HCV-infected individuals. To this aim, liver biopsies from 20 HIV/HCV co-infected patients and from 24 HCV-infected patients, similar for demographic features, HCV viral load and genotypes, and as well as for liver histology, were analysed. None of the patients had previously been treated with IFN. Thirteen co-infected patients were on triple combination antiretroviral treatment at the time of biopsy: 10 patients were receiving two nucleoside reverse transcriptase inhibitors (NRTI) plus one protease inhibitor, two were receiving two NRTI plus one non-NRTI, and one patient was receiving a combination of three NRTI.

Total RNA was extracted from liver biopsy, and analysed by limiting dilution reverse transcriptase–polymerase chain reaction, by using primers specific for IFN-α, IFN-γ, IFNAR-1 and PKR, as previously described [3]. Possible relationships between mRNA levels for IFN and HCV and HIV viral load, CD4 cell counts and liver damage were also investigated.

The results, shown in Table 1, indicate that in HCV/HIV-co-infected patients intra-hepatic IFN-α mRNA levels are upregulated compared with HCV-mono-infected patients, whereas IFN-γ, IFNAR-1 and PKR are profoundly downregulated, because in all biopsies from co-infected patients they were under the detectable levels. We further observed a positive correlation

<table>
<thead>
<tr>
<th>Gene</th>
<th>HCV/HIV co-infected</th>
<th>HCV mono-infected</th>
<th>P</th>
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</thead>
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<tr>
<td>IFN-α</td>
<td>1866 (73–10 417) 20/20</td>
<td>227 (4–6360) 24/24 b</td>
<td>0.001*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>&lt; 15 (NA) 0/20</td>
<td>53 (16–200) 11/24 c</td>
<td>0.002**</td>
</tr>
<tr>
<td>IFNAR-1</td>
<td>&lt; 15 (NA) 0/20</td>
<td>72 (20–408) 14/24 c</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>PKR</td>
<td>&lt; 15 (NA) 0/20</td>
<td>91 (16–288) 12/24 c</td>
<td>&lt; 0.001**</td>
</tr>
</tbody>
</table>

aRatio to β-actin × 10−3.
bMinimum detection level 3.
cMinimum detection level 15.
**Mann–Whitney test.
*Fisher’s exact test.
between IFN-α mRNA levels and the HIV-RNA viral load (r = 0.526, P = 0.018, in Spearman rank sum test). In co-infected patients, similar mRNA levels for IFN-α were found in patients with HCV viraemia higher or lower than 500 000 IU/ml, and they were not related to alanine aminotransferase levels or CD4 cell counts. Furthermore, no different IFN-α mRNA levels were observed in co-infected patients with absent/mild versus moderate/severe fibrosis. This is at variance with mono-infected patients, in whom IFN-α mRNA levels were higher in patients with a lower extent of fibrosis [3].

The upregulated IFN-α mRNA levels in the liver of HCV/HIV-co-infected patients and their relationship with the HIV viral load are in agreement with the chronic activation of the IFN system observed in HIV-infected patients, leading to the presence of circulating IFN-α [4].

In the present study we also showed that, in the liver of HCV/HIV patients, mRNA for IFN-α, IFNAR-1 and PKR are virtually absent. As IFN-γ is one of the main upregulators of IFNAR-1 [5] it is likely that the absence of mRNA for both factors in the liver of co-infected patients may be causally related.

The presence in these patients of upregulated IFN-α mRNA, together with a parallel absence of mRNA for IFNAR-1 indicate that, in spite of a strong activation of IFN-α expression, driven presumably by HIV, there is an impaired ability to respond to IFN-α action, because of the lack of expression of its receptor. This is also supported by the virtual absence of mRNA for the main IFN-α effector protein (PKR).

These results may have important implications regarding the pathogenesis of the liver damage and therapeutic regimes to be used in co-infected patients.

For the first issue, data from our previous study suggest that IFN-α may exert a possible protective role against the development of fibrosis [3]. In co-infected patients, in whom the response to IFN-α seems to be impaired, despite upregulated IFN-α mRNA levels, the reported accelerated progression towards liver fibrosis may be accounted for by the inability to mount a proper response.

For the second issue, intra-hepatic expression levels of type I IFN receptor have been related to the rate of sustained response to treatment in HCV-infected patients [6,7], and the presence of soluble IFN receptors in HCV-infected patients is considered a negative prognostic factor for therapy outcome [8]. In this respect, although initial data indicated a similar rate of response to IFN therapy in mono-infected and co-infected patients, a recent study indicated a reduced response to combined regimens of IFN plus ribavirin in the latter [9]. In view of this finding, it would be necessary to carry out a careful re-assessment of the optimal therapeutic regimens to be used in co-infected patients, which would be able to circumvent their intrinsic inability to mount an adequate response to IFN-α in the liver.

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References

Effect on CD4 T-cell count of replacing protease inhibitors in patients with successful HIV suppression: a meta-analysis

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Protease inhibitor (PI) therapy may be associated with adverse events. Switching the PI component to a non-PI may reduce some side-effects, but the immune effects are unclear. In a meta-analysis using strict inclusion criteria, a randomized switch from PI was associated with impaired CD4 T-cell gains compared with maintaining PI (PI arm +32 cells, effect size 0.130, P = 0.05). Therefore, PI-based therapy results in superior CD4 T-cell gains compared with switching to a non-PI regimen.

Combination antiretroviral therapies promote immune recovery and viral suppression in HIV-infected patients [1]. Unfortunately, the side-effects of protease inhibitors (PI) often prompt the replacement of the PI with other drug classes. Studies have now demonstrated that switching the PI component to a non-nucleoside reverse transcriptase inhibitor (NNRTI) or to abacavir maintains viral suppression [2–7]; however, the immunological effects of such switches have not been fully evaluated. An emerging body of literature suggests that PI therapy may have superior immunological outcomes compared with therapies that do not include a PI [8]. To evaluate whether such a difference occurs, we performed a meta-analysis of prospective studies that replaced the PI component of virologically suppressive therapy with either an NNRTI or abacavir and analysed the CD4 T-cell outcome of such a switch.

A MEDLINE search was conducted from 1996 to 2002 using the MeSH terms: HIV-1, anti-HIV agents, HIV protease inhibitors, reverse transcriptase inhibitors, nevirapine, switch.mp and replace.mp.

The inclusion criteria were: (i) randomized, controlled clinical trials; (ii) HIV-1-infected adults treated with two nucleoside reverse transcriptase inhibitors and one PI with suppressed viral replication for greater than 6 months before the switch; (iii) random assignment to either the continuation of PI therapy, or switch to an NNRTI or abacavir while maintaining the two nucleoside reverse transcriptase inhibitors; and (iv) CD4 cell counts recorded at baseline (i.e. before the switch) and at the conclusion of the study.

We evaluated the mean increase in the CD4 lymphocyte count using the standard deviation (SD) associated measure of variation. In the cases in which only the medians of the CD4 cell increases were reported, these were used as estimates for the mean increases. Estimates of SD in other cases were imputed based on studies in which the SD or standard error (SE) estimates were available.

The effect size, which reflects the standardized mean difference between the two groups, was calculated as follows:

\[
\frac{\text{mean CD4 cell count of PI group} - \text{mean CD4 cell count of switch group}}{\text{standard deviation}}
\]

Effect size together with an estimate of SD was used to calculate the t-statistic using a two-sided P-value and a 95% confidence interval.

Thirty-eight potential studies were identified, of which seven met all the inclusion criteria. Of the seven eligible studies, five studies, which included data on 757 patients (80.5% of the total patients) demonstrated a superiority of PI continuation on CD4 cell counts (Fig. 1). In contrast, two studies with 183 patients (19.4% of the total patients) demonstrated superior CD4 T-cell gains by switching to a NNRTI (nevirapine or efavirenz) as opposed to continuing PI therapy. We questioned whether differences in CD4 T-cell outcomes were governed by different lengths of follow-up, but linear regression analysis of CD4 T-cell change versus the length of follow-up did not reveal co-association (R² = 0.0051, P = NS).

We therefore performed a meta-analysis of PI switch using all seven randomized studies. The calculation of weighted average effect size was performed for all seven trials. The mean gain of CD4 T cells in the patients who were maintained on PI was 66 ± 11 (mean ± SE) cells/ml, whereas the increase was 34 ± 7 cells/ml in the patients switched to NNRTI or abacavir. Pooled results from all seven trials revealed that switching from a PI-based therapy to a non-PI-based therapy (involving either NNRTI or abacavir) in patients who have achieved successful viral suppression on multi-drug therapy does result in diminished CD4 cell increases. The effect size (0.130) was statistically significant [P = 0.05, 95% confidence interval (CI) –0.002 to 0.261] with a mean difference of 32 cells in the PI maintenance arm.

The success of the PI class of antiretroviral agents has transformed HIV infection into a chronic but manageable disease for many patients [1]. Unfortunately, despite their success, the utility of PI-based regimens is often limited by a high daily pill burden, dietary restrictions and treatment-associated adverse events, often prompting a switch to more tolerable agents [9]. However, the immunological effect of such changes is unknown. Our data demonstrate that discontinuing a PI in favor of another drug is associated with impaired CD4 T-cell recovery.
The comparative gain of 66 ± 11 CD4 T cells/ml in the PI-treated patients compared with 34 ± 7 cells/ml in the patients switched to alternative therapy must be viewed within the appropriate context. First, this difference was apparent after as little as 4 months of follow-up. It is important that studies with longer follow-ups be reported in order to determine whether such differences will continue to expand, or stabilize. Second, it is unclear whether these changes vary inversely with baseline CD4 T-cell counts. Although others have suggested that this effect may be greatest in patients with lower baseline CD4 T-cell counts [9], our study did not permit such subgroup analyses. Third, even small increases in the CD4 T-cell number enhance clinical outcomes [10], even when immunological improvement is not accompanied by virological response [10].

Our results, combined with th in-vitro data and non-randomized clinical studies, support the suggestion of a superior effect of PI on the CD4 T-cell number, and indicate the need for further research to define how this information may be used to the benefit of patients infected with HIV. Although this difference may not be of clinical significance for patients with high baseline CD4 cell counts, it is possible that patients with severe immune depletion would experience clinical benefit from the additional CD4 T-cell increase associated with PI. Prospective analyses are required to assess the clinical benefit of PI versus non-PI-based therapy.

**Fig. 1.** CD4 T-cell outcomes in patients randomly assigned to continue protease inhibitor-based therapy (solid bars) or to switch to non-protease inhibitor-based therapy (open bars), stratified by study. ABC, Abacavir; EFV, efavirenz; NVP, nevirapine.

Randomly assigned to continue therapy; □ switch to non-protease inhibitor-based therapy.

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References


Hormonal contraception and risk of HIV-1 acquisition: results of a 10-year prospective study

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The use of hormonal contraception has been associated with an increased risk of HIV-1 in some studies but not in others. We analysed data from a 10-year prospective cohort study of female sex workers in Mombasa, Kenya. In multivariate analysis, women using the injectable contraceptive depot medroxyprogesterone acetate and women using oral contraceptive pills were at increased risk of HIV-1 acquisition compared with women using no contraceptive method.

Almost 150 million women worldwide use hormonal forms of contraception, many of whom are at some risk of HIV-1 [1]. Unlike barrier methods of contraception, hormonal methods offer no protection against sexually transmitted diseases (STD), including HIV-1, and some studies have suggested that hormonal contraceptive use may even increase the risk of HIV-1. A meta-analysis of 28 studies found a significant association between HIV-1 infection and oral contraceptive pill use, with the strongest effect for studies conducted in Africa [odds ratio (OR) 1.65, 95% confidence interval (CI) 1.09–2.52] [2]. Two prospective studies among sex workers in Kenya and Thailand reported elevated risks of HIV-1 among women using the injectable contraceptive depot medroxyprogesterone acetate (DMPA) [3,4]. However, other studies have found no relationship between either oral or injectable contraceptive use and incident HIV-1 [2,5]. Most studies of this topic have been limited by imprecision in the measurement of contraceptive exposure and its relationship to the timing of HIV-1 acquisition, as well as by potential confounding by factors such as concurrent STD and sexual behavior. There thus remain insufficient data to make recommendations to women regarding the effect of contraceptive choices on the risk of HIV-1.

In 1993, we initiated a prospective open cohort study of HIV-1 acquisition among HIV-1-seronegative women attending a prostitute clinic in Mombasa, Kenya. Study procedures have previously been detailed [3]. At approximately monthly follow-up visits, sexual behavior and contraceptive use were recorded, risk reduction counselling was completed, free condoms were provided, and laboratory screening for HIV-1 and STD was performed.

In 1998, we reported that women in this cohort who used DMPA had a twofold (95% CI 1.3–3.1) greater risk of acquiring HIV-1 compared with women using no hormonal method, after controlling for sexual behavior, condom use, and STD [3]. In that analysis, oral contraceptive pill use was associated with increased HIV-1 risk, although this relationship did not reach statistical significance. Here we report an updated analysis from this cohort, now including 10 years of prospectively collected data, including 248 women who seroconverted to HIV-1.

As in our previous analysis, we estimated an exposure interval of 115 days for hormonal contraception in order to account for the time from infection until the
detection of HIV-1 antibodies at a clinic visit, as well as for persistence of the effect of contraception if discontinued [3]. The comparison group was women who did not use contraception or who had had a tubal ligation. At some time during follow-up, 378 women used DMPA, 276 used oral contraceptive pills, 24 used Norplant, and 31 used an IUD.

Between February 1993 and January 2003, 1498 women were enrolled in the cohort, of whom 1272 (85%) returned for follow-up. The median duration of follow-up was 60 days for STD and other genital infections [3]. Condom use was analysed as a separate covariate because condoms were used by many in the cohort for STD prevention, often in addition to another method for pregnancy prevention. Multivariate models also controlled for sexual behavior, demographic characteristics, and the occurrence of STD.

The median age at enrollment was 26 years (IQR 22–31). Sexual activity was relatively low in this group [median one (IQR 1–2) sexual partner and two (IQR 1–3) sexual encounters per week], because most participants (74%) had primary employment as barmaids and supplemented their income with commercial sex work. None reported injection drug use and only three (<1%) practised anal sex, making heterosexual vaginal intercourse the principal HIV-1 risk factor for virtually all participants.

We used multivariate Cox proportional hazards models to analyse the association between hormonal contraceptive use and incident HIV-1 infection. DMPA use was associated with a significantly increased risk of HIV-1 acquisition [hazard ratio (HR) 1.8, 95% CI 1.4–2.4; Table 1], similar to our previously reported finding. The use of oral contraceptive pills was also associated with a significantly increased HIV-1 risk (HR 1.5, 95% CI 1.0–2.1). Women who used the implantable contraceptive Norplant were at increased risk of HIV-1, although this was not statistically significant. There was no increased HIV-1 risk among women using an intrauterine device, suggesting that our results were not a result of residual confounding among women using an effective modern contraceptive method. In a separate model, we included only those clinic visits that occurred after July 1997 (the cut-off for our previously published analysis), and found similar results as in the model covering the entire 10-year period (for DMPA, HR 1.9, 95% CI 1.2–2.9, and for oral contraceptive pills, HR 1.8, 95% CI 1.0–3.1).

<table>
<thead>
<tr>
<th>Contraceptive method</th>
<th>Period N = 779</th>
<th>Period N = 1272</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>None/tubal ligation</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DMPA</td>
<td>2.0</td>
<td>1.3–3.1</td>
</tr>
<tr>
<td>Oral contraceptive pills</td>
<td>1.3</td>
<td>0.8–2.2</td>
</tr>
<tr>
<td>Norplant</td>
<td>Not done</td>
<td>1.6</td>
</tr>
<tr>
<td>IUD</td>
<td>Not done</td>
<td>1.1</td>
</tr>
</tbody>
</table>

CI, Confidence interval; DMPA, depot medroxyprogesterone acetate; IUD, intrauterine device; HR, hazard ratio.

aPreviously published analysis [3].

The contraceptive method was analysed as a time-dependent covariate. The comparison group was women who did not use contraception or who had had a tubal ligation. At some time during follow-up, 378 women used DMPA, 276 used oral contraceptive pills, 24 used Norplant, and 31 used an IUD.

bMultivariate Cox proportional hazards model adjusted for demographic characteristics, sexual behavior, and sexually transmitted diseases (STD), as previously described [3].

cMultivariate Cox proportional hazards model adjusted for the following covariates: age, duration of prostitution, parity, work place, number of sex partners/week, condom use, vaginal douching with soap products, and the presence of STD or other genital tract infections before the acquisition of HIV-1 (genital ulcer disease, positive cervical culture of Neisseria gonorrhoeae, mucopurulent cervical discharge, microscopic detection of cervicitis, bacterial vaginosis, trichomoniasis, and Candida vaginitis). In order to reflect changing sexual behavior over the course of follow-up, each woman’s average number of sexual partners and average condom use for each year of study participation were calculated. Continuous variables were dichotomized at the median.

dIn the previous analysis, women who used an IUD or Norplant were excluded because of small numbers.

Table 1. Multivariate analyses of contraceptive method and risk of HIV-1 acquisition.
These results suggest that the use of both injectable and oral contraception may increase the risk of HIV-1 acquisition, independent of sexual behavior and STD exposures. Our study was conducted among African prostitutes, and our results may be most applicable to women at high-risk of HIV-1. However, although the rate of partner change for this cohort was high, the average sexual frequency was similar to that reported in surveys among general populations of African women. As the majority of women in this cohort used condoms at least sporadically for STD protection, irrespective of hormonal contraceptive use, our results may be less confounded by patterns of condom use than studies performed among lower-risk populations, such as women attending family planning clinics. Moreover, our data included monthly measurements of HIV-1 status, contraceptive use, sexual behavior, and STD, thus minimizing the potential for bias caused by the misclassification of either outcome or exposures. Given the widespread use of hormonal contraception in areas of high HIV-1 prevalence, our findings are concerning. Regardless of the method women choose for pregnancy prevention, healthcare providers must emphasize that condoms are the only method proved to prevent HIV-1 transmission. Women who use hormonal contraception, especially those at high risk of HIV-1, should be especially encouraged to use condoms consistently.

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Acceptability and uptake of a package to prevent mother-to-child transmission using rapid HIV testing in Abidjan, Côte d’Ivoire

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The participation of HIV-1-infected pregnant women in a programme of prevention of mother-to-child transmission (MTCT) of HIV in Abidjan is described. Prenatal counselling with a rapid HIV test was proposed to 14 067 pregnant women, and acceptance was 89.4%. The return rate for results was 74.2%. The HIV-1 prevalence was 11.1%, and 26.2% of HIV-infected women started the prevention of MTCT programme. To increase the uptake, we recommend community mobilization and the strengthening of male involvement.

Every day 1900 children acquire HIV-1 infection from their mother in Africa. Short-course antiretroviral prophylaxis is the best confirmed measure to prevent mother-to-child transmission (MTCT) of HIV in this context. Nevertheless, despite the rapid implementation of pilot public health programmes [1], the uptake
of services for the prevention of MTCT remains low in Africa. Approximately 20% of all HIV-infected pregnant women identified through counselling and testing took prevention of MTCT antiretroviral drugs in west Africa [2,3]. The factors influencing participation in prevention of MTCT programmes have not been yet clearly identified. The ANRS 1201/1202 Ditrame Plus therapeutic cohort was launched in May 2000 in Abidjan, Côte d'Ivoire, to assess the effectiveness in prevention of MTCT programme combining a short-course antiretroviral regimen of zidovudine plus nevirapine and postpartum interventions. Here, we describe the frequency of missed opportunities for prevention of MTCT and study the profile of HIV-positive women who did not participate in this programme.

The Ditrame Plus project progressively reached six community-based antenatal clinics. All pregnant women (age ≥ 18 years) attending these antenatal clinics were provided pre-test counselling. All women accepting the HIV test signed an informed consent document. Their partners were also offered free HIV testing if they were informed by them. HIV testing was conducted using on-site rapid HIV testing in a serial strategy (Determine and Genie II). Women were considered infected with HIV type 1 (HIV-positive) if both tests were positive. If the results of the two rapid tests were discordant, an enzyme-linked immunosorbent assay test was used. The HIV test result was available within 24 h and was provided by the same counsellor as the pre-test counselling.

Informed and consenting HIV-positive pregnant women were offered free of charge the prevention of MTCT package of the Ditrame Plus project: antiretroviral prophylaxis (zidovudine plus nevirapine) from 36 weeks of gestation until delivery, alternatives to predominant breastfeeding (formula feeding at birth or exclusive breastfeeding for 3 months followed by rapid weaning) and mother and child cotrimoxazole prophylaxis. Regression logistic analysis was used to identify the risk factors for HIV+ pregnant women who did not return to receive test result and the characteristics of those who did not initiate the PMTCT package despite being informed of their HIV status.

From May 2000 to October 2002, 14,067 pregnant women received pre-test counselling. The acceptability of rapid HIV testing was 89.4%. The prevalence of HIV-1 infection (HIV-1 infection alone and dually reactive HIV-1+2) was 11.1% [95% confidence interval (CI) 10.5–11.6%; n = 1396]. The overall acceptability of HIV testing defined by the acceptance of both pre-test and post-test counselling was 9340/14 067 (66.4%). Of the 1396 HIV-1-infected women, 1023 (73.3%) came back for post-test counselling to receive their result. A similar proportion (74.3%) was observed for uninfected women (P = 0.38). No difference was found in HIV-1 prevalence between those who received their HIV test result and those who did not (10.9 versus 11.5%, P = 0.39).

Among the 1023 pregnant women who were informed of their HIV-1-seropositive status, only 366 started the peripartum intervention. There are two possible ways of reporting the uptake of this first component of the prevention of MTCT package: 366/1023 (35.8%) among pregnant women who were informed of their HIV serostatus and 366/1396 (26.2%) when considering as the target all HIV-1-infected pregnant women diagnosed through the antenatal testing process. The socioeconomic features of the 373 women who did not return to get their test result were similar except for the age of those of the 1023 women who received it in univariate analysis (Table 1). Women less than 25 years of age remained 1.3 times more likely than older women to refuse post-test counselling (95% CI 1.0–1.6) in multivariate analysis.

Among the 1023 HIV-infected women who knew their HIV status, the uptake of the prevention of MTCT package was significantly lower in univariate (Table 1) than multivariate analysis in illiterate women [odds ratio (OR) 1.6; CI 1.2–2.3] and in women living with a partner (OR 1.5 CI 1.1–2.0).

This observational survey conducted within a large-scale programme found that the acceptability of a prevention of MTCT package remains largely insufficient in a context of high HIV prevalence, despite the availability of rapid HIV testing on site and a background of the conduct of prevention of MTCT research with antiretroviral drugs [3,4]. Only one-third of HIV-positive women ended up initiating the proposed prevention of MTCT package. Our results unfortunately remain in accordance with the last published reports, despite the evolving context in favour of the prevention of MTCT [2–4]. In this study, there were several reasons to explain the low uptake of the package, but the refusal to believe the result given by the counsellor and the high risk of social stigmatization are key factors to consider early in the sequence of actions needed.

The present study found that a low education level was associated with a poor uptake of the prevention of MTCT package in HIV-infected women who knew their status. Particular attention must be paid to illiterate HIV-positive women during counselling in order to explain as clearly as possible the different options and stress the expected benefit to their children. The women who had been living with their partner accepted the prevention of MTCT package less
frequently than those who did not. In Kenya, only a third of HIV-positive women informed their partners of their test results because of stigma, domestic violence and disruption of the relationship. To prevent negative reactions of men, Gaillard et al. [5] recommended couple-counselling and enhanced partner involvement in MTCT prevention programmes.

The high frequency of missed opportunities documented in the first 2 years of our programme calls for a plan of community mobilization to reduce stigmatization. In this African urban setting, the involvement of community leaders is important to obtain their cooperation and participation in the design and conduct of such actions and is now being seriously considered. There is no universal solution to the successful implementation of a prevention of MTCT package in poor settings. Parameters to be considered among others in operational research programmes leading to the successful prevention of MTCT include: the background community awareness on the prevention of MTCT, the quality of antenatal and obstetric care, the training of healthcare providers, the strengthening of male involvement, and women’s empowerment and education.

**Table 1. Profile of HIV-1-infected pregnant women eligible for the prevention of mother-to-child transmission of AIDS programme (N = 1396) according to their acceptance of their HIV test result and of the prevention of mother-to-child transmission package in Abidjan, Côte d’Ivoire (ANRS 1201/1202 Ditrame Plus Project, 2002).**

<table>
<thead>
<tr>
<th></th>
<th>Did not return for test result (n = 1373)</th>
<th>Received test result (n = 657)</th>
<th>Included in DP and received ART (n = 366)</th>
<th>Risk of not returning to receive ART (n = 657)</th>
<th>Risk of not receiving the prevention of MTCT packageb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 years</td>
<td>167 (44.8)</td>
<td>259 (39.4)</td>
<td>137 (37.4)</td>
<td>1.28 (1.01–1.63)</td>
<td>1.08 (0.83–1.41)</td>
</tr>
<tr>
<td>≥ 25 years</td>
<td>206 (55.2)</td>
<td>398 (60.6)</td>
<td>229 (62.6)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>203 (54.4)</td>
<td>319 (48.6)</td>
<td>148 (40.4)</td>
<td>1.32 (0.95–1.82)</td>
<td>1.70 (1.21–2.40)</td>
</tr>
<tr>
<td>Primary school</td>
<td>105 (28.2)</td>
<td>228 (34.7)</td>
<td>131 (35.9)</td>
<td>0.89 (0.62–1.27)</td>
<td>1.38 (0.97–1.96)</td>
</tr>
<tr>
<td>College or high school</td>
<td>65 (17.4)</td>
<td>110 (16.7)</td>
<td>87 (23.7)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Previous pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65 (17.4)</td>
<td>99 (15.1)</td>
<td>46 (12.6)</td>
<td>1.27 (0.93–1.75)</td>
<td>1.23 (0.84–1.80)</td>
</tr>
<tr>
<td>Yes</td>
<td>308 (82.6)</td>
<td>558 (84.9)</td>
<td>320 (87.4)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Previous child still alive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>112 (30.0)</td>
<td>175 (26.6)</td>
<td>91 (24.9)</td>
<td>1.22 (0.93–1.59)</td>
<td>1.09 (0.82–1.47)</td>
</tr>
<tr>
<td>Yes</td>
<td>261 (70.0)</td>
<td>482 (73.4)</td>
<td>275 (75.1)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Income activity</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>158 (42.4)</td>
<td>290 (44.1)</td>
<td>163 (44.5)</td>
<td>0.93 (0.73–1.17)</td>
<td>0.98 (0.76–1.24)</td>
</tr>
<tr>
<td>Yes</td>
<td>215 (57.6)</td>
<td>367 (55.9)</td>
<td>203 (55.5)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Living with partner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>121 (29.8)</td>
<td>147 (22.4)</td>
<td>114 (31.2)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>262 (70.2)</td>
<td>510 (77.6)</td>
<td>252 (68.8)</td>
<td>0.80 (0.62–1.05)</td>
<td>1.57 (1.17–2.09)</td>
</tr>
</tbody>
</table>

ART, Antiretroviral prophylactic drug regimen (zidovudine plus nevirapine); CI, confidence interval; DP, ANRS 1201/1202 Ditrame Plus project; MTCT, mother-to-child transmission; OR, odds ratio.

aUnivariate analysis.

bRisk of not receiving antiretroviral prophylactic drug regimen in pregnant women who received HIV test result (n = 1023).

**ANRS 1201 Ditrame Plus Study Group**

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This study was presented in part at the 14th International Conference on AIDS (July 2002, Barcelona, Spain) [Abstract ThPe7778], and the presenting author (Didier Koumavi Ekouévi) received the IAS Young Investigator Award for Track D: Prevention Science.

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