POPULATION PHARMACOKINETICS OF EFAVIRENZ IN HIV PATIENTS:

INFLUENCE OF THE CYP2B6 GENOTYPE

Running title: INFLUENCE OF CYP2B6 GENOTYPE ON PHARMACOKINETICS OF EFAVIRENZ

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ABSTRACT

A population pharmacokinetic model of efavirenz has been developed from therapeutic drug monitoring data in HIV-positive patients using a non-linear mixed effect model (NONMEM).

The efavirenz plasma concentrations (375) of 131 patients were analyzed using HPLC-UV. Pharmacokinetic parameters were estimated according to a one-compartment model. The effects of sex, age, total body weight, height, body mass index and HIV treatment were analysed. In a subgroup of 32 patients, genetic polymorphisms of CYP2B6, CYP3A4 and MDR1 were also investigated.

Efavirenz oral clearance and the apparent volume of distribution were 9.50 liters/h and 311 liters respectively. The model included only the effect of CYP2B6 polymorphisms on efavirenz clearance; this covariate reduced the inter-subject variability of clearance by about 27%. Patients showing G/T and T/T CYP2B6 polymorphisms exhibited efavirenz clearances of about 50% and 75% lower than those observed in the patients without these polymorphisms (G/G). Accordingly, to obtain EFV steady state concentrations within of therapeutic range (1-4 mg/liter), it would be advisable to implement a gradual reduction in dose to 400 or 200 mg/day for intermediate or poor metabolizers patients, respectively. However, the remaining interindividual variability observed in the pharmacokinetic parameters of the model highlights the need for dose individualization to avoid inadequate exposure to efavirenz and suggests that these recommended doses be used with caution and confirmed by therapeutic drug monitoring and clinical efficacy. The population model can be implemented in pharmacokinetic clinical software for dosage optimization using Bayesian approach.
INTRODUCTION

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that has demonstrated appropriate efficacy and safety in the treatment of HIV-1 infection in several clinical studies. It is therefore often used in highly active antiretroviral therapy (HAART) implemented for the treatment of both naïve and experienced patients (25).

The metabolism of EFV includes hydroxylation by cytochrome P450 (CYP) 2B6 and, to a lesser extent, by CYP3A4 isoenzymes, followed by glucuronidation. Accordingly, the interindividual variability in its clearance—and hence its steady-state concentrations—will be related to variability in the activity of these cytochromes. These isoenzymes may undergo induction or inhibition, which must be taken into account when co-administering other drugs that act as substrates for these particular isoforms. Autoinduction is also seen for both of them; this is completed in approximately 28 days and elicits a significant decrease in the half-life of EFV (1). Furthermore, the pharmacokinetic (PK) variability may be partly explained in terms of the polymorphisms present in these isoenzymes as well as the proteins involved in drug transport (19), both of which affect its activity. The MDR-1 gene (Multi-drug resistance) encodes P-glycoprotein (P-gp), which is important in the transport of different substrates, including some antiretroviral drugs, although the influence of polymorphisms in this gene on plasma EFV levels has not been clearly established and remains under debate (16, 48).

The therapeutic drug monitoring (TDM) approach for the optimisation and individualisation of drug administration is of huge interest in the treatment of HIV because
it seems able to increase the efficiency of treatment and, possibly, lower the adverse effects
of antiretrovirals. EFV fulfils many of the criteria for TDM, including a possible correlation
between its plasma concentrations and the pharmacological effect, measured by CD4 cell
counts and viral load, as well as toxicity (11, 27, 30). This correlation is better with toxicity
than with efficacy (12). Additionally, many acceptable analytical assays are currently
available for this drug, and its kinetic disposition displays high inter-patient and low intra-
patient variabilities (11). TDM is also considered a good tool for estimating adherence.

Non-adherence is one of the main problems associated with the emergence of resistant
viruses (22, 44, 47) and has a high incidence: 33-60% (13, 38, 43).

Nevertheless, viral resistance mutations to HAART, which lead to poorly defined
therapeutic ranges, is one of the main problems hampering the TDM of these drugs. In fact,
relatively well defined therapeutic ranges have only been established for naïve patients.
Accordingly, the accepted therapeutic range for EFV -1 to 4 mg/liter (3-13 µmol/liter) (11, 
27) - must be interpreted with caution because it may change, depending on patient status
(combination regimens with other antiretrovirals, previous exposure to antiretrovirals or
not, resistance mutations, etc.). Indeed, some investigators (40) have suggested that the
lower limit for the therapeutic range should be raised to 2.3 mg/liter (7 µmol/liter). Despite
this limitation, there is great interest in TDM as a rational approach for improving HAART,
and many trials have assessed the feasibility of concentration-controlled therapy studies,
evaluating its impact and confirming its benefits (2, 8, 14, 18, 43). In fact, the TDM of
antiretrovirals has been included as part of the diagnostic set-up for HIV-infected patients
in the national guidelines of different countries such as France, the United Kingdom and the
Netherlands. Recently, Dahri and Ensom (12), advice the use of a previously published
decision-making algorithm (15), to determine if TDM is warranted for antiretrovirals. However, further studies in clinical setting will need to be conducted before such an approach can be recommended for widespread use.

Population PK enable the estimation of PK parameters from sparse data, such those from TDM, and hence permits information about the population of interest to be collected with a minimum of blood sampling from each patient. This strategy also permits the identification of the sources of variability able to explain inter-patient differences, a key factor in this kind of drug therapy; demographics, gene expression, clinical status and concomitant therapy are the main factors to be investigated. In this sense, population PK modelling has become a valuable tool for identifying and quantifying variability in the exposure to antiretrovirals (5).

Although several population PK analyses of EFV in HIV-positive patients have been published to date (11, 24, 25, 31, 33, 34, ), only some of them have analyzed the influence of the genotype of CYP2B6 (11, 31), the main enzyme involved in EFV metabolism. The aim of the present study was to develop a population PK model of EFV from sparse data (collected with TDM) in HIV-positive patients, using nonlinear mixed effect modelling (NONMEM). This methodology was used to evaluate the effects of age, total body weight (TBW), height, sex, body mass index (BMI) and the CYP2B6, CYP3A4 and MDR1 genotypes, on the PK profile of EFV.

METHODS
Patients and treatment

The analysis was conducted in 131 HIV-infected subjects treated in the outpatient unit of the Pharmacy Service of the University Hospital of Salamanca, from October 2005 to April 2007.

The inclusion criteria were: a) confirmed HIV infection; b) treatment initiated with EFV at least three months before patient inclusion in the study (unchanged dosage for at least 1 month); c) adherence to the treatment regimen higher than 90%; d) age equal to or greater than 18 years and e) no comedication with known drug inducers.

Adherence was assessed using dispensation records, the Simplified Medication Adherence Questionnaire (SMAQ) (26) and coefficients of variation (CV) of the mean EFV plasma concentration/dose ratio in each patient of less than 30%, according to previously observed intrapatient variability (11).

Most patients received EFV at 600 mg/day, with the exception of some who received 400 or 800 mg/day once daily (mean ± SD: 597.2 ± 33.3 mg/day) as part of their HAART regimen, and all of them had at least two EFV plasma concentrations for analysis. The demographic and clinical characteristics of patients included in the study are shown in table 1.
The study was subjected to approval by the ethics committee of the University Hospital of Salamanca and the patients gave written informed consent for genetic testing.

All patients, who were enrolled in a Pharmaceutical Care Program, were receiving EFV associated with two nucleoside reverse transcriptase inhibitors (NRTI), and a protease inhibitor (PI), boosted or not with ritonavir, was only added in three cases.

**Sampling, analytical assays and genetic analyses**

Patients were included in a TDM program and one blood sample was obtained during each visit to the Hospital. Plasma samples for measuring drug concentrations were collected at steady-state (more than 4 weeks after the initiation of EFV treatment), usually at the mid-point of the dosage interval. The times after ingestion (mean ± SD: 10.3 ± 1.8 h) were recorded and a mean of 2.9 samples per patient was obtained (ranging from 2 to 7).

EFV concentrations were assessed quantitatively with high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (9). This method was validated over the 0.5 to 10 mg/liter range using 600 µl of plasma. The recovery of EFV from human plasma was 107.4%. Within and between-day precisions, expressed as CV, were always < 5.7% for all the internal quality controls (0.5, 2.0 and 10.0 mg/liter). The limit of quantification was 0.25 mg/liter and the specificity of the 21 drugs most used in HIV patients was tested. Our analysis laboratory participates in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection (Dutch association for Quality assessment in Therapeutic Drug Monitoring and Clinical Toxicology [KKGT]), and successful results have been obtained.
In order to characterise the genetic polymorphisms of CYP2B6, CYP3A4 and MDR1, the patients’ blood samples were analysed using the PHARMACHIP® developed by Progenika, whose methodology is based on specific allele oligonucleotide probes imprinted on a glass support (42).

Covariates

To identify possible correlations between EFV oral clearance (CL/F) and the demographic and treatment characteristics of the patients, the following covariates were collected: sex, age, weight, height and HIV treatment. In addition, the genetic polymorphisms of CYP2B6, CYP3A4 and MDR1 were analysed. Because of the high cost of this, only 32 patients of the total group analysed (n = 131), chosen according the magnitude of their estimated EFV trough steady-state plasma concentrations (C_{ss min}), were genotyped: all patients with high (C_{ss min} > 4 mg/liter; n = 15) and low (C_{ss min} < 1 mg/liter; n = 9) concentrations and only 8 patients with concentrations within the therapeutic range (C_{ss min}: 1 - 4 mg/liter), the latter chosen randomly from the 107 remaining patients. Figure 1 shows this selection process and the mean age and weight of the genotyped patients, most of them Caucasians. The total number of EFV plasma concentrations was 158 in this subset of patients.

Population pharmacokinetic analysis

A PK population model was developed using NONMEM program (v. VI, University of California, San Francisco, USA), described elsewhere. A one-compartment open kinetic
model with first-order absorption and elimination (specified to NONMEM by the ADVAN2 and TRANS2 routines) was assumed. Thus, the fixed-effect PK parameters estimated directly with the specifications of this model were CL/F and the apparent distribution volume (V/F). The absorption rate constant (k_a) was fixed to 0.3 h^{-1}, following the study of Csajka et al. First-order conditional estimation (FOCE) with Laplace approximation was used throughout.

Both additive (θ_j = θ' + η_{θj}) and exponential (θ_j = θ' exp η_{θj}) error models were tested to describe interindividual variability, where θ_j is the estimate for a PK parameter in the j^{th} individual as predicted by the model; θ' is the population mean of the PK parameter, and η_{θj} represents the random variable with zero mean and variance ϖ^2. Covariance was also estimated. It should be noted that the first-order method used in this analysis approximates the exponential error model as a proportional error model. The terms for interindividual variability were included only for CL/F and V/F. Additionally, additive (C_{ij} = C'_{ij} + η_i) and exponential (C_{ij} = C'_{ij} exp η_i) error models were tested to estimate residual variability, where C_{ij} and C'_{ij} are the observed and predicted EFV concentrations for the j^{th} individual at time i, respectively, and η_i is the additive error (with zero mean and variance σ^2). To elucidate the preliminary relationships between a PK parameter obtained using a Bayesian maximum a posteriori estimation (POSTHOC option in NOMEM) and covariates, a graphic approach to exploratory data analysis and the stepwise generalized additive model (GAM) implemented in Xpose were used. The inclusion of a fixed-effect parameter in the basic model quantifies the relationship between a PK parameter and a covariate, and allows it to be known whether that covariate significantly improves the ability of the model...
to predict the observed concentration-time profile. The objective function value (OFV) difference between two hierarchical models is asymptotically $\chi^2$-distributed, with degrees of freedom (d.f.) equal to the difference in the number of parameters between the two models, and it should be at least 3.84 (if d.f. = 1) in order to achieve the desired level of significance of $\alpha = 0.05$. Other diagnostic criteria for the retention of a covariate in the model were: a reduction in unexplained interindividual variability for the associated PK parameter; an improvement in the graphic diagnostic model, evaluated by randomly distributed weighted residuals; a closer relationship between the predicted and observed concentrations, and that the 95% confidence interval, estimated using standard errors (SEs), should not include a zero value. A further criterion was that the percentage estimation error (EE) of fixed and random parameters should not be higher than 25 and 50% respectively (3). The full model thus generated was then subjected to backwards elimination, where each model parameter was fixed to a zero value, using a more stringent criterion of statistical significance ($\alpha = 0.01$).

**Model Validation**

The suitability of the final model was evaluated using pseudo-residuals, a validation approach proposed by Mesnil et al (28). Monte Carlo simulation conducted with NONMEM was applied to mimic the mean EFV concentrations in adult patients receiving standard doses of EFV (600 mg/day) by assigning the individual characteristics included in the final model (CYP2B6 genotypes) to the simulated population. One thousand random concentrations were generated for each simulated patient and the pseudo-residuals were
RESULTS

In the basic model, both interindividual and residual variabilities were best described by proportional structures. In this EFV model without covariates, the mean population estimates for CL/F, V/F and their interindividual CVs were 9.22 liters/h, 295.0 liters, 50.30% and 79.75%, respectively. The CV of residual variability was 19.70%.

Graphic exploratory analysis of the relationship between the individual Bayesian CL/F and V/F estimated with NONMEM (POSTHOC option) using the covariates analysed by GAM revealed that TBW and BMI were weakly correlated with them. Additionally, the patients showing G/T and T/T CYP2B6 polymorphisms exhibited a drug CL/F around 50% and 75% lower, respectively, than patients without these polymorphisms. These results were confirmed when this discrete covariate -CYP2B6 polymorphisms- was included in the full population model, as well as when the model was developed only in the 32 genotyped patients. According to this exploratory analysis, no other covariate was found to significantly correlate with the individual Bayesian PK parameters. In fact, when age, sex, CYP3A4, MDR1 and HIV treatment were included in the model, the magnitude of the estimates was negligible and the percentage of associated SE was greater than 100%, indicating the lack of statistical and clinical significance of these covariates. However, the
effects of sex, BMI, TBW, height, CYP2B6, CYP3A4 and MDR1 polymorphisms were
analysed in the different population models assayed.

Table 2 summarizes the statistical analysis of some covariates tested with NONMEM
according to this preliminary analysis and shows the differences in the OFV with respect to
the basic model and the variations in the interindividual variability of CL/F and V/F
expressed as CV (%). The influence of the TBW and BMI on either CL/F or V/F did not
contribute significantly to the goodness of fit, and indeed the OFV increased with respect to
the basic model when they were included in the model. Only the effect of CYP2B6
genotypes on CL/F contributed to a significant decrease in OFV. Thus, the final regression
model, whose values are summarized in table 3, can be defined as follows:

\[
\text{CL/F (liters/h)} = \theta_1 \times e^{-0.03 \times \text{CYP2B6}}
\]

\[
\text{V/F (liters)} = \theta_2
\]

where \(\theta_1\) and \(\theta_2\) are the estimated coefficients for CL/F and V/F, respectively, \(\theta_3\) is the fixed
parameter relative to CYP2B6 polymorphisms (which took values 0, 1 or 2 when the
patients had wild-type (G/G), heterozygote (G/T) and homozygote (T/T) genotypes,
respectively). In this model, the estimate of the CV for interindvidual variability in CL/F
was 36.47% vs. 50.30% when the covariates were not incorporated into the model (basic
model). Regarding V/F, although none of the covariates could be included in the final
model, its CV\(_{V/F}\) was reduced from 79.75% in the basic model to 55.14% in the final one.
Residual variability was decreased by about 25% from the basic model. Additionally,
scrutiny of the scatterplot of weighted residuals (WRES) vs. predicted concentrations
obtained from the final model revealed a significant improvement in its pattern (random
distribution) with respect to the basic one, in agreement with the OFV decrease (OFV difference = 175.438; p<0.01). Fixed-effect parameters were estimated with a SE of less than 20%. All the random effects had SE values of less than 35%.

Figure 2 shows the scatterplots of the measured EFV concentrations vs. those predicted by the basic and the final models, obtained with the full population (N=131) and with the genotyped subgroup (N=32). These plots show the improvement in fit obtained in both situations with the final model, reflected as a tighter and more random scatter around the identity line. Furthermore, the correlation coefficients of the linear regression between the observed vs. fitted concentrations with the final models were 0.690 (full population; p<0.05) and 0.687 (genotyped population; p<0.05); i.e., significantly better than the 0.07 and 0.03 calculated when EFV plasma concentrations were estimated with the basic models.

In order to evaluate the final model, pseudo-residuals were computed using 1000 samples per individual simulated by Monte Carlo. The QQ-plot is shown in Figure 3, which depicts the quantiles of the normalized pseudo-residuals vs. the theoretical quantiles from a normal distribution; the assumption of this distribution appears reasonable, since deviations from the identity line (x=y) show minimal departures from the expected distribution. A p-value of 0.494 was found, which is higher than the empirical one (0.0037), and hence the model tested can be considered adequate.

DISCUSSION
The clinical usefulness of patient-specific PK data for antiretrovirals has been evaluated in an effort to develop strategies for monitoring drug exposure in HIV-infected patients (29). The goal of the present study was to investigate the population PK parameters of EFV estimated from TDM data in order to implement them in clinical PK software for dosage optimization.

Although the kinetics of EFV seems to be better described by a two-compartment model (4, 24, 45), in most studies (11, 25, 31, 33, 34) a one-compartment model has been assumed. In view of this, and of the poor design (data from TDM), here a one-compartment model that appears to describe the data adequately was used. Although sampling was only performed at certain discrete and previously defined times (sparse data), CL/F and V/F could be estimated with acceptable SEs.

Although the literature reports very different values for $k_a$ (from 0.18 to 1.39 h$^{-1}$) (31, 33), a $k_a$ value of 0.3 h$^{-1}$ was fixed in the model in agreement with Csajka et al (11), which is the most consistent study estimating $k_a$ owing to the number of patients and sampling times used. However, being aware of the limitation of fixing this parameter, we tested other models in which $k_a$ was fixed at other values found in the literature but no significant differences in the estimation of CL/F were observed. These results are consistent with those reported by Wade et al. (46), who found that misspecification of $k_a$ does not markedly affect the ability to adequately estimate the CL/F, the main PK parameter for drug dosage optimization. Meal composition also seems to affect EFV absorption (32), but in the present study the effect of a high-fat diet could not be taken into account because of the
different dietary habits of the patients and the impossibility of establishing a protocol for its
analysis. In any case, the effect of diet was minimised since in most patients drug
administration was performed at least two hours after the evening meal (in agreement with
the recommendations of the Pharmaceutical Care Program).

Knowledge of the different factors affecting the PK of a given drug is critical in
decisions regarding dosage. TBW and BMI, which are representative indices of body size
and which are usually well correlated with drug PK parameters, were not seen to exert any
significant influence on the CL/F and V/F of EFV, despite the weak relation observed in the
preliminary graphic analysis. In fact, the OFV was significantly increased (> 30 units) with
respect to the basic model when these covariates were included in the model (table 2). Most
PK studies carried out on EFV to date have failed to demonstrate a relationship between
TBW and the PK parameters (11, 24, 25, 31, 33), except for a recent publication (41), in
which this covariate was included as factor influencing EFV plasma concentrations. The
relatively narrow age range of the group studied (41.8 ± 9.1 yr), most corresponding to
adult patients (only 4 > 65 yr), was probably the reason why age could not be included in
the model.

Sex (86 males/45 females), which was relatively well represented in our population,
does not appear in the final model either, since the statistical criteria required for its
inclusions were not fulfilled. Previously published results for this covariate are
contradictory. Thus, whereas some investigators have reported a decrease in the CL/F in
women (4, 31), other authors have failed to find such a difference (11, 25, 33); so studies
with large numbers of women are required to attempt to establish the true influence of sex,
since most studies have included a significantly lower percentage of women than men.

Other studies using the same population approach (NONMEM) have also failed to reveal any significant relationship between demographic covariates (age, sex and TBW) and the PK parameters of EFV (11, 24, 25, 33). The mean estimated values for CL/F found by those authors ranged from 8.82 to 11.70 liters/h, similar to those observed in our population (9.50 liters/h). Another recent population model developed in black patients with a low number of EFV plasma concentrations analyzed also afforded a similar value of CL/F (9.4 liters/h), although this model included the sex as a covariate (31).

The estimated value for V/F, 311 liters in the proposed model, is similar to the values reported by Pfister and Csajka (282 and 252 liters, respectively), although other studies have reported widely varying values, ranging from 150 to 421 liters (31, 34). This broad range of values could be explained in terms of a probable degree of uncertainty in the estimation of V/F owing to the nature of the data (sparse data) usually analysed in most studies. In fact, in our study V/F was also estimated from a limited sampling at a single time point; this is why we tried to fix this parameter to the bibliographic values. However, poorer fits were obtained and greater uncertainty in CL/F estimation.

It is foreseeable from our results and those of other studies that genetic factors (20, 34) contribute significantly to interindividual variability. Differences in PK due to the CYP2B6 polymorphisms have been reported for several drugs, including EFV, that are mainly metabolized by this isoenzyme (7, 37, 39).
Of the 15 patients with abnormally elevated EFV levels, 12 (80%) had CYP2B6 G516T polymorphism (6 T/T and 6 G/T) and only 20% did not have it. Although only 32 patients were genotyped (24.4% from the total population studied), which is the main limitation of this study, these findings again support the important effect of the genotypes of this isoenzyme on EFV clearance. Thus, the G/T (intermediate-metabolizers) and T/T (poor-metabolizers) genotypes modified CL/F by factors of 0.53 (95% CI: 0.44 - 0.65) and 0.28 (95% CI: 0.22 - 0.35), respectively, with respect to the G/G (extensive metabolizers) genotype. Accordingly, CYP2B6 genotypes could partly account for the large interindividual variability in EFV PK and identify individuals at risk of extremely elevated EFV plasma levels.

Other researchers have observed the effect of CYP2B6 genotypes on the CL/F of EFV but, using the same methodology, the quantitative influence on this PK parameter has only been identified by Nyakutira (2008) in black patients (31). This author found ratios of EFV clearance for intermediate and poor metabolizer patients with respect to extensive metabolizers of 0.77 (95% CI: 0.46 - 1.1) and 0.42 (95% CI: 0.21 - 0.63), respectively. These values are higher than those found by us, although our mean values lie within the 95% CI proposed by this author. However, the higher clearances estimated in this study could be justified in terms of the use of concomitant medication, including the inducer rifampicin, a first-line drug in tuberculosis-coinfection, which was not recorded in that study, even though this co-infection affected more than 60% of the patients analyzed. Thus, rifampicin was probably present in these patients, since this antibiotic has been shown to...
elicit increases of more than 30% in EFV clearance because of its induction effect on the CYP2B6 and CYP3A4 enzymes (6).

Since only 4 patients had the CYP3A4*1B polymorphism, three of them heterozygous (*1/*1B) and one of them homozygous (*1B/*1B), it was not possible to establish a relationship between this polymorphism and PK parameters in the final model. The low presence of the CYP3A4*1B polymorphism and its minor role in EFV metabolism could account for this. Previous studies aimed at evaluating the effect of CYP3A4*1B, CYP3A5*3 and CYP3A5*6 on EFV PK have also been unable to detect any influence (16, 21).

On the basis of statistical criteria, described in the Material and Methods section, some of them shown in table 2, polymorphisms in MDR1 could not be included in the final model, although a slight increase in EFV CL/F was observed in the preliminary graphic analysis. In this sense, previous studies addressing the relationship between the C3435T MDR1 gene and EFV plasma concentrations have afforded contradictory results (35, 36).

Although the incorporation of CYP2B6 polymorphism covariate in the final model reduced the interindividual variabilities of the CL/F and V/F parameters, these were still seen to have significant values (36.47% and 55.14% respectively). It is difficult to compare these findings with those obtained in other similar studies because the variabilities observed were attributed to different PK parameters of the model. Thus, Nyakutira estimated an interindividual variability for CL/F of 76%: significantly higher than that observed in our
study and in other publications (11, 24). Nevertheless, all PK variability was attributed to 
this parameter and the variability in other PK parameters such as V/F was ignored. Another 
example is the study of Csajka, who analyzed sparse data and attributed all the PK 
variability (54.6%) to bioavailability and ignored the probable variability of the remaining 
kinetic parameters estimated. Accordingly, although the results on interindividual 
variability in the PK parameters show some differences, all the above studies point to a 
relatively broad variability in the disposition kinetics of EFV.

The residual variability, expressed as CV, decreased from 19.70% to 14.70% from the 
basic to the final model, which indicates low variability within patients: an essential 
prerequisite for TDM. This residual variability was lower than that established by other 
authors (11, 24) but similar to that observed by Nyakutira (18%). This low value could be 
attributed to a closer follow-up of our patients, which were included within a 
Pharmaceutical Care program that addressed not only their adherence (assessed and 
considered as an exclusion criterion) but also their drug administration times with respect to 
the ingestion of food. In light of these results, in the future, a CV of the mean EFV plasma 
concentration/dose ratio in each patient of 20% could be used in our patients as a new 
adherence criterion instead of the 30% considered initially; this more restrictive criterion, 
would possibly contribute to a better knowledge about adherence, which is essential to 
achieve success in antiretroviral therapy.

All parameters of the population model were estimated with acceptable precision since 
the SE were less than 20% and 35% for the fixed-effect and random parameters,
respectively.
Although the results have been only compared with those obtained in studies using NONMEM (11, 24, 25, 31, 33), in general the estimated PK parameter values are in agreement with the results from earlier published studies, regardless of the methodology employed (34). Additionally, the results obtained with Monte Carlo simulation support the suitability of the model. Nevertheless, it would be desirable to perform an external validation study in a new group of patients with similar characteristics, although the absence of consensus concerning the usefulness of the TDM of antiretroviral agents makes the collection of this a slow process.

The final model proposed, although valid, has some limitations that should be noted: 1) In most cases a standard fixed dose of 600 mg/day was used, 2) a limited number of EFV concentrations per patient (range: 2-7) was used, usually collected at the mid-point of the dosage interval (sparse data from TDM); this is why mixed-effect models were used to adequately characterize the population pharmacokinetic, 3) the assumption of a one-compartment pharmacokinetic model, because this model is the one most widely used for this drug (11, 25, 31, 33, 34) and the nature of data prevents the use of more complex models and 4) owing to the high costs of genetic analysis, and the fact that this tool has not yet been introduced routinely in clinical practice, only 25% of the patients were genotyped.

According to the PK parameters of the population model, and bearing in mind the effect of CYP2B6 genotypes on CL/F, to obtain EFV steady-state concentrations close to the mean value of therapeutic range (1-4 mg/liter) it would be advisable to implement a gradual decrease in the dose to 400 or 200 mg/day for intermediate or poor metabolizer
patients, respectively. These low doses have also been proposed and their efficacy has been demonstrated in another study carried out in Japanese patients (17). However, the remaining interindividual variability in the PK profile observed in this study suggests that these recommended doses should be used with caution and confirmed by TDM and clinical efficacy. This is why we recommend implementing our PK population model in clinical software which, using Bayesian algorithms, permits EFV dosage to be optimized with a minimum number of drug plasma levels once adherence has been ensured. Thus, pharmacogenetics and PK combined with TDM should be used to guide EFV dosages. However, since genotyping has not been introduced into routine clinical practice, clinicians should initially be guided by the phenotype assessed through the plasma EFV concentrations obtained in TDM, which may also be used as a selection criterion concerning the patients to be genotyped in order to confirm that the cause of supratherapeutic concentrations is genetic and not attributable to other factors. Additionally, genetic information may prove to be useful for the “a priori” dosing of drugs whose kinetic profile is governed by isoenzymes and carriers encoded by genes susceptible to polymorphism. Finally, to confirm these preliminary results quantifying the influence of genetic factors, further prospective studies, with larger data sets, should be carried out.
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REFERENCES


Table 1. Demographic and clinical characteristics of the patients included in the study.

<table>
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<th>VARIABLE</th>
<th>Mean ± SD</th>
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<td>No. of patients (male/female)</td>
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<tr>
<td>No. of plasma concentrations</td>
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<td>Ethnicity (Caucasian/black)</td>
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<tr>
<td>Daily dose (mg/day)</td>
<td>597.2 ± 33.3</td>
</tr>
<tr>
<td>EFV plasma concentration (mg/liter)</td>
<td>3.30 ± 2.05</td>
</tr>
<tr>
<td>Plasma data per patient</td>
<td>2.9 ± 1.3</td>
</tr>
</tbody>
</table>

**FREQUENCY**

| Genotyped patients:                        | 32 (24.4)       |
| CYP2B6 (G/G)                                | 53.1            |
| (G/T)                                       | 28.1            |
| (T/T)                                       | 18.8            |
Table 2. Summary of the analysis of the influence of the covariates explored in the study on the pharmacokinetic parameters of the model.

<table>
<thead>
<tr>
<th>Covariates analysed</th>
<th>OFV (DOFV)</th>
<th>CV&lt;sub&gt;CL/F&lt;/sub&gt; (%)</th>
<th>CV&lt;sub&gt;V/F&lt;/sub&gt; (%)</th>
<th>σ (%)</th>
<th>Results and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basic model</td>
<td>340.477</td>
<td>50.30</td>
<td>79.75</td>
<td>19.70</td>
<td>Model without covariates</td>
</tr>
<tr>
<td>2. Does TBW influence CL/F?</td>
<td>409.108 (68.631)*</td>
<td>47.64</td>
<td>106.77</td>
<td>21.33</td>
<td>No</td>
</tr>
<tr>
<td>4. Does TBW influence V/F?</td>
<td>360.713 (20.236)*</td>
<td>44.27</td>
<td>77.65</td>
<td>19.25</td>
<td>No</td>
</tr>
<tr>
<td>5. Does sex influence CL/F?</td>
<td>382.694 (42.217)*</td>
<td>67.75</td>
<td>71.90</td>
<td>16.58</td>
<td>No. Unacceptable EE for the fixed parameter associated with the covariate (95% CI includes zero)</td>
</tr>
<tr>
<td>6. Does sex influence V/F?</td>
<td>382.359 (41.882)*</td>
<td>37.02</td>
<td>216.33</td>
<td>15.62</td>
<td>No. The parameter associated with the covariate is negligible (0.98)</td>
</tr>
<tr>
<td>7. Does CYP2B6 polymorphism influence CL/F?</td>
<td>165.039 (-175.438)*</td>
<td>36.47</td>
<td>55.14</td>
<td>14.70</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Does CYP3A4 polymorphism influence CL/F?</td>
<td>162.376 (-2.663)**</td>
<td>38.08</td>
<td>65.04</td>
<td>14.63</td>
<td>No. Unacceptable EE for the parameter associated with the covariate (95% CI includes zero)</td>
</tr>
<tr>
<td>9. Does MDR1 polymorphism influence CL/F?</td>
<td>161.375 (-3.664)**</td>
<td>37.81</td>
<td>60.99</td>
<td>15.26</td>
<td>No. Unacceptable EE for the parameter associated with the covariate (95% CI includes zero)</td>
</tr>
</tbody>
</table>

*With respect to the basic model; **With respect to model 7. EE: Estimation error; DOFV: Difference of objective function value (OFV); σ: residual variability expressed as CV.
Table 3. Parameter estimates and their standard errors (SE) for the final population model proposed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_1$ (liters/h)</td>
<td>9.50</td>
<td>4.06</td>
</tr>
<tr>
<td>$\theta_2$ (liters)</td>
<td>311</td>
<td>10.76</td>
</tr>
<tr>
<td>$\theta_3$</td>
<td>0.638</td>
<td>15.35</td>
</tr>
<tr>
<td>CV_{CL/F} (%)</td>
<td>36.47</td>
<td>15.58</td>
</tr>
<tr>
<td>CV_{V/F} (%)</td>
<td>55.14</td>
<td>34.74</td>
</tr>
<tr>
<td>$\sigma$ (%)</td>
<td>14.70</td>
<td>15.71</td>
</tr>
</tbody>
</table>

$\sigma$: residual variability expressed as CV

**Final model:**

$CL/F = \theta_1 \times e^{-\theta_3 \times CYP2B6}$

$V/F = \theta_2$
LEGENDS OF FIGURES

Figure 1: Patient selection for genetic analysis.

Figure 2: Observed (Cobs) vs. predicted plasma concentrations of EFV (Cpred) in the whole sample (A, B and C) and in the genotyped sample (A’, B’ and C’). A, A’: population concentrations predicted with basic model; B, B’: population concentrations predicted with final model; C, C’: individual concentrations predicted with final model.

Figure 3: QQ-plot of the pseudo-residuals in the simulated patients vs. the uniform distribution: the observed values are plotted against the theoretical quantiles for a uniform distribution over [0,1].
Efavirenz treated patient
N=131

Patients with $C_{\text{min}}^{\text{ss}} < 1 \text{ mg/L}$
N=9
(51.1 ± 14.3 years; 65.1 ± 11.4 Kg)

Patients with $C_{\text{min}}^{\text{ss}}$ between 1 and 4 mg/L
N=107
(43.0 ± 3.4 years; 63.8 ± 11.1 Kg)

Patients with $C_{\text{min}}^{\text{ss}} > 4 \text{ mg/L}$
N=15
(43.0 ± 3.4 years; 63.8 ± 11.1 Kg)

Random selection

Patients with $C_{\text{min}}^{\text{ss}}$ between 1 and 4 mg/L
N=8
(40.3 ± 3.9 years; 67.5 ± 12.8 Kg)