Population HIV-1 Dynamics in Vivo: Applicable Models and Inferential Tools for Virological Data from AIDS Clinical Trials

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SUMMARY

In this paper we introduce a novel application of hierarchical nonlinear mixed-effect models to HIV dynamics. We show that a simple model with a sum of exponentials can give a good fit to the observed clinical data of HIV-1 dynamics (HIV-1 RNA copies) after initiation of potent antiviral treatments, and can also be justified by a biological compartment model for the interaction between HIV and its host cells. This kind of model enjoys both biological interpretability and mathematical simplicity after reparameterization and simplification. A model simplification procedure is proposed and illustrated through examples. We interpret and justify various simplified models based on clinical data taken during different phases of viral dynamics during antiviral treatments. We suggest the hierarchical nonlinear mixed-effect model approach for parameter estimation and other statistical inferences. In the context of an AIDS clinical trial involving patients treated with a combination of potent antiviral agents, we show how the models may be used to draw biologically relevant interpretations from repeated HIV-1 RNA measurements and demonstrate the potential use of the models in clinical decision-making.

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1. Introduction

1.1 Background

The development of assay techniques for quantifying HIV-1 RNA in HIV-1 infected patients makes it possible to use viral load (HIV-1 RNA copies) as a surrogate marker to accelerate AIDS clinical trials. Recently some deterministic HIV-1 dynamic models have been developed to describe the interaction between HIV and its host cells in individual patients (Perelson, Kirschner and De Boer, 1993; Schenzle, 1994; Kirschner and Perelson, 1995; Kirschner, 1996; Phillips, 1996; De Boer and Boucher, 1996; Nowak and Bangham, 1996). A stochastic model is also proposed by Tan and Wu (1998).

However, most of the models developed by biomathematicians and biologists are too complicated and contain too many unknown parameters to be used to analyze real clinical data. Recently several simplified models have been proposed and applied to real virological data from clinical trials (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996 and 1997). These studies of HIV dynamics have led to a new understanding of the pathogenesis of HIV infection. However, various assumptions have to be made in order to simplify these models; some of these assumptions may not be valid in practice. The estimation methods used in these studies are not efficient and not flexible enough to deal with sparse data. Although biomathematicians and theoretical biologists have worked on mathematical dynamic models of HIV since the end of the 1980's (Merrill, 1987; Anderson and May, 1989; Perelson, 1989), few statisticians have been involved in this effort. We expect that this paper can stimulate more research in this field among statisticians.

HIV dynamic models provide a global picture of the virus elimination and production process during antiviral treatments for each individual patient. Thus in evaluating the efficacy of anti-HIV treatments and understanding HIV infection pathogenesis, it is of great interest to estimate viral dynamic parameters for the whole population and for each individual patient in an AIDS clinical trial.

1.2 A Clinical Data Example

In AIDS Clinical Trial Group (ACTG) Protocol 315, fifty-three HIV infected patients were treated with potent antiviral drugs (ritonavir, 3TC and AZT). Plasma HIV-1 RNA was repeatedly quantified on Days 2, 7, 10, 14, 21, 28, and Weeks 8, 12, 24 and 48 after initiation of treatment. The Nucleic Acid Sequence-Based Amplification assay (NASBA)

was used to measure plasma HIV-1 RNA. This assay offers a lower limit of detection of 100 RNA copies per ml plasma. We present repeated measurements of viral load from four of these patients in Figure 1. The viral load in all other patients follow a similar pattern, i.e., after initiation of antiviral treatment, a fast decay of plasma virus followed by a slower decay (commonly referred to as two phase decay). The investigators of the study were interested in the following scientific questions: (1) what is the mechanism behind the two phases of virus decay? (2) what is the biological meaning of the decay rates of the two phases? (3) how can we characterize viral dynamics in the patient population and intra- and inter-subject variations? (4) how can we estimate the decay rates of these two phases for each individual patient and use this information in making clinical decisions for individuals? (5) how can we use the results of modeling and estimation in clinical practice?

Place of Figure 1

The purposes of this paper are, (1) to introduce a viral dynamic model (Section 2.1) and to simplify it to an estimable function, allowing the mechanism of two-phase viral decay and its biological meaning to be explained by simple and estimable HIV-1 dynamic models (Section 2.2); (2) to demonstrate the use of the hierarchical nonlinear mixed-effect model statistical framework as a basis for estimation of population and individual viral dynamic parameters (Section 3); and (3) to show how the models may be used to draw biologically relevant interpretations and aid in clinical decision-making within the context of the data from ACTG 315 (Section 4).

2. HIV Dynamic Models and Simplifications

2.1 A Model of HIV Dynamics

We propose a mathematical model for HIV dynamics by considering the following cell and virus compartments: (1) uninfected target cells, such as T cells, macrophages, lymphoid mononuclear cells (MNCs), and tissue langerhans cells, which are possible targets of HIV-1 infection; (2) "mysterious" infected cells, cells other than T cells, such as tissue langerhans cells and microglial cells whose behavior is not completely known; (3) long-lived infected cells, such as macrophages, that are chronically infected and long-lived; (4)

latently infected cells, which contain the provirus but are not producing virus immediately, and only start to produce virus when activated; (5) productively infected cells, which are actively producing virus; (6) infectious virus, virus that is functional and capable of infecting target cells; (7) noninfectious virus, virus that is dysfunctional and cannot infect target cells. We denote the concentration of the variety of these cells and virus by $T, T_m, T_s, T_l, T_p, V_I$, and V_{NI}, respectively.

Without the intervention of antiviral treatment, uninfected target cells may either decrease due to HIV infection or be in an equilibrium state due to balancing between regeneration and proliferation of uninfected target cells and HIV infection. Some uninfected target cells (T) are infected by infectious virus (V_I) and may become mysterious infected cells (T_m), long-lived infected cells (T_s), latently infected cells (T_l) or productively infected cells (T_p) with proportions of $\alpha_m kV_I, \alpha_s kV_I, \alpha_l kV_I$, and $\alpha_p kV_I$, respectively, where $\alpha_m + \alpha_s + \alpha_l + \alpha_p = 1$. Latently infected T cells may be stimulated to become productively infected cells with a rate of δ_l. Infected cells, T_m, T_s and T_p, are killed by HIV at the rates of δ_m, δ_s and δ_p, respectively, after producing an average of N virions per cell during their lifetimes. Infected cells, T_m, T_s and T_l may also die at the rates of μ_m, μ_s and μ_l, respectively, without producing virus. We assume that the proportion of noninfectious virus produced by infected cells is η, without the intervention of protease inhibitor (PI) antiviral drugs. The elimination rates for infectious virus and noninfectious virus are assumed to be the same, say c.

We assume that the antiviral therapy consists of one or more protease inhibitor (PI) drugs and reverse transcriptase inhibitor (RTI) drugs. We model the effect of RTI drugs by reducing the infection rate from k_0 to $(1-\gamma)k_0$, where $0 \leq \gamma \leq 1$ (Herz, et al., 1996). Parameter γ reflects the RTI drug efficacy (Bonhoeffer, Coffin, and Nowak, 1997). If $\gamma = 0$, the RTI drugs have no effect; if $\gamma = 1$, the RTI drugs are perfect and completely block the HIV infection. The PI drugs are assumed to be so potent that the production of infectious virions is almost blocked except for a small fraction. To account for compartments where the PI drugs cannot reach and persistent virus that the PI drugs cannot completely block the production of, we consider an additional virus production term with a constant (average) rate, P, in the model. If only a small fraction of persistent virus can escape from the attack of PI drugs, it may be considered as a Poisson process, and can thus be modeled by a constant production in a deterministic model. Thus, after initiation

of combination treatment with PI and RTI drugs, the HIV dynamic model is

$$\begin{aligned}
\tfrac{d}{dt}T_m &= (1-\gamma)\alpha_m k_0 T V_I - \delta_m T_m - \mu_m T_m, \\
\tfrac{d}{dt}T_s &= (1-\gamma)\alpha_s k_0 T V_I - \delta_s T_s - \mu_s T_s, \\
\tfrac{d}{dt}T_l &= (1-\gamma)\alpha_l k_0 T V_I - \delta_l T_l - \mu_l T_l, \\
\tfrac{d}{dt}T_p &= (1-\gamma)\alpha_p k_0 T V_I + \delta_l T_l - \delta_p T_p, \\
\tfrac{d}{dt}V_I &= (1-\eta)P - cV_I, \\
\tfrac{d}{dt}V_{NI} &= \eta P + N\delta_m T_m + N\delta_s T_s + N\delta_p T_p - cV_{NI}.
\end{aligned} \tag{1}$$

where $\alpha_m + \alpha_s + \alpha_l + \alpha_p = 1$.

Ideally, it is unlikely that the target cell density T is constant; Perelson et al. (1993) have represented T by the following nonlinear differential equation:

$$\frac{d}{dt}T = s + r_T T\left(1 - \frac{T + T_m + T_s + T_l + T_p}{T_{\max}}\right) - k_1 V_I T - \mu_T T \tag{2}$$

where s is a regeneration rate. The term $r_T T\left(1 - \frac{T+T_m+T_s+T_l+T_p}{T_{\max}}\right)$ is a logistic proliferation rate. The term $k_1 V_I T$ models the rate at which infectious virus infects a target cell, and μ_T is the death rate of target cells; see Perelson et al. (1993) for details. Due to the nonlinearity introduced by (2), the system of differential equations (1) is analytically intractable. Fortunately, however, the $CD4^+$ T cell count changes much more slowly than viral load, and the T cell count recovers slightly during the first 2 to 4 weeks of antiviral treatment and then reaches steady-state rapidly. Thus, a reasonable simplification is to approximate this recovery process from (2) by $T = T_0 + T_r(1 - e^{-rt})$. This simplification may not be ideal, but carries the advantage that the system of equations (1) may be solved analytically; moreover, we have investigated this simplification by simulation for scenarios similar to that of the ACTG 315 data, and found the approximation to be quite good. The final solution for the total virus $V = V_I + V_{NI}$ has the form (see Appendix),

$$\begin{aligned}
V(t) &= P_0 + P_1 e^{-\lambda_1 t} + P_2 e^{-\lambda_2 t} + P_3 e^{-\lambda_3 t} + P_4 e^{-\lambda_4 t} + \\
&\quad (P_5 + P_6 t)e^{-\lambda_5 t} + P_7 e^{-\lambda_6 t} + P_8 e^{-\lambda_7 t},
\end{aligned} \tag{3}$$

where $P_i, i = 0, \ldots, 8$ are functions of the model parameters in (1) (details in Appendix), $\lambda_1 = \delta_p, \lambda_2 = \delta_m + \mu_m, \lambda_3 = \delta_s + \mu_s, \lambda_4 = \delta_l + \mu_l, \lambda_5 = c, \lambda_6 = r$, and $\lambda_7 = c + r$. At time $t = 0$, $V(0) = \sum_{i \neq 6} P_i$. Parameter P_i represents the initial viral production rate, and parameter λ_i represents the exponential decay rate of virus in the corresponding

compartment. Also notice that, if P in (1) cannot be approximated by a constant due to dependence on the density of infected cells, the decay rates $\lambda_1, ..., \lambda_7$ would not be exactly the elimination rates of infected cells. Instead they would be complicated functions of all parameters in (1). We shall explore this problem in detail in the future.

As suggested by Herz et al. (1996) and others (Perelson et al., 1996 and 1997; Wu et al., 1997 and 1998b), several phases of HIV-1 dynamics may exist after initiation of antiviral treatments. There are several hours of intracellular and pharmacological delay after the first dose of antiviral drugs; thereafter the viral decay will follow the function (3). First there is a transition phase reflecting the decay of free virus and productively infected cells (Perelson et al., 1996; Herz et al., 1996). This is followed by a rapid exponential decay which mainly reflects the decay of productively infected cells. The decay becomes slower after several weeks, which may reflect the decay of long-lived infected cells and/or latently infected cells (Perelson et al., 1997), or may be due to the constant production P_0 discussed in the last section (Wu et al., 1997). We believe that other decay phases may also exist, but may not be identified due to the limited sensitivity and accuracy of current HIV-1 RNA assays. We illustrate various phases of HIV-1 dynamics in Figure 2.

Place of Figure 2

2.2 Simplified Models for Clinical Data

There are some difficulties in applying this model to real data analysis. First, we have to estimate the intracellular and pharmacological delay as shown in Figure 2, which requires frequent measurement data on viral load and pharmacokinetics in the first several hours after initiation of antiviral treatment (Herz et al., 1996; Perelson et al., 1996). This kind of data is very expensive and rarely available in clinical trials unless the trial is specifically designed to gather it. However, we can see from (3) that, if there is an intracellular/pharmacological delay period of t_d, the solution is the same with the parameters P_i replaced by $P_i e^{\lambda_i t_d}$. Therefore we can ignore the delay and still correctly estimate the parameters λ_i, which medical investigators are mostly interested in due to their biological correlation with treatment efficacy and turnover rates of virus and infected cells. The estimate of the P_i's may be biased, but not very seriously, since t_d is only several hours (Perelson et al., 1996).

Secondly, formula (3) is still too complicated and contains too many parameters. In theory, if very accurate measurements are available over a very long time, all parameters in the model (3) can be identified (Godfrey, 1983). However, due to measurement noise, poor assay sensitivity, and limited sampling schedules, only a small number of exponentials can be realistically fitted to the repeated measurements of total virus. If some of the exponential decay rates, $\lambda_{1i}, \ldots, \lambda_{qi}$, are not well separated, it may also cause unidentifiability problems.

Therefore we propose a procedure to simplify model (3) in order to estimate the important and clinically interesting parameters based on measurements of total viral load, $V = V_I + V_{NI}$. This procedure is motivated by the exponential curve peeling method (Anderson, 1983; Van Liew, 1967) which fits a multiple-exponential curve piecewise in a backward order. However, our method involves approximating the multiple-exponential curve forwards. The basic idea is illustrated in the following discussion.

First we consider that HIV-1 RNA data are obtained frequently from initiation of antiviral treatment up to the first rapid decay phase (Figure 2). In this case, the data only allow us to model the productively infected cells and virus compartments (Perelson et al., 1996; Wu, Ding and DeGruttola, 1998a). However, unlike Perelson et al. (1996) and Wu et al. (1998a), in which the other infected cell compartments such as T_m, T_s, and T_l are simply ignored, here we propose to approximate these ignored compartments by a constant virus production. Since the ignored infected cells have a relatively long half-life, the virus decay due to these compartments will be slow in a short time period, and remain approximately constant. Therefore model (1) can be simplified as

$$\begin{aligned} \tfrac{d}{dt}T_p &= k^*TV_I - \delta_p T_p, \\ \tfrac{d}{dt}V_I &= (1-\eta)P - cV_I, \\ \tfrac{d}{dt}V_{NI} &= \eta P + P^* + N\delta_p T_p - cV_{NI}, \end{aligned} \tag{4}$$

where P^* accounts for virus production from the ignored compartments, T_m, T_s, and T_l, and T can also be assumed as a constant for the short period. Then an analytic solution for (4) is

$$V(t) = P_0 + P_1 e^{-\delta_p t} + (P_2 + P_3 t)e^{-ct}. \tag{5}$$

Note that the number of parameters to be estimated is reduced to six. Following Perelson, et al. (1996, 1997), the number of parameters may be further reduced by assuming a

quasi-steady state condition before treatment; however, this condition may not always be realistic in actual clinical trials (Perelson et al., 1997). Moreover, simplified models using steady-state conditions lead to serious nonlinearity in parameters, which causes difficulties and inefficiency in parameter estimation and other statistical inferences (Ratkowsky, 1983).

When the viral load data are available for a longer period, more infected cell compartments may be considered (Perelson et al. 1997; Wu et al., 1997 and 1998b). However, due to the limited accuracy and sensitivity of current HIV-1 RNA assays, perhaps only one more compartment can be realistically identified; the virus production from other compartments may again be approximated by a constant P^*. Simplifying in a manner similar to that used in above, we can obtain the total virus load in the form

$$V(t) = P_0 + P_1 e^{-\delta_p t} + P_2 e^{-\lambda t} + (P_3 + P_4 t) e^{-ct}, \tag{6}$$

where λ is a possibly confounded clearance rate of long-lived and latently infected cells (Perelson et al., 1997; Wu et al., 1998b). However, we also recall that the clearance of free virus has been estimated to be very rapid (less than 6 hours of half-life, see Perelson et al. 1996). Hence the term due to the clearance of free virus, $(P_3 + P_4 t)e^{-ct}$ in model (6), will be negligible compared to other terms due to the clearance of infected cells after a few days of treatment. Thus the model can be further simplified as

$$V(t) = P_0 + P_1 e^{-\delta_p t} + P_2 e^{-\lambda t}, \qquad \text{for} \quad t \geq t_c, \tag{7}$$

where t_c is the time required for the term $(P_3 + P_4 t)e^{-ct}$ to become negligible.

In general, if viral dynamics data for only a segment of the treatment time are available from a clinical trial, we may simplify the viral dynamic model by considering the following: (i) we may ignore some terms associated with the faster decays (e.g. free virus and productively infected cells) as in the above examples, if they are negligible in the modeling time period based on results from similar studies or published literature; (ii) we may approximate slower decays (e.g. long-lived infected cells or mysterious infected cells) by constants if they are slow enough in the modeling time period; (iii) if the sensitivity and accuracy of HIV-1 RNA assays do not allow us to accurately estimate all compartment terms, it is better to neglect some compartments or replace them by simpler terms such as a constant. Sometimes we may intentionally use only a segment of the viral dynamic

data even when we have the complete data, since this may help to simplify the model, and thus some important parameters can be more efficiently estimated (Wu et al., 1997); (iv) Statistical model selection criteria such as AIC or BIC may be used to simplify the models.

The single exponential models (Ho et al. 1995; Wei et al. 1995) and bi-exponential models (Wei et al. 1995 and Wu et al. 1998b) are special cases of our model (7) arrived at by further ignoring some slowly decaying compartments and the constant term. But these oversimplified models sometimes may yield misleading results (Wu et al., 1997). Thus we recommend using a constant term to approximate the slowly decaying compartments unless some hard evidence or statistical model selection methods show that it is unnecessary.

3. Hierarchical Nonlinear Mixed-Effect Model Approach

The structure of data such as those from ACTG 315 is that of repeated viral load measurements on each of a number of subjects. Because the viral dynamic process described by the models in Section 2 takes place within a given subject, and the process may differ among subjects, a natural statistical framework is that of the hierarchical nonlinear mixed effect model (Davidian and Giltinan, 1995). Specifically, we consider the following general model:

Stage 1. Intra-patient variation in viral load measurement:

$$y_{ij} = log(V(t_{ij}, \boldsymbol{\beta}_i)) + e_{ij}, \quad \boldsymbol{e}_i|\boldsymbol{\beta}_i \sim (\mathbf{0}, \boldsymbol{R}_\mathbf{i}(\boldsymbol{\beta}_\mathbf{i}, \boldsymbol{\xi})), \tag{8}$$

where y_{ij} is the log-transform of the total viral load measurement for the ith patient and at the jth time point t_{ij}, $i = 1, \ldots, m; j = 1, \ldots, n_i$. The log-transformation of raw data is used to stabilize the variance (it is also more normally distributed). Model (3) can be re-written as

$$V(t_{ij}, \boldsymbol{\beta}_i) = \sum_{\ell=0}^{k} e^{\theta_{\ell i} - \lambda_{\ell i} t_{ij}}, \tag{9}$$

where the θ's are the log-transformations of the P's, the coefficients of exponentials in the last section. With the log-transformation, the new parameters, $\theta_{\ell i} = \log(P_{\ell i})$, are of similar scale as the λ's, the exponential decay rates. Denote the dynamic parameters for the ith patient by $\boldsymbol{\beta}_i = [\theta_{0i}, \ldots, \theta_{ki}, \lambda_{1i}, \ldots, \lambda_{qi}]'$. The within-patient

random error $\boldsymbol{e}_i = [e_{i1}, \ldots, e_{in_i}]'$ has a zero mean and covariance matrix $\boldsymbol{R}_i$ conditional on $\boldsymbol{\beta}_i$.

Stage 2, Inter-patient variation:

$$\theta_{0i} = \theta_0 + b_{0i}, \quad \ldots \quad \theta_{ki} = \theta_k + b_{ki}, \tag{10}$$

$$\lambda_{1i} = \lambda_1 + b_{(k+1)i}, \quad \ldots \quad \lambda_{qi} = \lambda_q + b_{(k+q)i}. \tag{11}$$

Population parameters are $\boldsymbol{\beta} = [\theta_0, \ldots, \theta_k, \lambda_1, \ldots, \lambda_q]'$. Random effects are $\boldsymbol{b}_i = [b_{0i}, \ldots, b_{(k+q)i}]' \sim (\boldsymbol{0}, \boldsymbol{D})$.

For the real data analysis in the next section, we used a diagonal variance-covariance matrix $\boldsymbol{R}_i$ (uncorrelated within-patient measurements). A more complicated structure may be considered in the future. All the dynamic models we fitted to the ACTG 315 data in the next section were carried out within this statistical framework. The real data support that the θ's and λ's are likely to be normally distributed. The log-transformed parameter, $\theta_{\ell i} = \log(P_{\ell i})$, can also ensure the positivity of the parameters and improve the estimation stability.

A number of inferential procedures are available for fitting hierarchical nonlinear mixed effects models, see Davidian and Giltinan (1995) for an overview. These methods allow estimation of population parameters $\boldsymbol{\beta}$ and $\boldsymbol{D}$ as well as prediction of individual parameters $\boldsymbol{\beta}_i$ via empirical Bayes techniques. Because the data from HIV dynamic studies may be sparse for some patients, we favor methods based on linearization of the model in the random effects. A number of software implementations are in widespread use (Roe, 1997); for analysis of the data from ACTG 315, we used the Splus function NLME, which implements the method of Lindstrom and Bates (1990). For such procedures, model selection criteria such as Akaike's (AIC) and Schwarz's Bayesian (BIC) information criteria have been advocated for comparison of competing models; for the particular structure of viral dynamic models, Wu et al. (1998a) found BIC to be more reliable.

4. Analysis of ACTG 315 Data

The modeling and inferential methods of the previous sections were applied to the data from ACTG 315. Of the 53 patients, 7 either dropped out from the study or showed

profiles that did not correspond to the pattern of the rest of the patients, exhibiting no response to the antiviral drugs due to poor absorption of the drugs, noncompliance, drug resistance, or other unknown reasons. The remaining subjects had between 3 and 9 viral load measurements; data from four representative patients are shown in Figure 1. It is worth noting that the HIV-1 RNA assay has a limit of detection of 100 copies. For patients with measurements below detectable level, we imputed the values to be 50 (=1.7 in $\log_{10}$ scale). Since viral rebound due to drug resistance and noncompliance is not taken into account in our models, the data after viral rebound are excluded from our analysis. Considering that, after a long time period, the data are likely to be contaminated and influenced by factors such as drug resistance, noncompliance, and assay detection limits, and model assumptions may not hold, we stopped using the data after 12 weeks.

As proposed in Section 2, since the viral load data are available only after 2 days following the initiation of treatment, the exponential decay term related to the clearance of free virus, e^{-ct}, can be ignored. Based on the estimate $c = 3\ day^{-1}$ in Perelson et al. 1996, the terms containing e^{-ct} account for less than 1% of the total viral load after 2 days and account for less than 0.0001% of the total viral load after 7 days.

We considered the following two simplified models based on the suggestions in Section 2, since both models can capture two observed phases of virus decay.

$$V(t) = P_0 + P_1 e^{-\delta_p t}, \tag{12}$$

$$V(t) = P_1 e^{-\delta_p t} + P_2 e^{-\lambda_l t}. \tag{13}$$

The AIC and BIC were used to compare the two models. On the basis of AIC and BIC values (see Table 1), Model (13) is preferred. Attempts to fit more complex models were not fruitful, most likely because the data are not rich enough to identify more compartments. From the fit of (13), estimates of individual profiles based on empirical Bayes methods, available in the NLME software, are shown for 4 patients in Figure 1; the model seems to provide a good fit. A summary of estimation results from both models is given in Table 1. Standard diagnostic plots of observed vs. fitted values based on the population estimate of $\boldsymbol{\beta}$, residuals vs. fitted values based on this estimate, and residuals based on empirical Bayes estimates of the $\boldsymbol{\beta}_i$ for each patient (Pinheiro and Bates, 1995) revealed no anomalies indicating gross lack-of-fit.

Place of Table 1

The results from the preferred model (Table 1) are very important for understanding the biological mechanism and pathogenesis of HIV-1 infection. The individual estimates of the viral dynamic parameters can be used for clinical decisions in the treatment of individual patients. First, the population estimates of the two decay rates, $\hat{\delta_p} = 0.442 \pm 0.029, \hat{\lambda_l} = 0.032 \pm 0.003$, represent the turnover rates of productively infected cells and long-lived and/or latently infected cells, respectively. The corresponding half-lives [$\log(2)/\delta_p$ and $\log(2)/\lambda_l$] of these two virus compartments can be calculated as 1.57 ± 0.103 days and 21.66 ± 2.03 days respectively. These estimated life-times of infected cells are believed to be shorter than those of normal uninfected cells, although some debates still exist. Undoubtedly, these estimation results would help immunologists and virologists to understand the cell kinetics and the interaction mechanisms of HIV-1 and its target cells.

Second, the ratio, $P_2/(P_1+P_2)$ gives an approximate estimate of the proportion of virus produced by long-lived and/or latently infected cells in the total virus pool. Our estimate is 1.1%, which indicates that most of the virus is produced by productively infected cells, and only a small proportion is produced by long-lived and/or latently infected cells. Thus antiviral therapies should mainly target the productively infected cells, although the compartment of long-lived/latently infected cells cannot be ignored.

Third, the individual estimates of the decay rates show that the interpatient variations are large. The estimates of δ_p range from 0.17 to 0.64, with an estimated CV of 31% (the corresponding half-life ranges from 1 day to 4 days). The estimates of λ_l range from 0.0014 to 0.0534, with an estimated CV of 47% (the corresponding half-life ranges from 13 days to 483 days). The large interpatient differences indicate that each individual patient should be treated differently in clinical decisions. For example, the eradication times for the two compartments, productively infected cells and long-lived and/or latently infected cells, can be approximated by $\log P_1/\delta_p$ and $\log P_2/\lambda_l$. The ranges of estimates of the eradication time for these two compartments are (14, 71) days and (158, 2688) days. If the virus is eradicated, patients may stop taking expensive and toxic antiviral drugs. The estimates of eradication time based on our models and methods give an estimate for minimum treatment time. This information, combined with other information such

as the presence of virus in other reservoirs, may assist clinicians to make decisions and predictions regarding when to stop treatment. More medical discussion of these findings can be found in Wu et al. (1998b).

5. Summary and Discussion

In this paper we presented a model for HIV-1 dynamics in vivo, in which we considered both RTI and PI antiviral drug effects and all the possible infected cell and virus compartments. In order to apply this model to clinical trials, we proposed a procedure to simplify the model for HIV-1 RNA data in different phases of virological response. Without making an assumption of steady-state before treatment, we reduced the model to applicable models for clinical data by reparameterization and reasonable approximation. We fitted this model to clinical data within the hierarchical nonlinear mixed effects framework. We are currently developing SAS macros and Splus functions for fitting HIV dynamic models using these methods.

Note that many mathematical functions such as polynomial functions, some power functions (Ratkowsky, 1990) or piecewise linear functions may fit the viral dynamic trajectory equally well or even better. Statisticians usually select a model for their data based on the convenience and goodness-of-fit to the data. However, without a biological interpretation and justification, a mathematical model may not be useful. We believe that a good model should have the following features: (i) good fit to the observed data; (ii) reasonable biological interpretation; (iii) conceptual and computational simplicity; (iv) applicability to low-quality and less frequent clinical data; (v) convenience for statistical inferences and interpretation; (vi) lower costs economically and computationally. The simplified models for HIV-1 dynamics proposed in this paper enjoy all these features. Although biological compartment models are sometimes considered complex, the models proposed in this paper can also be treated as simple statistical models to trace the trajectory of viral load. Then these models can still be used for prediction or other purposes, but their biological interpretation may not be valid.

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APPENDIX

Solutions of the Full Dynamic Model for Antiviral Treatment with Combination of PI and RTI Drugs

From (1), the solution for V_I is straightforward by integration,

$$V_I(t) = (1-\eta)P/c + [V_I(0) - (1-\eta)P/c]e^{-ct}.$$

Plug the above result to equations for T_m, T_s and T_l and using $T = T_0 + T_r(1-e^{-rt})$ we easily obtain the following solutions by integration and some algebra.

$$\begin{aligned}
T_m(t) = &\ b_{m0} + b_{m1}e^{-\lambda_m t} + b_{m5}e^{-ct} + b_{m7}e^{-rt} + b_{m8}e^{-(c+r)t} \\
T_s(t) = &\ b_{s0} + b_{s2}e^{-\lambda_s t} + b_{s5}e^{-ct} + b_{s7}e^{-rt} + b_{s8}e^{-(c+r)t} \\
T_l(t) = &\ b_{l0} + b_{l3}e^{-\lambda_l t} + b_{l5}e^{-ct} + b_{l7}e^{-rt} + b_{l8}e^{-(c+r)t},
\end{aligned}$$

where

$$\begin{aligned}
b_{m0} = &\ (1-\gamma)\alpha_m k_0(T_0+T_r)(1-\eta)P/(c\lambda_m) \\
b_{m1} = &\ T_m(0) - b_{m0} - b_{m5} - b_{m7} - b_{m8} \\
b_{m5} = &\ (1-\gamma)\alpha_m k_0(T_0+T_r)[V_I(0)c - (1-\eta)P]/[c(\lambda_m - c)] \\
b_{m7} = &\ -(1-\gamma)\alpha_m k_0 T_r(1-\eta)P/[c(\lambda_m - r)] \\
b_{m8} = &\ -(1-\gamma)\alpha_m k_0 T_r[V_I(0)c - (1-\eta)P]/[c(\lambda_m - c - r)].
\end{aligned}$$

And b_{si} and b_{li}, $i = 0, 5, 7, 8$ have the same expressions as b_{mi}'s with α_m and λ_m replaced by α_s, λ_s, α_l and λ_l accordingly, and

$$\begin{aligned}
b_{s2} = &\ T_s(0) - b_{s0} - b_{s5} - b_{s7} - b_{s8} \\
b_{l3} = &\ T_l(0) - b_{l0} - b_{l5} - b_{l7} - b_{l8}
\end{aligned}$$

Then the solution for T_p is, (by plug the solution of T_l into equation (1))

$$T_p(t) = \ b_{p0} + b_{p3}e^{-\lambda_l t} + b_{p4}e^{-\delta_p t} + b_{p5}e^{-ct} + b_{p7}e^{-rt} + b_{p8}e^{-(c+r)t}$$

where $b_{p4} = T_p(0) - b_{p0} - b_{p3} - b_{p5} - b_{p7} - b_{p8}$ and

$$\begin{aligned}
b_{p0} = &\ [(1-\gamma)\alpha_p k_0(T_0+T_r)(1-\eta)P/c + \delta_l b_{l0}]/\delta_p \\
b_{p3} = &\ \delta_l b_{l3}/(\delta_p - \lambda_l) \\
b_{p5} = &\ [(1-\gamma)\alpha_p k_0(T_0+T_r)(V_I(0) - (1-\eta)P/c) + \delta_l b_{l5}]/(\delta_p - c) \\
b_{p7} = &\ [-(1-\gamma)\alpha_p k_0 T_r(1-\eta)P/c + \delta_l b_{l7}]/(\delta_p - r) \\
b_{p8} = &\ [-(1-\gamma)\alpha_p k_0 T_r(V_I(0) - (1-\eta)P/c) + \delta_l b_{l8}]/(\delta_p - c - r).
\end{aligned}$$

Plug all the above solutions into equation (1), we are left with a first-order linear differential equation for V_{NI}. Hence the solution for V_{NI} is

$$\begin{aligned}
V_{NI}(t) = &\ P_0^* + P_1 e^{-\lambda_m t} + P_2 e^{-\lambda_s t} + P_3 e^{-\lambda_l t} + P_4 e^{-\delta_p t} \\
&\ P_5^* e^{-ct} + P_6 t e^{-ct} + P_7 e^{-rt} + P_8 e^{-(c+r)t}.
\end{aligned}$$

where $P_5^* = V_{NI}(0) - P_0^* - P_1 - P_2 - P_3 - P_4 - P_7 - P_8$ and

$$
\begin{aligned}
&P_0^* = (N\delta_p b_{p0} + N\delta_m b_{m0} + N\delta_s b_{s0} + \eta P)/c \\
&P_1 = N\delta_m b_{m1}/(c - \lambda_m) && P_2 = N\delta_s b_{s2}/(c - \lambda_s) \\
&P_3 = N\delta_p b_{p3}/(c - \lambda_l) && P_4 = N\delta_p b_{p4}/(c - \delta_p) \\
&P_6 = N\delta_p b_{p5} + N\delta_m b_{m5} + N\delta_s b_{s5} \\
&P_7 = N(\delta_p b_{p7} + \delta_m b_{m7} + \delta_s b_{s7})/(c - r) && P_8 = -N(\delta_p b_{p8} + \delta_m b_{m8} + \delta_s b_{s8})/r
\end{aligned}
$$

Finally we get the total virus load, $V(t) = V_I(t) + V_{NI}(t)$, as

$$
\begin{aligned}
V(t) = \; & P_0 + P_1 e^{-\lambda_m t} + P_2 e^{-\lambda_s t} + P_3 e^{-\lambda_l t} + P_4 e^{-\delta_p t} \\
& + (P_5 + P_6 t) e^{-ct} + P_7 e^{-rt} + P_8 e^{-(c+r)t}.
\end{aligned}
$$

where $P_0 = P_0^* + (1 - \eta)P/c$ and $P_5 = V(0) - P_0 - P_1 - P_2 - P_3 - P_4 - P_7 - P_8$.

Table 1: Estimation Results (SE represents the standard error)

Effects	Parameters	Model (12)	Model (13)
	δ_p (SE)	0.216 (0.011)	0.442 (0.029)
Fixed	λ_l (SE)	-	0.032 (0.003)
Effects	$\log P_0$ (SE)	11.023 (0.222)	-
	$\log P_1$ (SE)	5.530 (0.192)	12.142 (0.240)
	$\log P_2$ (SE)	-	7.624 (0.252)
Standard	δ_p	0.035	0.137
Deviation	λ_l	-	0.015
of	$\log P_0$	1.284	-
Random	$\log P_1$	1.100	1.397
Effects	$\log P_2$	-	1.545
σ		0.381	0.267
Loglikelihood		-230.469	-162.151
AIC		480.938	354.302
BIC		518.838	411.013

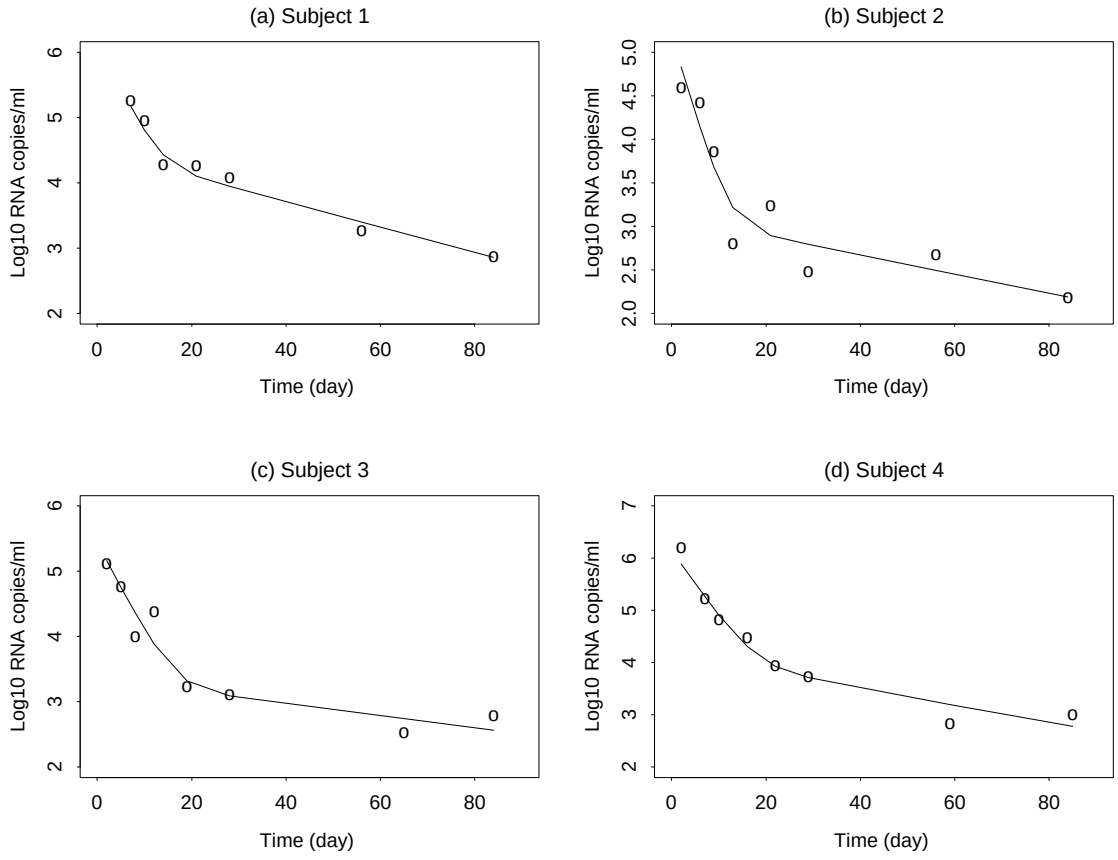

Figure 1: Plasma HIV-1 RNA copies ($\log_{10}$ scale) and fitted curves of simplified model, $V(t) = P_1 e^{-\delta_p t} + P_2 e^{-\lambda_l t}$ for 4 patients, where the circles are the observed values, and the solid lines are fits of the four individual profiles based on empirical Bayes estimates of individual-specific model parameters.

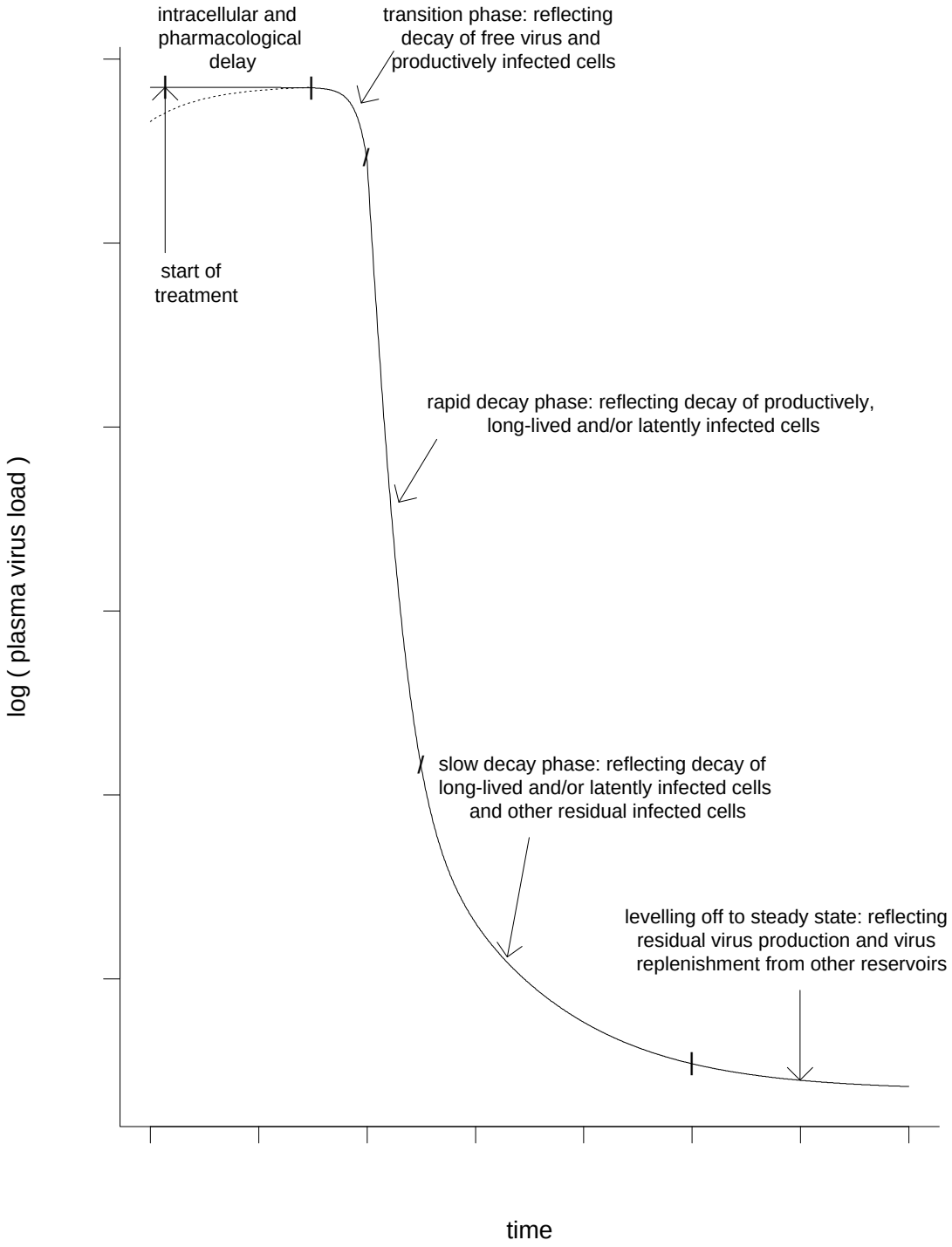

Figure 2: Illustration of the different phases of plasma viral dynamics following antiviral drug treatment. If the data on the transition phase and rapid decay phase are available, the suggested model is $V(t) = P_0 + P_1 e^{-\delta_p t} + (P_2 + P_3 t)e^{-ct}$. If the data on the rapid decay phase and slow decay phase are available, the suggested model is $V(t) = P_0 + P_1 e^{-\delta_p t} + P_2 e^{-\lambda t}$. If the data on the slow decay phase and leveling off phase are available, the suggested model is $V(t) = P_0 + P_2 e^{-\lambda t}$. The suggested models should be refined by the model selection procedure. In the phase of intracellular and pharmacological delay, the dotted line denotes non-steady-state case before treatment.