

Drug resistance degree is associated with duration of ARV exposure and predicted by baseline CD4 and gender in HIV-infected patients failing first-line WHO-recommended ARV regimen: a cross-sectional viral load survey of a cohort in Cameroon (Médecins sans Frontières-Ministry of Health)

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Background

The growing access to antiretroviral therapy (ART) in resource-limited countries has raised concern about resistance to initial regimen and its effect on second-line therapy. The absence of viral load monitoring limits early detection of virologic failure, thus favouring the accumulation of drug resistance-associated mutations. The extent of this resistance accumulation is still poorly reported, especially for non-B and non-C subtypes. The accumulation of NRTI-resistance mutation may confer wide cross-resistance within NRTI class; increasing number of NNRTI-resistance mutations may jeopardize response to second-generation NNRTIs such as etravirine.

Aim of the study

Our aim was to investigate if there was a relationship between the extent of resistance and the duration of ART exposure; we also sought baseline characteristics of failing patients able to predict the extent of resistance and describe the consequences on second-line NRTI and NNRTI options.

Methods

Setting

In 2001, Médecins sans Frontières (MSF) started an ART program, in collaboration with PRESICA project (IRD-UMR 145 Montpellier), the Military Hospital in Yaoundé and the Cameroon Ministry of Health. ART was offered according to WHO recommendations and national guidelines.

Cross-sectional survey

From 15 September 2006 through 15 May 2007, a cross-sectional survey was conducted to assess the clinical, immunologic, virologic and adherence status of all the patients who had begun ART since at least 12 ± 2 months, and presented to the clinic in the study period. At the visit demographic, biometric, adherence data (using a modified visual analogue scale from 1 to 6) and blood samples were collected. CD4 and viral load quantification were done at Centre Pasteur du Cameroun in Yaoundé.

Resistance test

For HIV-RNA >250 copies/ml, RT gene was reverse transcribed and amplified with the nested primers pair MJ3/MJ4-NE1/A35; strands were sequenced and aligned with the HIV-1 HXB2 reference genome using the CEQ2000 software (Beckman-Coulter).

Sequences were electronically sent to the Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy, and analyzed with Stanford University resistance interpretation algorithm.

Ethical approval

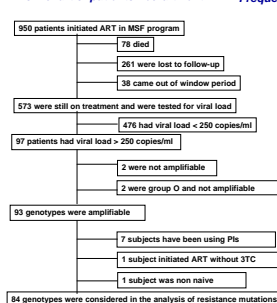
The study was approved by the MSF ethical board, the ethical board of France (Comité de protection des personnes – St. Germain en Laye, Paris), the ethical committee of Cameroon and the Ministry of Health of Cameroon. All participants gave their written informed consent, and received second-line ART according to their individual genotype result.

Statistical analysis

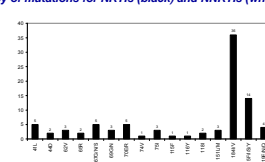
Only mutations with a frequency ≥5 have been considered in the analysis. Chi-square or Fisher's exact test were used to assess relationships between discrete variables; Cochran-Armitage test was applied to assess for trend along categories of ART duration. Nonparametric Mann-Whitney rank sum test or Kruskal-Wallis test were applied to compare values of independent distributions of continuous variables. Pearson correlation coefficient was calculated to assess linear correlation. At multivariate analysis, generalized linear model (GLM) and multiple logistic regression were applied to estimate the independent contribution of baseline covariates in predicting the number of mutations (for NRTIs or NNRTIs) or the presence of different mutation patterns. Significance tests were 2-sided; p-value ≤0.05 was considered statistically significant. The analyses were made using SAS software (version 8.2; SAS Institute).

Results

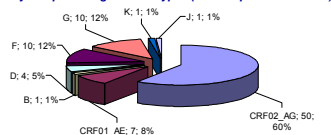
Flow-chart of patients' recruitment



Frequency of mutations for NRTIs (black) and NNRTIs (white)

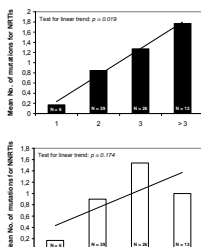


Frequency and percentages of subtypes (from sequenced isolates)



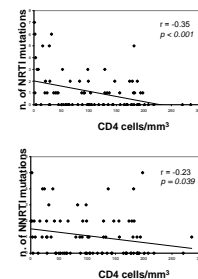
Relationship between mutations and duration of ART exposure

- 39/84: wild-type
- 45/84: ≥1 mutation
- 39/84: ≥1 mutation for both classes (NRTI and NNRTI)
- mean number of mutations: 2.13
- median exposure to ART: 2.18 years (range: 0.82-5.95)
- the mean number of mutation increased over time (figures on the right)
- among mutations considered in the analysis (M41L, M184V, T215F, T215Y, A98G, K103N, V108I, Y181C, G190A) only V108I showed a significant trend by time: it occurred not before 3 years of ART exposure (p for trend 0.001).



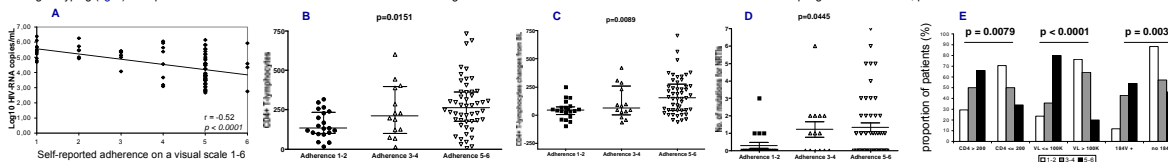
Relationship between baseline CD4 and mutations

The lower the CD4+ cell count at baseline, the higher was the number of mutations for NRTIs ($r = -0.35$; $p < 0.001$) and NNRTIs ($r = -0.23$; $p = 0.039$). Patients with baseline CD4+ cell count ≤50/mm³, as compared to those with CD4+ cell counts >50/mm³, had a higher mean number of mutations for NRTIs and NNRTIs (2.17 versus 0.66, $p = 0.001$; 1.65 versus 0.84, $p = 0.01$, respectively), and were more likely to have mutations at failure detection as shown in the table below.



Relationship between self-reported adherence and mutations

Eleven patients (13.6%) reported a score of 1, six (7.4%) a score of 2, six (7.4%) a score of 3, eight (9.9%) a score of 4, forty-six (56.8%) a score of 5 and four (4.9%) a score of 6. For three patients the adherence score was missing. Differences between men and women across all adherence levels were not statistically significant. Nevertheless, subjects with a score of 1 were 6 out of 24 (25%) among men, and 5 out of 57 (8.8%) among women. A higher adherence was associated with a lower viral load ($r = -0.52$; $p < 0.0001$, fig.A), a higher number of CD4 at the time of sampling ($p = 0.0151$, fig.B), a higher increase in CD4 from the baseline ($p = 0.0089$, fig.C) and a higher number of mutations for NRTIs ($p = 0.0445$, fig.D). Higher levels of adherence were also associated with CD4+ count > 200/mm³ and HIV-RNA < 100.000 copies/ml at the time of sampling, and with the presence of M184V mutation at genotyping (fig.E). The presence of M184V mutation was associated also with a higher mean relative increase in CD4 from baseline to the time of sampling: 1072% vs 176%, $p = 0.003$.



Multivariable analysis

- **Covariates:** age, baseline BMI, baseline CD4 (≤ or >50 cells/mm³), gender and WHO stage.
- **GLM:** the mean number of mutations for NRTIs and NNRTIs was higher for subjects with baseline CD4 ≤50/mm³ (2.10 versus 0.56, $p < 0.0001$; 1.65 versus 0.76, $p = 0.005$, respectively), and the mean number of mutations for NNRTIs was higher for women with respect to men (1.60 versus 0.81, $p = 0.015$).
- **Logistic regression:** baseline CD4 ≤50/mm³ was predictive of having at least one mutation for NRTIs, at least one mutation for NNRTIs, at least one mutation for both classes, and other specific mutations as shown in table beside; being a woman was predictive of having at least one mutation for NRTIs (OR = 4.05, 95% CI: 1.19-16.67, $p = 0.034$) and at least one mutation for both classes (OR = 4.95, 95% CI: 1.44-20.83, $p = 0.017$).

We tried to determine the impact of mutations on future possible options among the NRTI class (didanosine and tenofovir) and the NNRTI class (etravirine). A baseline CD4+ cell count ≤50 cells/mm³ was a strong independent predictor of resistance to didanosine and etravirine (see table beside).

Table. Relationship between baseline CD4+ cell counts ≤50/mm³ or >50/mm³ and various degrees of drug resistance.

Outcome measure Data are no. (%), unless otherwise indicated	Total n = 84	BL CD4+ ≤50 cells/mm ³ n = 23	BL CD4+ >50 cells/mm ³ n = 61	Univariable Logistic regression		Multivariable Logistic regression	
				odds ratio	95% CI	adjusted odds ratio*	95% CI
≥1 mutation for NRTIs	40 (47.6%)	17/23 (73.9%)	23/61 (37.7%)	4.68	1.68-14.57	7.49	2.20-32.14
≥1 mutation for NNRTIs	44 (52.4%)	17/23 (73.9%)	27/61 (44.3%)	3.57	1.29-11.04	4.25	1.36-15.48
≥1 mutation for both classes	39 (46.4%)	16/23 (69.6%)	23/61 (37.7%)	3.77	1.39-11.14	6.10	1.80-25.53
Selection of M41L	5 (6.0%)	4/23 (17.4%)	1/61 (1.6%)	12.6	1.3-120.0	0.007	NA
Selection of M184V	35 (41.7%)	15/23 (65.2%)	20/61 (32.8%)	3.8	1.4-10.6	0.007	5.49
Selection of T215Y	6 (7.1%)	4/23 (17.4%)	2/61 (3.3%)	6.2	1.05-36.6	0.026	NA
Selection of Y181C	16 (19.0%)	10/23 (43.5%)	6/61 (9.8%)	7.0	2.2-22.9	<0.001	7.57
≥1 TAM	14 (16.7%)	9/23 (39.1%)	5/61 (8.2%)	7.20	2.15-26.79	0.002	8.45
≥1 TAM-I or Q151M	9 (10.7%)	6/23 (26.1%)	3/61 (4.9%)	6.82	1.62-35.12	0.011	7.12
Resistance to didanosine	11 (13.1%)	4/23 (17.4%)	7/61 (11.5%)	6.23	1.67-26.41	0.008	6.36
Resistance to tenofovir	4 (4.8%)	1/23 (4.3%)	3/61 (4.9%)	NA	NA	NA	NA
Resistance to etravirine [†]	24 (28.6%)	12/23 (52.2%)	12/61 (19.7%)	4.45	1.60-12.84	0.005	4.718

The cut-off for resistance was the score of ≥30, according to Stanford algorithm. BL = baseline; CI = Confidence Intervals; NA = not applicable for small sample size. *Adjustment was made for age, baseline body mass index, gender and WHO stage. [†]Subjects with ≥3 etravirine resistance associated mutations (RAMs) were 2, while subjects with a Stanford score for etravirine ≥30 were 24.

Conclusions

Failing patients with baseline CD4≤50/mm³ are at higher risk of extensive drug-resistance, that increases over time of ART exposure when virologic monitoring is not available. Earlier ART initiation (before profound CD4+ count decrease), and targeted VL testing, might be considered to preserve options for second-line regimens in resource-limited settings.