

ORIGINAL RESEARCH

Increased risk of early virological failure in non-European HIV-1-infected patients in a Dutch cohort on highly active antiretroviral therapy

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Objective
To compare early and late responses to highly active antiretroviral therapy (HAART) in European and non-European HIV-1 infected patients in a Dutch cohort.

Methods
We retrospectively analysed the response to HAART of 216 previously treatment-naive HIV-1-infected patients using the University Medical Centre Utrecht HIV database. African ($n = 51$), Asian ($n = 7$), and Central/South American ($n = 6$) patients were classified as non-European, and others as European ($n = 152$). Early failure was defined as a viral load that remained above 400 HIV-1 RNA copies/mL after 6 months of treatment with HAART. Late-phase failure was determined in patients who were successfully treated in the early phase and was defined as two consecutive viral load measurements above 400 copies/mL, a new AIDS-defining event or death.

Results
In the early phase, four of 152 (2.6%) European and eight of 64 (12.5%) non-European patients failed HAART. A significant increased risk of virological failure in the early phase of treatment was observed for non-Europeans as compared to Europeans (odds ratio 4.6; 95% confidence interval 1.1–20.2). Low serum drug levels in the absence of resistant virus were often seen at the time of early failure. No difference in late-phase failure was observed between the two groups (adjusted hazard ratio 0.6; 95% confidence interval 0.3–1.2).

Conclusions
Non-European patients had a 4.6 times higher risk of virological failure than their European counterparts in the first 6 months of treatment with HAART. This failure seemed to be associated with low serum drug levels at the time of failure. However, if HAART was successful in the early phase, response rates in the late phase were similar for Europeans and non-Europeans.

Keywords: adherence, antiretroviral therapy, epidemiology, HIV, resource poor, resistance, subtype

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Introduction

New HIV diagnoses in resource-poor countries, especially in sub-Saharan Africa, have made the largest contribution to the rise in new HIV diagnoses worldwide, and so European countries are faced with a significant increase in the numbers of patients from these countries [1].

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A recent report on the epidemiology of 4117 patients with HIV infection and AIDS from 22 clinics in the Netherlands showed a clear increase in the percentage of patients from HIV endemic regions (i.e. Africa, the Caribbean or Latin America), from 6% of new HIV diagnoses registered in 1985 to nearly 40% in 2001 [2]. Heterosexual African women have made the largest contribution to this increase in new HIV diagnoses among non-Europeans. In the United Kingdom, a similar trend was observed in a study from London which showed that, although there had been a reduction in the percentage of newly diagnosed HIV-infected patients who were white,

from 72 to 48% between 1994 and 2000, the proportion of Africans newly diagnosed with HIV in this centre almost doubled in this period, from 24 to 45% [3].

Although access to antiretroviral treatment is very restricted in resource-poor countries, in European countries there should be equal access to highly active antiretroviral therapy (HAART) and health care facilities for all HIV-infected patients. However, it has been reported that non-European HIV-1-infected patients in European countries have a worse response to HAART than their European counterparts [4].

Despite the changing demography of HIV-infected patients in Western countries, surprisingly few data are available on the success of HAART in patients originating in HIV endemic countries compared with their white European counterparts. There are, however, many reasons to expect differences in outcome of HIV infection between European and non-European patients, including differences in HIV subtypes, resistance patterns, pharmacokinetics and adherence [5,6].

We examined the responses to HAART of antiretroviral treatment-naive HIV-infected patients in the period from August 1996 to October 2002, to investigate whether non-European HIV-infected patients have an equivalent response to HAART to European HIV-infected patients in the Netherlands. In addition, we determined the subtype, resistance patterns and serum drug levels of all patients who failed HAART in the first 6 months to explore possible reasons for failure in both groups.

Patients and methods

Patients

We used the University Medical Centre Utrecht HIV monitoring database, part of the former AIDS Therapy Evaluation National Centre (ATHENA), Stichting (foundation) HIV Monitoring (SHM) database, to conduct a retrospective cohort study on patients who visited our centre from August 1996 to October 2002 [7]. All patients gave written informed consent to the use of anonymous information about their course of HIV infection. The start date for inclusion was the time at which HAART was introduced in August 1996. We included HIV-1-infected patients aged ≥ 18 years who were treatment-naive when they started HAART. For each patient, there was a minimum duration of follow up of 7 months, with three or four monthly measurements of viral load (HIV RNA) and CD4-cell count. HIV-infected women who received HAART temporarily during pregnancy and delivery were excluded from the cohort ($n = 5$). Patients born in Africa, Asia and Central and South America were classified as non-European. European patients were born and raised in Europe. All non-European citizens were combined into one group to enhance the statistical power.

HAART was defined as a combination of at least three antiretroviral drugs that included either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTIs). HAART was started when the patient met the national guidelines for starting antiretroviral treatment [8,9]. We used an intent-to-continue treatment approach and thus ignored subsequent changes to treatment, including treatment interruptions and terminations.

Outcome measures

Early phase

Patients failed in the first early phase of HAART if viral load remained above 400 HIV-1 RNA copies/mL of serum after 6 months treatment.

Late phase

Late-phase failure was determined in patients who were initially successful in responding to treatment in the early phase. We defined late-phase failure as the occurrence of one of the following events: (1) at least two consecutive HIV-1 RNA measurements of more than 400 copies/mL; (2) a new AIDS-defining event CDC (centres for disease control) stage B or C HIV stage [9]; or (3) an HIV-related death. Cut-off points and definitions were chosen in accordance with other HAART efficacy studies [10].

Measurements

Patient information continuously stored in the monitoring database is derived from medical charts filled out during the first attendance of a patient at a clinic or hospital, from laboratory viral load and CD4-cell count data, and from additional clinical data collected during the follow-up period. At baseline, the following data were collected for each study subject using the database: date of birth, gender, country of birth, AIDS-defining events, CD4-cell count, viral loads with dates and assay used, and type of treatment with start and stop dates. AIDS-defining events were recorded according to the 1993 revised classification system for HIV infection [8,9]. Viral load was measured using the Cobas Amplicor HIV-1 Monitor test (Roche, Woerden, The Netherlands; version 1.0 until mid-1998, and thereafter version 1.5), with a lower limit of detection of 400 copies/mL. After the introduction of the ultra-sensitive test (HIV S-RNA test) in February 2000, a detection level of 50 copies/mL was used.

When a patient failed treatment in the early phase, analysis of HIV genotype profiles was performed on blood taken from the patient before treatment was started, to determine whether resistance-conferring mutations were present. Genotypic drug resistance determinations for the protease and reverse transcriptase genes were performed using the HIV-1 ViroSeq genotyping kit (Abbott Laboratories, Chicago, IL). Interpretation of the genotype profiles for the presence of resistance-conferring mutations was performed using RETROGRAM [11,12].

Drug serum levels were measured in serum taken from the patient when early-phase failure was diagnosed. Determination of indinavir, nelfinavir, saquinavir and ritonavir in plasma was performed by reverse-phase high-performance liquid chromatography [13].

Statistical analysis

In the univariate analysis, we determined the significance of differences in mean age, mean CD4-cell count and mean viral load (measured on a $\log_{10}$ scale) between the two groups using Student's *t*-test. Differences in gender, CDC class and therapy class were determined using Pearson's χ^2 tests. We applied multivariable logistic regression modelling to assess the association between patients' origin and early virological failure independent of potential confounding variables.

Only patients who did not fail treatment in the early phase were included in the analysis of the late phase. We used Kaplan–Meier product limit methods to construct the survival curves for both ethnic groups. Cox proportional hazard modelling was applied to assess the relative risk of developing an outcome for non-Europeans compared with Europeans, adjusted for age, sex, CDC stage, CD4-cell count, viral load, type of antiretroviral treatment at baseline and difference in follow-up period. We defined the period at risk as the interval between the first date of a viral load under 400 copies/mL plasma and the first date of the occurrence of an outcome. We censored subjects who did not develop one of the outcomes during follow-up as of the time that they were lost to follow-up or the end of the study period (1 October 2002). We defined as lost to follow-up those patients who were untraceable during the follow-up period or who moved to another region. There was no significant deviation from the proportional hazards assumption. Odds ratios (ODs) and hazards ratios (HRs) with their corresponding 95% confidence intervals (CIs) were calculated as approximations of relative risks.

Results

Data on 216 patients, 152 European and 64 non-European, could be analysed. The majority of non-Europeans came from Africa ($n = 51$; 18% Ethiopia, 12% Angola, 10% Zaire and 60% other African countries), with the others coming from Asia ($n = 7$) and Central or South America ($n = 6$).

There was a significant difference in baseline characteristics for sex, initial treatment and median age (Table 1). The HIV-infected patients in the non-European group were more often female, were younger in age at the time of HAART initiation and more often had HAART containing a NNRTI instead of a PI in their initial treatment schedule. Mean baseline viral loads were similar for the two groups; 5.08 vs. 4.96 $\log_{10}$ copies/mL plasma for European and non-European patients, respectively. Also, mean baseline CD4 cell counts were comparable; 223 vs. 229 cells/μL for European and non-European patients, respectively.

Early phase

There was a significant difference in response to HAART in the first 6 months of treatment between European and non-European HIV-infected patients. Virological failure in the early phase of treatment occurred in four of the 152 European patients (2.6%) and in eight of the 64 non-European patients (12.5%). Using logistic regression analysis, the risk for failure was 4.6 times higher for non-Europeans than for Europeans, independent of age,

Table 1 Baseline characteristics of European and non-European HIV-infected patients ($n = 216$)

	European ($n = 152$)	Non-European ($n = 64$)	OR (95% CI)	Mean difference (95% CI)	*P*-value
Sex female*	16 (10.5%)	29 (45.3%)	7.0 (3.5–14.4)		<0.001
Baseline CDC stage C†	46 (30.3%)	13 (20.3%)	0.6 (0.3–1.2)		0.134
Initial treatment PI‡	123 (80.9%)	43 (67.2%)	0.5 (0.3–0.9)		0.029
Age (years) (median)	40.4	31.5		8.9 (6.5–11.4)	<0.001
Mean viral load (SD)§	5.08	4.96		0.2 (–0.06 to 0.30)	0.183
Mean CD4 cell count (SD)¶	223 (192)	229 (177)		6 (–61 to 50)	0.838

*vs. male; †CDC stage C vs. A/B; ‡two nucleoside reverse transcriptase inhibitors (NRTIs) with one protease inhibitor (PI) vs. a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen; §$\log_{10}$ copies/mL; ¶cells/μL.
OR, odds ratio (risk of being positive for the parameter for non-Europeans compared to Europeans); CI, confidence interval; SD, standard deviation.
Follow up [median (range)]: European: 30.5 (6–73) months; non-European: 18 (6–74) months.

Table 2 Characteristics of the 12 patients who failed highly active antiretroviral therapy (HAART) in the first 6 months of treatment

Patient	Origin*	Gender	Baseline CD4 (cells/μL)	Baseline viral load (copies/mL)	Baseline CDC	Antiretroviral therapy		Treatment interruptions	HIV subtype	Baseline primary mutations	ARV toxicity at time of failure	Drug serum levels
						Initial	At failure					
1	NE	Male	79	186800	A	NFV, ZDV, 3TC	ddI, NVP, d4T	Not known	B	–[†]	–	NFV adequate
2	NE	Female		364000	C	d4T, IDV, ddI	d4T, IDV, 3TC	Not known	Not known	?[‡]	–	SQV[§] undetectable
3	NE	Female	156	29760	A	NFV, ZDV, 3TC	NFV, ZDV, 3TC	Not known	Non-B	–	–	NFV adequate
4	NE	Male		175440	A	IDV, ZDV, 3TC	IDV, ZDV, 3TC	Not known	Non-B	–	–	IDV undetectable
5	NE	Female	32	765000	C	RTV, SQV, ZDV, 3TC	RTV, SQV, ZDV, 3TC	Probably	Non-B	–	–	RTV, SQV undetectable
6	NE	Male	390	34400	A	NFV, ZDV, 3TC	NFV, ZDV, 3TC	Not known	B	–	–	Not possible
7	NE	Female	398	52300	A	NFV, ZDV, 3TC	–	Yes	B	–	+	No treatment during failure
8	NE	Male	97	547120	A	SQV, ZDV, 3TC	SQV, ZDV, 3TC	Not known	Non-B	+ 98G[¶]	–	SQV undetectable
9	E	Male	510	48000	A	IDV, ZDV, 3TC	–	Yes	B	–	+	No treatment during failure
10	E	Male	186	750000	A	NFV, ZDV, 3TC	NFV, ZDV, 3TC	Not known	B	+ 67N, 219Q, 69D[#]	–	NFV borderline
11	E	Male	196	300000	A	NVP, NFV, ZDV, 3TC	NFV, ZDV, 3TC	Not known	Not known	–[††]	–	NFV adequate
12	E	Female	345	412000	A	NFV, ZDV, 3TC	–	Yes	B	–	–	NFV undetectable

Frequently low drug serum levels and/or treatment interruptions among HIV-1-infected patients who fail HAART.
*NE, non-European; E, European.
[†]Genotyping of HIV at time of failure showed a virus with mutations resulting in resistance to 3TC and NFV (30 N, 90 M and 184 V).
[‡]No sample available for genotype testing.
[§]No sample available for drug level measurement in the first year. Under treatment with SQV, d4T and 3TC 1 year after initiation of HAART, SQV was undetectable in the serum of the patient.
[¶]Possible non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance-conferring mutation.
[#]ZDV resistance; antiretroviral therapy less effective.
[††]Genotyping of HIV at time of failure showed a virus with mutations resulting in resistance to NFV (30 N) and 3TC (184 V).
CDC, centres for disease control. NFV, nelfinavir; ZDV, zidovudine; 3TC, lamivudine; ddI, didanosine; d4T, stavudine; IDV, indinavir; NVP, nevirapine; SQV, saquinavir; RTV, ritonavir.

sex, type of initial treatment and baseline viral load and CD4 cell count (OD 4.6; 95% CI 1.1–20.2; $P = 0.042$). The unadjusted OR was 5.3 (95% CI 1.4–21.9; $P = 0.007$).

Table 2 shows some of the characteristics of the 12 patients who failed in the early phase of HAART. In at least seven of these 12 patients (58%), drug serum levels were undetectable and/or treatment was interrupted on the patient's own initiative. Only one of these seven patients had a baseline resistance-conferring mutation for NNRTI. This patient was not treated with a NNRTI-containing regimen. Of the remaining five patients, three had adequate drug serum levels at time of failure. Of these three patients with adequate drug levels, one had resistance to the given HAART prior to initiation of HAART and two had developed a resistant virus under treatment in the first 6 months.

Eleven of the 12 early failing patients finally achieved undetectable viral loads under treatment. In seven of these 11 patients, the viral load remained undetectable.

Late phase

After 6 months of treatment, 204 (94%) HIV-infected patients had a successful response to HAART. Median follow-up in the late phase was 30.5 (range 1–73) months for European and 18.0 (1–74) months for non-European patients. Of the 148 European patients who had successful responses to HAART in the early phase, 48 (32.4%) failed in the late phase; 42 had virological failure, two developed a new AIDS-defining event and four died. Of the 56 non-European patients who had a successful response to HAART in the early phase, 11 (19.6%) failed in the late phase; nine had virological failure and two died from HIV-related causes.

The risk of failure in the late phase was not statistically significantly different in European and non-European patients after adjustment for differences in duration of follow up, age, sex, type of initial treatment, baseline viral load and CD4 cell count (HR 0.6; 95% CI 0.3–1.2; $P = 0.14$).

Discussion

HAART failure in the first 6 months of treatment occurred significantly more often in non-European than in European HIV-infected patients in our Dutch cohort. If HAART was successful in the first 6 months, failure rates became similar in the two groups.

Some important baseline characteristics differed between the two groups. Non-Europeans were significantly more often female, were younger and used more frequently a NNRTI-containing regimen. However, the association between early failure and origin of patients remained highly significant after adjustment for these differences in the multivariate analysis.

Recent studies did not find any significant evidence of a gender difference in virological, immunological or clinical outcomes after starting HAART [14,15].

It is unlikely that the younger age of the non-European patients contributed to the increased risk of treatment failure, because older age at presentation has been found to be associated with shortened survival and increased risk of disease progression under treatment [15,16]. The more frequent use of NNRTI-containing regimens in the non-European group is not likely to have influenced the outcome either, because studies comparing PI-containing regimens with NNRTI-containing regimens have shown no differences in immune reconstitution [17]. The response of patients infected with African subtypes of HIV-1 to HAART appears to be independent of regime and HIV-1 clade [18].

The strength of this study is that baseline mean viral load and CD4 cell count were similar in the two groups and so could not have influenced the results. It has been reported that a low CD4 cell count at initiation of therapy and high viral load at baseline are dominant prognostic factors in patients starting HAART and are associated with a higher risk of disease progression [15,19]. Two previous studies investigating differences in response to HAART between African and white patients were limited because of significantly lower CD4 cell counts at baseline in the African HIV-infected patients [20,21].

Possible explanations for the difference in early failure rates between the two groups include differences in resistance to HAART at the start of therapy, different HIV subtypes with different susceptibility to antiretroviral treatment, and differences in pharmacokinetics and adherence.

Resistance to antiretroviral therapy is known to play an important role in therapeutic failure. Recently, it was reported that the proportion of new HIV infections in North America that involve drug-resistant virus is increasing [22]. In this study, we only genotyped HIV in the 12 patients who failed HAART in the early phase.

Mutations causing resistance prior to the initiation of HAART were detected in one of the eight non-European patients and in one of the four European patients. In one of these two patients, mutations in the NNRTI region were detected at baseline. However, no NNRTI was included in the initial HAART regime of this patient. Therefore, the presence of the NNRTI resistance-conferring mutations did not contribute to therapeutic failure. In conclusion, only one of the 12 early treatment-failing patients had mutations causing resistance prior to the initiation of HAART.

Because of the lack of information about HIV genotypes in the patients who responded successfully to treatment, these results confirm the occurrence of resistant virus

among treatment-naive HIV-infected patients, but are not conclusive regarding the role of resistance in increased early virological failure among non-Europeans.

The findings of studies on the effect of HIV subtype on response to antiretroviral therapy are conflicting. It is known that European patients are more often infected with HIV-1 subtype B and that Africans are more often infected with non-B HIV-1 subtypes, as was also found in our treatment-failing patients. Whether this compromises the response to therapy was not determined conclusively in previous studies. A Spanish study showed that, in a clinic in Madrid, polymorphisms at the positions involved in PI resistance occurred more often in non-B HIV subtypes, suggesting that this may play a role in virological failure when treating patients with non-B HIV subtypes with PI-containing regimens [5]. However, a Belgian study found no difference in viral load response to a nelfinavir-containing regimen in treatment-naive HIV-1-infected patients between B and non-B subtypes [23]. Because the non-European group in our study is a mixed group, no inferences can be made with regard to the role of B vs. non-B responses.

NNRTI-containing regimens instead of PI-containing regimens were more often prescribed to non-European patients in our cohort. However, the only early treatment-failing non-European patient with a resistant virus showed mutations in the NNRTI region, and this patient was treated with a PI-containing regimen.

An interesting question is the influence of race on pharmacokinetics. Lower antiretroviral drug serum levels among black individuals, compared with white individuals, as a result of differences in metabolism have not been described in the literature. Recently, however, differences in the clearance of efavirenz between races have been described.

An American study reported that black and Hispanic patients had a 32% slower clearance of efavirenz compared with Caucasians [24]. A CYP2B6 allelic variant that may occur more commonly in black than in white individuals is associated with approximately 3-fold higher plasma efavirenz levels in homozygote genotypes [25]. This may explain the susceptibility to efavirenz central nervous system side-effects and the increased rate of efavirenz discontinuation. In our 12 early treatment-failing patients, we found no elevated drug serum levels. This does not exclude the possibility of earlier discontinuation of HAART because of toxicity. In two of the 12 early treatment-failing patients, side effects were mentioned in the period of treatment failure. These two patients, one European and one non-European, discontinued treatment. Neither of them was on treatment with efavirenz. In conclusion, we found no indication of an influence of pharmacokinetics in our cohort. However, the toxicity of HAART may influence adherence overall.

Excluding immunological and virological associated influences as probable causes of early treatment failure, the most likely reason for early treatment failure is poor adherence. Although our study was not designed to determine the exact cause of treatment failure, the frequent undetectable drug levels and treatment interruptions among most early treatment-failing patients, and especially among non-Europeans, as well as the absence of pre-existent resistance to prescribed antiretroviral drugs among these patients suggest that poor adherence to treatment was the main cause of early virological failure. Most of the non-European patients were asylum-seeking refugees from African countries. Language barriers and cultural differences make communication and health education difficult tasks.

Once patients had responded successfully to HAART and had been on treatment for some time, with perhaps a consequent improved understanding of the disease and its treatment, there was no difference in the frequency of treatment failure. The hypothesis that poor medication knowledge is associated with lower medication adherence was supported in a recent study of HIV-infected patients who had recently started HAART or had switched to another regimen [6]. In that study, the ethnic origin of the patients was not defined.

Our data contrast with the findings of two earlier studies. Frater *et al.* found no significant difference in initial response to HAART. However, after 9 months of antiretroviral therapy, they found a relative increase in viral load in the African cohort [4]. They contributed this late failure to poor adherence by excluding other causes of failure based on findings in other studies. In contrast with their study, in our cohort we examined the early failures more closely and found evidence for poor adherence in low serum antiretroviral medication levels. A Danish study found no major differences between white and non-white patients with respect to virological, immunological or clinical responses to HAART, but the non-white patients started receiving treatment after a longer period of latency, which might also be interpreted as early poor outpatient adherence [21].

The few studies, including ours, comparing response to HAART between European and Non-European HIV-infected patients so far are retrospective studies in which information about adherence is often absent. In view of the important role poor adherence seems to play in treatment failure among non-European HIV-1-infected patients, a prospective study to determine the factors that cause poor adherence should be performed. Different methods for examining adherence have been described [9,26]. Close

observation and repeated drug serum level measurements might elucidate the influence of possible differences in pharmacokinetics on virological failure and adherence. When the determinants of poor adherence in non-European HIV-1-infected patients have been revealed, an intervention study may indicate how to decrease treatment failure among non-European HIV-1-infected patients in European countries.

In conclusion, non-European HIV-1-infected patients more often failed treatment during the first 6 months of HAART. However, once a successful response to HAART had been achieved in the early phase, response rates in the late phase were similar for Europeans and non-Europeans. Efforts to increase adherence to HAART may improve the prognosis of non-European HIV-1-infected patients in European countries.

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