Biometrics 000, 000–000 DOI: 000
000 0000

Maximum likelihood estimation of long term HIV dynamic models and antiviral response

Marc Lavielle

INRIA Saclay, France

and

Adeline Samson

CNRS UMR8145, Université Paris Descartes, France

and

Ana Karina Fermin

Université de Nanterre, France

and

France Mentré

INSERM U738 and Université Paris-Diderot, France

Summary: HIV dynamics studies, based on differential equations, have significantly improved the knowledge of HIV infection. While first studies use simplified short-term dynamic models, recent works consider more complex long-term models combined with a global analysis of whole patients data based on nonlinear mixed models. This approach increases the accuracy of the HIV dynamic analysis. However statistical issues remain given the complexity of the problem. We propose to use the SAEM (Stochastic Approximation EM) algorithm, a powerful maximum likelihood estimation algorithm, to simultaneously analyze the HIV viral load decrease and the CD4 increase in patients using a long-term HIV dynamic system. We apply the proposed methodology to the prospective COPHAR2 - ANRS 111 trial. Very satisfactory results are obtained with a model with latent CD4 cells. The 10 parameters, 7 with between patient variability, of this model defined with five differential equations are well estimated. We show that the efficacy of nelfinavir is reduced compared to indinavir and lopinavir

Key words: HIV dynamics, Non linear mixed effects models, SAEM algorithm, Model selection

[1]This work was supported by the French ANR (Agence Nationale de la Recherche).

1. Introduction

Understanding variability in response to antiretrovial treament in HIV patients through modelling is an important challenge. Several HIV dynamic models were proposed to describe the decrease in viral load and the increase in CD4 cells under treatment and nonlinear mixed effect models (NLMEM) are appropriate to estimate the parameters of these models and their inter-patient variability. These dynamic HIV systems usually describe the interaction between several types of CD4 cells and two types of virions (infectious or not). They are defined as nonlinear differential systems and have generally no closed-form solutions. As the available data are the measurements of total number of CD4 (the sum of all the types of CD4) and the total number of virions (infectious and not infectious), the components of the differential system are partially observed, complicating the estimation of the parameters characterizing each component.

Several analyses of HIV dynamic models through NLMEM are published (Wu et al., 1998; Fitzgerald et al., 2002; Putter et al., 2002; Wu and Zhang, 2002; Wu, 2004; Wu et al., 2005; Huang et al., 2006; Guedj et al., 2007). The first analyses of viral load dynamic through modelling, using standard nonlinear regression or mixed models, considered a short time period and thus assumed that the concentration of non-infected CD4 cells was constant (Perelson et al., 1996; Wu et al., 1998; Ding and Wu, 2001). In this case, the differential system becomes linear with an analytical solution. Another simplified approach is to assume that the initiated therapy inhibits any new infection, which is unrealistic. Under that assumption, the system can be solved explicitly (Wu et al., 1998; Putter et al., 2002). Putter et al. (2002) proposed the first simultaneous estimation of the viral load and CD4 dynamics based on a differential system under this assumption, but had to focus only on the first two weeks of the dynamic after initiation of an anti-retroviral treatment. These two assumptions

are unsatisfactory when studying long-term response to anti-retroviral treatment for which the use of complete models expressed through differential equations is mandatory.

Maximum likelihood estimation of NLMEM is complex because the likelihood has no closed form, even for simple models. The first published approaches using complex ODE HIV models and NLMEM adopted a Bayesian statistical point of view. Putter et al. (2002); Wu et al. (2005); Huang et al. (2006) estimated parameters of ODE system using Markov Chain Monte Carlo (MCMC) algorithms to give posterior distributions of the parameters, given informative prior distributions on each ODE parameter. The choice of informative prior distributions is the main drawback of this approach. Furthermore, Bayesian algorithms can be very slow to converge, especially in this complex context. Wu et al. (2005) adjusted only viral load (i.e. the CD4 were not taken into account) with a dynamic system describing the long-term HIV dynamics and considering drug potency, drug exposure/adherence and drug resistance during chronic treatment of HIV-1 infected patients. However, the authors used a simplified model which does not consider separately the compartments of HIV-producing infected cells and the latent cells and do not decompose the virus compartment into infectious and non-infectious virions as proposed by Perelson et al. (1996); Perelson and Nelson (1997).

For maximum likelihood estimation in NLMEM, the first algorithms were based on approximations such as linearization (Pinheiro and Bates, 1995) or Laplace approximation (Wolfinger, 1993). However these algorithms may lead to inconsistent estimates (Ding and Wu, 2001). More recently algorithms based on Gaussian quadrature have been proposed (Guedj et al., 2007), however these algorithms are cumbersome and were not applied to problems with more than three random effects. Other new algorithms, more suited to problems with several random effects are stochastic EM algorithms. Among them, the Stochastic Approximation EM algorithm (SAEM) has convergence results (Delyon et al., 1999; Kuhn and Lavielle, 2005) also studied for models defined by ODE (Donnet and Samson, 2007). It

is implemented in the MONOLIX software and has been mainly applied for the analysis of pharmacokinetics models (see Lavielle and Mentré (2007) for example).

Another complexity of the analysis of viral load data is left censoring, as the experimental devices are not able to measure low level of viral load with sufficient accuracy. When viral load are below a given limit, namely the limit of quantification (LOQ), the exact value of the viral load is unknown. The proportion of subjects with viral load below LOQ has increased with the development of highly active anti-retroviral. Although it is known that when ignored this censoring may induce biased parameter estimates (Samson et al., 2006; Thiébaut et al., 2006), several authors did not take into account this problem (Ding and Wu, 2001; Wu et al., 2005). Conversively, Guedj et al. (2007); Hughes (1999); Jacqmin-Gadda et al. (2000) proposed different approaches to handle accurately the censored viral load data. Samson et al. (2006) extended the SAEM algorithm to perform adequate maximum likelihood estimation for left-censored data.

Given the drawbacks of published statistical approaches proposed for the estimation of HIV dynamic parameters, the first objective of this work is to estimate parameter of HIV dynamic models with the SAEM algorithm. A second objective is to propose and apply statistical approaches for studying model identifiability and model selection in this context. We apply this approach to the data obtained in patients of the clinical trial COPHAR2 - ANRS 111 (Duval et al., 2009) initiating an antiviral therapy with two nucleoside analogs (RTI) and one protease inhibitor (PI). We analyze simultaneously the HIV viral load decrease and the CD4 increase based on a long-term HIV dynamic system. We consider three biological models and compare them with respect to their ability to represent HIV-infected patients after initiation of reverse transcriptase inverse (RTI) and protease inhibitor (PI) drugs therapy. We also compare the different treatments used in this trial.

This article is organized as follows. Section 2 presents three mathematical models for long-

term HIV dynamics. In Section 3 we discuss nonlinear mixed effects models and estimation with the SAEM algorithm, model selection, model identifiability and covariate testing. We then provide the results obtained in the COPHAR II-ANRS 134 clinical trial using MONOLIX in Section 4. Section 5 concludes this article with some discussion.

2. Mathematical models for HIV dynamics after treatment initiation

In this section, we present three nonlinear ordinary differential systems modeling the interaction of HIV virus with the immune system of the human body after initiation of antiviral treatment containing reverse transcriptase inhibitor (RTI) and protease inhibitor (PI).

The most basic of these models includes four components :noninfected CD4 cells and infected CD4 cells, infectious viruses and noninfectious viruses (Perelson and Nelson, 1997; Perelson, 2002). The second model differentiates the noninfected CD4 cells into activated and quiescent noninfected (De Boer and Perelson, 1998; Guedj et al., 2007). The third model differentiates the infected CD4 cells into latently and actively infected cells (Funk et al., 2001).

2.1 *The basic dynamic model*

Let T_{NI}, T_I and V_I denote the concentration of target noninfected CD4 cells, productively infected CD4 cells and infectious viruses, respectively. Following Perelson et al. (1996), it is assumed that CD4 cells are generated through the hematopoietic differentiation process at a constant rate λ. The target cells are infected by the virus at a rate γ per susceptible cell and virion. Noninfected CD4 cells die at a rate μ_{NI} whereas infected ones at a rate μ_I. Infected CD4 cells produce virus at a rate p per infected cell. The virus are cleared at a rate μ_V. In absence of treatment, interactions between CD4 cells and viruses are thus described as:

$$\begin{aligned} \frac{dT_{NI}}{dt} &= \lambda - \gamma\, T_{NI} V_I - \mu_{NI} T_{NI} \\ \frac{dT_I}{dt} &= \gamma\, T_{NI} V_I - \mu_I T_I \\ \frac{dV_I}{dt} &= p\, T_I - \mu_V V_I \end{aligned} \tag{1}$$

Two additional parameters η_{RTI} and η_{PI} are introduced to model the effect of antiviral therapy containing RTI and PI. RTI prevents susceptible cells from becoming infected through inhibition of the transcription of the viral RNA into double-stranded DNA. η_{RTI} denotes the proportion of susceptible cells prevented to be infected and is valued between 0 and 1. A value of $\eta_{\text{RTI}} = 1$ corresponds to a completely effective drug that results in preventing all new infections of CD4 cells. PI leads to the production of noninfectious viruses V_{NI} which is modeled trough an additional equation. V_{NI} are produced at a rate $\eta_{\text{PI}} p$. η_{PI} is a proportion between 0 and 1 and $\eta_{\text{PI}} = 1$ corresponds to a completely effective drug that results in preventing all new infection of CD4 cells. It is assumed that infectious and non infectious viruses die at the same rate μ_V.

Under combined PI and RTI action, the system (1) becomes:

$$\begin{aligned} \frac{dT_{NI}}{dt} &= \lambda - (1-\eta_{\text{RTI}})\gamma\, T_{NI} V_I - \mu_{NI} T_{NI} \\ \frac{dT_I}{dt} &= (1-\eta_{\text{RTI}})\gamma\, T_{NI} V_I - \mu_I T_I \\ \frac{dV_I}{dt} &= (1-\eta_{\text{PI}}) p\, T_I - \mu_V V_I \\ \frac{dV_{NI}}{dt} &= \eta_{\text{PI}}\, p\, T_I - \mu_V V_{NI} \end{aligned} \tag{2}$$

It is assumed that before the treatment initiation, the system has reached an equilibrium state. From (2), the steady state values for uninfected CD4 cells, infected CD4 cells, infectious

and noninfectious virions are given by:

$$\begin{aligned} T_{NI}(0) &= \frac{\mu_I \mu_V}{p\,\gamma} \\ T_I(0) &= \frac{\mu_V V_I(0)}{p} \\ V_I(0) &= \frac{\lambda - \mu_{NI} T_{NI}(0)}{\gamma\, T_{NI}(0)} \\ V_{NI}(0) &= 0 \end{aligned} \tag{3}$$

The measured viral load is the total viral load $V = V_I + V_{NI}$ and the measured CD4 cell count is the total $T = T_{NI} + T_I$. This basic model is called $\mathcal{M}_B$. Its parameters and their definitions are summarized in Table 1.

2.2 *The quiescent dynamic model*

Guedj et al. (2007) proposed a more elaborated model which distinguishes quiescent CD4 cells, T_Q, target (activated) noninfected cells, T_{NI}, and infected T cells, T_I. In this model, only activated CD4 cells can become infected with HIV, and quiescent cells are assumed to be resistant to infection. Quiescent CD4 cells T_Q are generated through the hematopoietic differentiation process at a constant rate λ. For adults, as the CD4 cell compartment is largely maintained by self-renewal, the dynamic model allows the quiescent CD4 cells to become activated at a low constant rate α_Q. Quiescent CD4 cells are assumed to die at a rate μ_Q, and to appear by the deactivation of activated noninfected CD4 cells at a rate ρ. Therefore, activated noninfected CD4 cells appear by activation of quiescent cells at rate α_Q and revert to the quiescent stage at rate ρ.

The system of differential equations describing this model after initiation of antiviral

treatment is written as:

$$\begin{aligned}
\frac{dT_Q}{dt} &= \lambda + \rho\, T_{NI} - \alpha_Q T_Q - \mu_Q T_Q \\
\frac{dT_{NI}}{dt} &= \alpha_Q T_Q - (1-\eta_{\mathrm{RTI}})\gamma\, T_{NI} V_I - \rho T_{NI} - \mu_{NI} T_{NI} \\
\frac{dT_I}{dt} &= (1-\eta_{\mathrm{RTI}})\gamma\, T_{NI} V_I - \mu_I T_I \\
\frac{dV_I}{dt} &= (1-\eta_{\mathrm{PI}}) p\, T_I - \mu_V V_I \\
\frac{dV_{NI}}{dt} &= \eta_{\mathrm{PI}}\, p\, T_I - \mu_V V_{NI}.
\end{aligned} \tag{4}$$

As proposed by Perelson et al. (1996), it is assumed that newly produced viruses are fully infectious before the introduction of a PI treatment and that before the treatment initiation, the system has reached an equilibrium state. From (4), the steady state values are given by:

$$\begin{aligned}
T_Q(0) &= \frac{\lambda + \rho\, T_{NI}(0)}{\alpha_Q + \mu_Q} \\
T_{NI}(0) &= \frac{\mu_V\, \mu_{NI}}{\gamma\, p} \\
T_I(0) &= \frac{\mu_V\, V_I(0)}{p} \\
V_I(0) &= \frac{\alpha_Q\, T_Q(0)}{\gamma\, T_{NI}(0)} - \frac{\rho + \mu_{NI}}{\gamma} \\
V_{NI}(0) &= 0
\end{aligned} \tag{5}$$

The measured viral load is the total viral load $V = V_I + V_{NI}$ and the measured CD4 cell count is the total $T = T_Q + T_{NI} + T_I$. This quiescent model is called $\mathcal{M}_Q$. Its parameters and their definitions are summarized in Table 1.

2.3 *The latent dynamic model*

The third model takes into account the fact that not all CD4 cells actively produce virus upon successful infection (see Funk et al. 2001). This is reflected by splitting the infected cell pool into actively and latently infected cells. Uninfected CD4 cells are infected by the virus, as previously, at a rate $(1-\eta_{\mathrm{RTI}})\gamma V_I$. But only a proportion π of this infected cells are activated CD4 cells, T_A, and a proportion $(1-\pi)$ are latently infected CD4 cells, T_L. The

latently infected CD4 cells die at a rate μ_L and become activated at a rate α_L. The actively infected cells T_A die at a rate μ_A and only these cells produce virus particles.

The system of differential equations describing this model after initiation of antiviral treatment is written as:

$$\begin{aligned}
\frac{dT_{NI}}{dt} &= \lambda - (1-\eta_{\mathrm{RTI}})\gamma\, T_{NI} V_I - \mu_{NI} T_{NI} \qquad (6)\\
\frac{dT_L}{dt} &= (1-\pi)(1-\eta_{\mathrm{RTI}})\gamma\, T_{NI} V_I - \alpha_L T_L - \mu_L T_L\\
\frac{dT_A}{dt} &= \pi(1-\eta_{\mathrm{RTI}})\gamma\, T_{NI} V_I + \alpha_L T_L - \mu_A T_A\\
\frac{dV_{\mathrm{I}}}{dt} &= (1-\eta_{\mathrm{PI}}) p\, T_A - \mu_V V_I \qquad (7)\\
\frac{dV_{\mathrm{NI}}}{dt} &= \eta_{\mathrm{PI}}\, p\, T_A - \mu_V V_{NI}.
\end{aligned}$$

As previously, it is assumed that before treatment initiation, the system has reached an equilibrium state. From (6), the steady state values are given by:

$$\begin{aligned}
T_{NI}(0) &= \frac{\mu_A \mu_V (\alpha_L + \mu_L)}{\gamma p (\alpha_L + \pi \mu_L)} \qquad (8)\\
T_A(0) &= \frac{\mu_V V_I(0)}{p}\\
T_L(0) &= \frac{(1-\pi)\gamma\, T_{NI}(0) V_I(0)}{\alpha_L + \mu_L}\\
V_I(0) &= \frac{\lambda - \mu_{NI} T_{NI}(0)}{\gamma\, T_{NI}(0)}\\
V_{NI}(0) &= 0.
\end{aligned}$$

The measured viral load is the total viral load $V = V_I + V_{NI}$ and the measured CD4 cell count is the total $T = T_{NI} + T_L + T_A$ This latent model is called $\mathcal{M}_L$. Its parameters and their definitions are summarized in Table 1. .

3. Statistical Methods

3.1 *The nonlinear mixed effects model*

Let N be the number of patients. For patient i, we measure n_i viral loads at times (t_{ij}), $j = 1, \ldots, n_i$ and m_i CD4 cells at times (τ_{ij}), $j = 1, \ldots, m_i$.

Let us define $v_i = (v_{i1}, \ldots, v_{i\,n_i})$ where v_{ij} is the observed $\log_{10}$ HIV viral load (cp/mL) for individual i at time t_{ij}, $i = 1, \ldots, N$, $j = 1, \ldots, n_i$, and $z_i = (z_{i1}, \ldots, z_{i\,m_i})$ where z_{ij} is the the CD4 cell count (cells/mm^3) for individual i at time τ_{ij}, $i = 1, \ldots, N$, $j = 1, \ldots, m_i$. The observed $\log_{10}$ viral load and the CD4 cell count of all patients are analyzed simultaneously using a nonlinear mixed effects model, where V and T are the total number of virus and CD4 cells:

$$\begin{aligned} v_{ij} &= \log_{10}(1000\, V(t_{ij};\psi_i)) + e_{V,ij} \\ z_{ij} &= T(\tau_{ij};\psi_i) + T(\tau_{ij};\psi_i)\, e_{T,ij} \end{aligned} \tag{9}$$

$$\begin{aligned} \psi_i &= h(\phi_i) \quad ; \quad \phi_i \sim \mathcal{N}(\mu, \Omega) \\ e_{V,i} &\sim \mathcal{N}(0, \sigma_V^2 I_{n_i}) \quad ; \quad e_{T,i} \sim \mathcal{N}(0, \sigma_T^2 I_{m_i}) \end{aligned}$$

Here, $(e_{V,ij})$ and $(e_{T,ij})$ represent the residual errors. We assume a constant error model for the log viral load concentration and a proportional error model for the CD4 concentration.

Different parameteric models for V and T were proposed in the previous section. Nonlinear mixed effects models means that these models are functions of a vector of individual parameters ψ_i. This vector ψ_i is assumed to be some transformation $h(\phi_i)$ of a Gaussian random vector ϕ_i with mean μ (the vector of fixed effects) and variance-covariance Ω (the covariance matrix of the random effects). The random individual parameters (ψ_i) are assumed to be independent of the residual errors $(e_{V,i}, e_{T,i})$.

For these models η_{RTI} and η_{PI} are inhibition parameters that take their values in $[0, 1]$. Then, they are defined as the logistic transformation of a Gaussian random variable. Similarly

for model $\mathcal{M}_Q$, π is defined as the logistic transformation of a Gaussian random variable. The others parameters are non-negative parameters. This constraint is satisfied by assuming a log-normal distribution, that is by defining these parameters as the exponential transformation of Gaussian random variables.

The observation model is complicated by the detection limit of assays. When some viral load data v_{ij} is below the limit of quantification LOQ, the exact value v_{ij} is unknown and the only available information is that $v_{ij} \leqslant LOQ$. These data are classically named left-censored data. Let denote $I_{\text{obs}} = \{(i,j)|v_{ij} \geqslant LOQ\}$ and $I_{\text{cens}} = \{(i,j)|v_{ij} \leqslant LOQ\}$ the index sets of respectively the uncensored and censored observations. Finally, we observe

$$v_{ij}^{obs} = \begin{cases} v_{ij} & \text{if} \quad (i,j) \in I_{\text{obs}} \\ LOQ & \text{if} \quad (i,j) \in I_{\text{cens}}. \end{cases}$$

3.2 *Parameters Estimation*

Let $\theta = (\mu, \Omega, \sigma_T^2, \sigma_V^2)$ be the set of unknown population parameters. Maximum likelihood estimation of θ is based on the likelihood function of the observations (v^{obs}, z):

$$l(v^{\text{obs}}, z\,;\,\theta) = \prod_{i=1}^{N} \int \int p(v_i^{\text{obs}}, v_i^{\text{cens}}, z_i, \phi_i; \theta)\, d\phi_i\, dv_i^{\text{cens}} \tag{10}$$

where $p(v_i^{obs}, v_i^{\text{cens}}, z_i, \phi_i; \theta)$ is the likelihood of the complete data $(v_i^{obs}, v_i^{cens}, z_i, \phi_i)$ of the i-th subject. As the random effects ϕ_i and the censored observations v_i^{cens} are unobservable and as the regression functions are nonlinear, the foregoing integral has no closed form. Therefore the maximum likelihood estimate is not available in a closed form.

We propose to use the Stochastic Approximation Estimation Maximisation (SAEM) algorithm, a stochastic version developed by Delyon et al. (1999) of the Expectation-Maximization algorithm introduced by Dempster et al. (1977). This algorithm computes the E-step of the EM algorithm through a stochastic approximation scheme. It requires a simulation of one realization of the non observed data in the posterior distribution at each iteration, avoiding the computational difficulty of independent samples simulation of the Monte-Carlo EM and

shortening the time consumption. SAEM algorithm is a true maximum likelihood estimation method, for which convergence results are proved: pointwise almost sure convergence of the estimate sequence to a local maximum of the likelihood is proved under conditions satisfied by models from the exponential family Delyon et al. (1999). The simulation of the non observed data in the posterior distribution is not direct for NLMM. Kuhn and Lavielle (2005) propose to combine the SAEM algorithm with a Markov Chain Monte Carlo method to realize this simulation step. Donnet and Samson (2007) propose a version of the SAEM algorithm adapted to mixed models defined by differential equations. The censoring of observed response presents an additional challenge in the analysis of NLMM. The SAEM algorithm also enables to take into account these left-censored viral load data accurately Samson et al. (2006). The combination of the two extensions of SAEM for differential equations and for left-censored data handling is used in the following analyzes.

3.3 *Model selection*

The purpose of model selection is to identify a model that best fits the available data set with a dimension as small as possible. The two most popular model selection criteria are the Akaike Information Criterion AIC and the Bayesian Information Criterion BIC. Both AIC and BIC have some theoretical foundations: Kullback-Leibler distance in information theory (for AIC), and integrated likelihood in Bayesian theory (for BIC). If the complexity of the true model does not increase with the size of the data set, BIC is the preferred criterion, otherwise AIC is preferred (Burnham and Anderson, 1998).

Let $P_{\mathcal{M}}$ be the number of parameters in the model $\mathcal{M}$. In the following, we will denote $l_{\mathcal{M}}$ the likelihood function of the observations (v^{obs}, z) and $\hat{\theta}_{\mathcal{M}}$ the maximum likelihood estimate of θ in model $\mathcal{M}$.

The AIC penalizes the minimized deviance of the model by 2 times the number of free parameters in the model, includind fixed effects and variance components. The BIC, on the

other hand, penalizes the minimized deviance by $\log(N)$ times the number of free parameters (we recall that N is the number of individuals).

$$\begin{aligned} AIC(\mathcal{M}) &= -2\log l_{\mathcal{M}}(v^{\text{obs}}, z; \hat{\theta}_{\mathcal{M}}) + 2P_{\mathcal{M}}, \\ BIC(\mathcal{M}) &= -2\log l_{\mathcal{M}}(v^{\text{obs}}, z; \hat{\theta}_{\mathcal{M}}) + \log(N)P_{\mathcal{M}}. \end{aligned}$$

A main difference between AIC and BIC is that the penalty term in BIC is much larger than in AIC. AIC favors a large model while BIC prefers a parsimonious model. Following the simulation results of Bertrand et al. (2008), the best model is defined here as the model with the lowest BIC. Using any of these two criteria requires to compute the log-likelihood of model $\mathcal{M}$.

Following (9), for any model $\mathcal{M}$, the likelihood l of the observations (v^{obs}, z) can be decomposed as follows (we omit the subscript $\mathcal{M}$) to simplify the notation).

$$\begin{aligned} l(v^{\text{obs}}, z; \theta) &= \int p(v^{\text{obs}}, z, \phi; \theta)\, d\phi && (11) \\ &= \int h(v^{\text{obs}}, z|\phi; \theta)\pi(\phi; \theta)\, d\phi && (12) \\ &= \int h(v^{\text{obs}}, z|\phi; \theta)\frac{\pi(\phi; \theta)}{\tilde{\pi}(\phi; \theta)}\tilde{\pi}(\phi; \theta)\, d\phi && (13) \end{aligned}$$

where π is the probability distribution density of ϕ and $\tilde{\pi}$ any absolutely continuous distribution with respect to π. Then, $l(v^{\text{obs}}, z; \theta)$ can be approximated via an Importance Sampling integration method:

(1) draw $\phi^{(1)}, \phi^{(2)}, \ldots, \phi^{(K)}$ with the distribution $\tilde{\pi}(\cdot; \theta)$,

(2) let

$$\hat{l}_K(v^{\text{obs}}, z; \theta) = \frac{1}{K}\sum_{k=1}^{K} h(v^{\text{obs}}, z|\phi^{(k)}; \theta)\frac{\pi(\phi^{(k)}; \theta)}{\tilde{\pi}(\phi^{(k)}; \theta)} \qquad (14)$$

Obviously, $\hat{l}_K(v^{\text{obs}}, z; \theta)$ is a consistant estimator of the observed likelihood: $\mathbb{E}(\hat{l}_K(v^{\text{obs}}, z; \theta)) = l(v^{\text{obs}}, z; \theta)$ and $\text{Var}(\hat{l}_K(v^{\text{obs}}, z; \theta)) = \mathcal{O}(K^{-1})$.

Furthermore, if $\tilde{\pi}$ is the conditional distribution $p(\phi|v^{\text{obs}}, z; \theta)$, the variance of the estimator

is null and $\hat{l}_K(v^{\text{obs}}, z; \theta) = l(v^{\text{obs}}, z; \theta)$ for any value of K. That means that an accurate estimation of $l(v^{\text{obs}}, z; \theta)$ can obtained with a small value of K if the sampling distribution is close to the conditional distribution $p(\phi|v^{\text{obs}}, z; \theta)$.

We recommend the following procedure: for $i = 1, 2, \ldots, N$, estimates empirically the conditional mean $\mathbb{E}(\phi_i|v_i^{\text{obs}}, z_i; \widehat{\theta})$ and the conditional variance-covariance matrix $\text{Var}(\phi_i|v_i^{\text{obs}}, z_i; \widehat{\theta})$ of ϕ_i as described above. Then, the $\phi_i^{(k)}$ are drawn with the sampling distribution $\tilde{\pi}$ as follows:

$$\phi_i^{(k)} = \mathbb{E}(\phi_i|v^{\text{obs}}, z; \widehat{\theta}) + \text{Var}^{\frac{1}{2}}(\phi_i|v^{\text{obs}}, z; \widehat{\theta}) \times T_i^{(k)}$$

where $(T_i^{(k)})$ is a sequence of *i.i.d.* random vectors and where the components of $T_i^{(k)}$ are independent variables distributed with a $t-$distribution with ν degrees of freedom.

The numerical results presented here were obtained with $\nu = 5$ d.f.

3.4 *Model identifiability*

In order to apply the HIV dynamic model for modeling viral responses and estimating individual dynamics parameters using viral load data from a clinical study, we need to resolve an important statistical problem, that is, the identifiability of model parameters. Generally, many of the variables in the model may not be measurable and parameters may not be identifiable. In practice, we will need a trade-off between the model complexity and the parameter identifiability based on the clinical data. If a model has too many components, it may be difficult to analyze. If a model is too simple, some important clinical factor cannot be incorporated, although the viral dynamic parameter can be identified and estimated. Various works on system identification of these nonlinear HIV models can be found for example in (Perelson and Nelson, 1997; Xia and Moog, 2003; Jeffrey and Xia, 2005; Guedj et al., 2007).

However, it is difficult to use mathematical tools to study experimental identifiability given a model and a dataset. Estimation of over-parameterized model is possible with the SAEM algorithm. But then a close inspection of the Fisher information matrix and the large standard errors of fixed effect is needed to help to choose which parameters can, indeed,

be accurately estimated. The identifiability property can be improved if values of some parameters are fixed to some known values.

3.5 *Covariate testing*

Other statistical issues in NLME models are the utilization of covariates to explain part of inter-individual parameter variability. Comparing models with and without covariates can be performed through model selection with the BIC criterion. For nested models, the likelihood ratio test can be applied by computing the log-likelihoods of the different nested models. Another approach is to use the Wald test from the estimated effects of covariates and their standard errors.

3.6 *The MONOLIX software*

MONOLIX is a free software, which implements a wide variety of stochastic algorithms such as Stochastic Approximation of EM (SAEM), Importance Sampling, MCMC, and Simulated Annealing, all dedicated to the analysis of nonlinear mixed-effects models. The objective of this new software are: a) parameter estimation by computing the maximum likelihood estimator of the parameters, without any approximation of the model and standard errors for the maximum likelihood estimator; b) model selection by comparing several models using some information criteria (AIC, BIC), testing hypotheses using the Likelihood Ratio Test, testing parameters using the Wald Test and c) Goodness of fit.

MONOLIX can be downloaded from the MONOLIX website (http://software.monolix.org) and version 2.4 was used in this work. We used the code BiM (release 2.0, April 2005) which implements a variable stepsize method for stiff initial value problems for ODEs.

4. Application to the COPHAR II - ANRS 134 trial

This methodology is applied to the anaysis of the COPHAR II- ANRS 134 trial an open prospective non-randomized interventional study.

4.1 *Material and Methods*

In the COPHAR II- ANRS 134 trial, 115 HIV-infected patients adults started an antiviral therapy with at least 2 RTI and one of three different PI. 48 patients were treated with indinavir (and ritonavir as a booster)(I), 38 with lopinavir (and ritonavir as a booster) (L) and 35 with nelfinavir (N). Patients were followed one year after treatment initiation. Viral load and CD4 cell count were measured at screening, at inclusion and at weeks 2(or 4), 8, 16, 24, 36 and 48. Plasma HIV-1-RNA were measured by Roche monitored with a limit of quantification of 50 copies/ml.

Observed viral load and CD4 cell count are displayed in Figure 1 which clearly shows a large inter-subject variability.

[Figure 1 about here.]

The results of this trial, with further details, are reported in Duval et al. (2009). The proportion of virological failure was higher in the nelfinavir group and similar for indinavir and lopinavir, although lopinavir is supposed to be a more potent PI now widely used.

We first compared the three models $\mathcal{M}_B$, $\mathcal{M}_Q$ and $\mathcal{M}_L$ using the BIC criterion. Then we studied the identifiability of the selected model and we tested the effect of the PI group by adding a covariate on η_{PI}..

The effect of the various PI were tested by using the following logit transformation:

$$\text{logit}(\eta_{\text{PI},i}) = \mu + \beta_{\text{TRT}_i} + b_i \tag{15}$$

where TRT_i is the PI administrated to patient i (L, I or N). The reference group is the lopinavir group ($\beta_L = 0$). We use the BIC criterion and the Wald test to study the difference in the 3 PI groups: no PI effect (LIN), only two groups: L vs. IN (L-IN) or LI vs N (LI-N), three groups L-I-N.

4.2 *Results*

.

[Table 1 about here.]

We first compared the three dynamic models. Here, for each model, the complete set of fixed effects summarized in Table 1 are estimated. Furthermore, variability on all the parameters is assumed without any correlation between the random effects. The population parameters were estimated with the SAEM algorithm and the log-likelihoods were estimated by Monte Carlo Importance Sampling. The BIC criteria computed for the three models are displayed in Table 2. According to this criterion, the latent model $\mathcal{M}_L$ is selected as the best model among the three candidate models and the second-best model is the quiescent model $\mathcal{M}_Q$. Furthermore, the best fits for both the viral load and the CD4 counts are also obtained with model $\mathcal{M}_L$ ($\sigma_V = 0.46$ and $\sigma_T = 0.25$).

[Table 2 about here.]

We then studied the identifiability of the $\mathcal{M}_L$, and we found that it is not possible, given the data set, to estimate both μ_V the rate of death of virions and p their rate of production. Indeed, when assuming variability on both μ_V and p the estimated standard errors of both fixed effect are very high and could not be estimated for their inter-patient variabilities (Table 3).

[Table 3 about here.]

Therefore for the three models, we assumed that μ_V do not vary across patients and is fixed to the value 30 /day as in (Ramratnam et al., 1999; Guedj et al., 2007).

Table 4 reports the BIC criterion and the estimated β's for the different merging of the PI group. The smallest BIC value is for LI-N implying a different effect of nelfinavir versus lopinavir and indinavir that are grouped. The estimated parameters of that model

are reported in Table 5. The efficacy of PI η_{PI} is 0.99 for lopinavir-indinavir and 0.75 for nelfinavir, i.e. nelfinavir efficacy for blocking of infectious viruses is 25% less important than for lopinavir and indinavir, which are boosted PI. The LRT for the nelfinavir effect is very significant ($p = 10^{-12}$ for comparison of models LIN and LI-N), no significant improvement were found when separating L and I (comparison of models LI-N vs L-I-N: $p = 0.32$). The Wald tests also agree that only β_N is significatively different from zero.

[Table 4 about here.]

[Table 5 about here.]

This model indeed provided good fits of both viral load and CD4 cells as can be seen on the visual predictive check (Figure 2) and on some individual fits for patients in each treatment group (Figure 3).

[Figure 2 about here.]

[Figure 3 about here.]

5. Discussion

This article proposes the SAEM algorithm to estimate parameters of nonlinear mixed model based on partially observed complex HIV differential systems. The estimation of the parameters of such a mixed model is a difficult statistical and computational challenge. The HIV differential systems defining these mixed models are non linear, consequently without any analytical solution.

Furthermore, the ODE system is generally *stiff*, the classic ODE solver such as Runge-Kutta being not adapted to solve numerically the system. We use the code BiM (release 2.0, April 2005) which implements a variable order-variable stepsize method for (stiff) initial value problems for ODEs.

The analysis of such data is also complicated by the left-censoring of the viral load data

due to the lower limit of detection of experimental devices, and it is well known that omitting to correctly handle this censored data provides biased estimates of dynamic parameters. The SAEM algorithm has theoretical convergence properties and is computationally efficient on these dynamic models. In this article we applied it to a clinical trial in HIV infection, using all the data (both viral load and CD4 measurements) obtained during 48 weeks of follow-up in naive patients starting a treatment while most studies of HIV dynamics model studied only viral load data during a shorter period (2-6 weeks) after the initiation of anti-retroviral treatment.

We compared several HIV dynamic models and show that the latent model was the best one using the BIC criterion. We also study the practical identifiability of this model from the standard errors. Using likelihood ratio test to compare the efficacy of the three studied PI, We found a signficant difference in the efficacy of nelfinavir compared to lopinavir or indinavir. This is in agreement with the results of the trial (Duval et al; 2009) in which virological failure was found in 33% of patients treated with nelfinavir and only in 5% of patients treated by indinavir or lopinavir. The HIV dynamic model used in this study has some limitations. First, it does not take into account the fact that HIV undergoes rapid mutation in the presence of anti-retroviral therapy. Of course, considering such phenomenon in the model may introduce many more parameters. We attempted to keep the model itself as simple as possible and the goodness of fit were satisfactory. Second, we consider a constant treatment effect, however, the effect of antiviral treatment may change over time, due to pharmacokinetics intra-patient variability, fluctuating patient adherence, emergence of drug resistance mutations and/or other factors. Huang et al. (2006) propose viral dynamic models to evaluate antiviral response as a function of time-varying concentrations of drug in plasma. A more elaborate model would thus promisingly include this additional extension. Nevertheless, these limitations do not offset the major findings from our modeling approach, although further improvement may

be brought. The SAEM algorithm is an useful tool for model development and parameter estimation in this context of HIV dynamics.

Acknowledgements

The authors would like to thank the scientific committee of COPHAR II-ANRS111 trial for giving us access to the data, especially the principal investigators Pr Dominique Salmon-Céron from Infectious Diseases Unit at Cochin Hospital, Paris and Dr Xavier Duval from Center for Clinical Investigation, Bichat Hospital,Paris, France.

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Figure 1. Observed viral load decrease (left) and CD4 increase (right) after treatment initiation in the three PI groups: lopinavir (top), indinavir (middle) and nelfinavir (bottom)

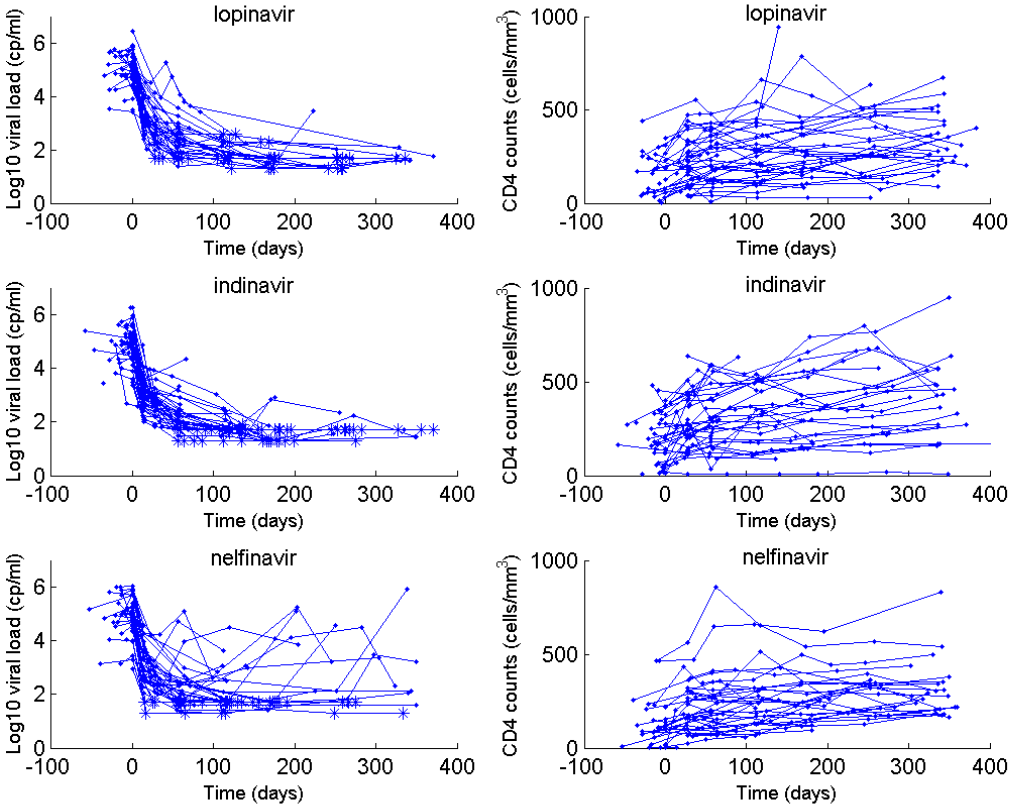

Figure 2. Visual predictive checks for the latent model $\mathcal{M}_L$. The observed viral loads and CD4 counts are displayed with dots, the predicted median with a solid line and a 90% prediction interval with dotted lines.

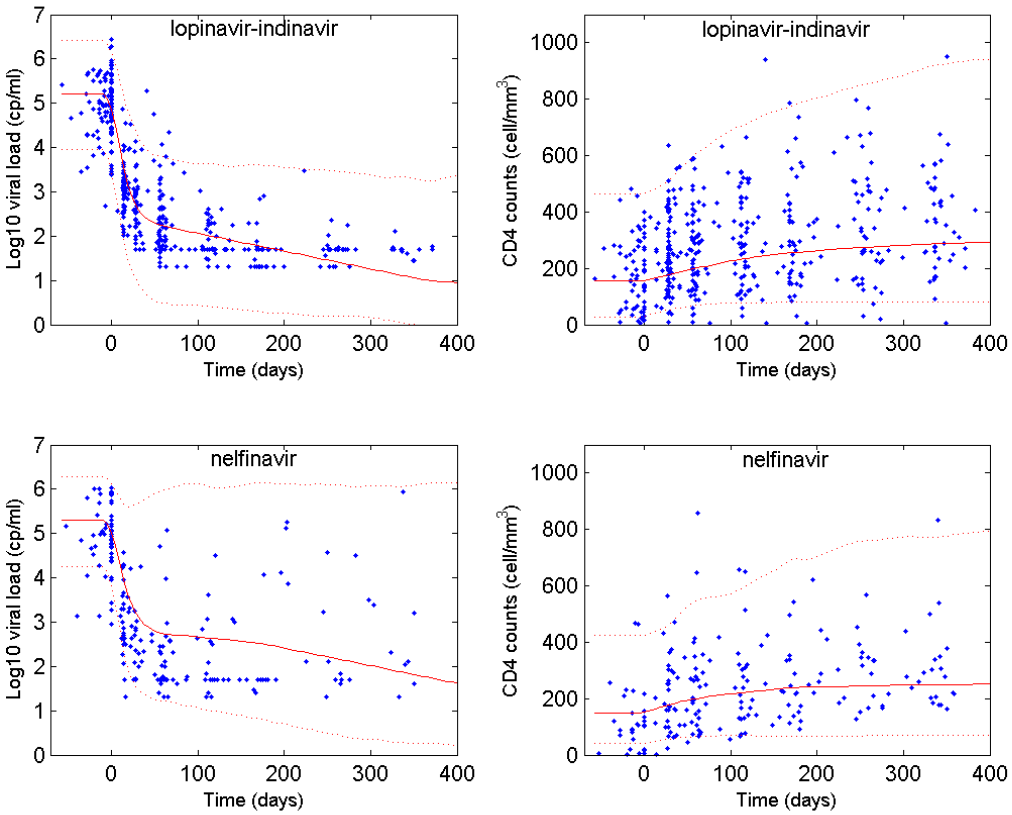

Figure 3. Examples of individual fits obtained with the latent model $\mathcal{M}_L$: ID=67 (lopinavir), ID=11 (indinavir) and ID=105 (nelfinavir). The + represent the non censored observations and the $*$ the limit of quantification.

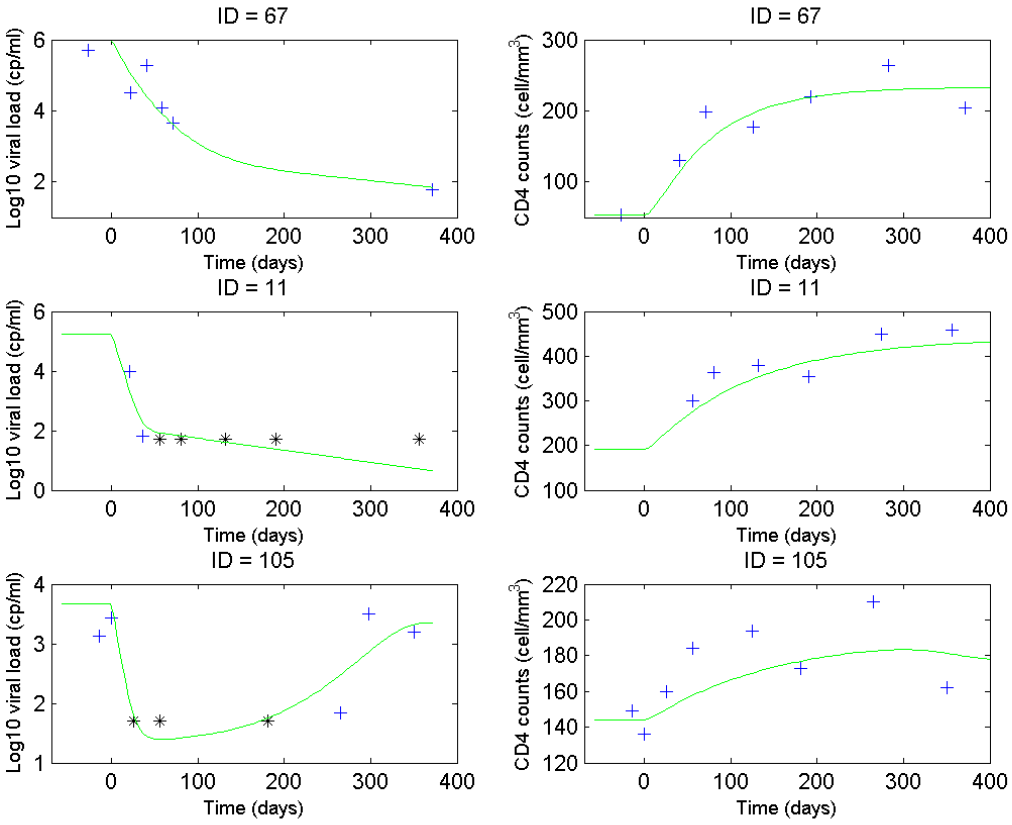

Table 1
Parameters of each HIV dynamic model ($\mathcal{M}_B$: basic model, $\mathcal{M}_Q$: quiescent model, $\mathcal{M}_L$: latent model

Parameter	unit	description	$\mathcal{M}_B$	$\mathcal{M}_Q$	$\mathcal{M}_L$
λ	cells/mm^3/day	Rate of production of infected CD4 cells	$\star$	$\star$	$\star$
γ		Infection rate of CD4 cell per virion	$\star$	$\star$	$\star$
μ_{NI}	day^{-1}	Death rate of uninfected CD4 cells	$\star$	$\star$	$\star$
μ_I	day^{-1}	Death rate of infected CD4 cells	$\star$	$\star$	
μ_Q	day^{-1}	Death rate of quiescent CD4 cells		$\star$	
μ_L	day^{-1}	Death rate for latently infected CD4 cells			$\star$
μ_A	day^{-1}	Death rate for actively infected CD4 cells			$\star$
μ_V	day^{-1}	Death rate of virions	$\star$	$\star$	$\star$
p		Number of virions production by CD4 cell	$\star$	$\star$	$\star$
ρ	day^{-1}	Rate of reversion to the quiescent state		$\star$	
α_Q		Activation rate of quiescent CDA cells		$\star$	
α_L		Activation rate of latently infected CD4 cells			$\star$
π		Proportion of infected CD4 cells that become activated			$\star$
η_{RTI}		Efficacy of NRTI	$\star$	$\star$	$\star$
η_{PI}		Efficacy of PI	$\star$	$\star$	$\star$
Number of parameters			8	11	11

Table 2
Comparison of the three dynamic models. The estimated standard errors are in parenthesis.

Model	$-2 \times$ log Likelihood	BIC	σ_V	σ_T
Basic model ($\mathcal{M}_B$)	9048(4)	9134(4)	0.67(0.03)	0.27(0.01)
Quiescent model ($\mathcal{M}_Q$)	8963(7)	9077(7)	0.62(0.03)	0.27(0.01)
Latently model ($\mathcal{M}_L$)	8644(6)	8758(6)	0.46(0.02)	0.25(0.01)

Table 3
Simultaneous estimation of μ_V and p in model $\mathcal{M}_L$, assuming variability on μ_V and p

θ	$\hat{\theta}$	SE (%)
p_0	650	240
μ_V	30.8	236
ω_{p_0}	0.665	NaN
ω_{μ_V}	0.621	NaN

Table 4

Comparison of different covariate models for PI group. The estimated standard errors are in parenthesis. Here, p_{β_N} (resp. p_{β_I}) is the p-value of the Wald test used for testing $\beta_N = 0$ (resp. $\beta_I = 0$).

Model	$-2\times$ log-likelihood	BIC	β_N	p_{β_N}	β_I	p_{β_I}
LIN	8646 (6)	8741 (6)				
LI-N	8635 (6)	8734 (6)	-5.6 (2.6)	0.045		
L-I-N	8631 (6)	8735 (6)	-4.9 (2.3)	0.036	-1.1 (4.0)	0.790

Table 5

Estimated fixed effects and standard deviations of the random effects for the latent model $\mathcal{M}_L$. The estimated standard errors are in parenthesis. See eq. (9) for the definition of the fixed and random effects.

	Parameter (S.E.) ;	Inter-patient variability (S.E.)
λ (cells/mm^3/day)	2.61 (0.25)	0.55 (0.044)
γ	0.0021 (0.0009)	0 (fixed)
μ_{NI} (day^{-1})	0.0085 (0.0010)	0.44 (0.073)
μ_L (day^{-1})	0.0092 (0.0009)	0 (fixed)
μ_A (day^{-1})	0.289 (0.016)	0.399 (0.047)
μ_V (day^{-1})	30 (fixed)	0 (fixed)
p	641 (110)	0.9 (0.13)
α_L	1.6e-5 (1.7e-6)	0.678 (0.33)
π	0.443 (0.038)	0.45 (0.047)
η_{RTI}	0.90 (0.17)	2.93 (1.8)
η_{PI}	0.99 (0.003)	3.19 (2)
β_N	-5.6 (2.6)	
σ_V	0.464 (0.024)	
σ_T	0.254 (0.009)	