Examining hepatotoxicity and CD4 recovery in concomitant antiretroviral and anti-tuburculosis treatment

Dionna Jacobson[1]*

Abstract
HIV-tuberculosis-co-infection remains to be a global issue as patients with HIV are at high risk of developing tuberculosis. Combination antiretroviral and anti-tuberculosis treatments used to combat co-infection have been effective in reducing mortality rates in these populations. However, the intrinsic hepatotoxicity associated with these drugs is not well characterized in combination therapies. Here, it was shown that risk of developing hepatotoxicity was greater with concomitant antiretroviral and anti-tuberculosis treatment as compared to antiretrovirals alone. Risk was also dependent on body drug exposure of antiretroviral drugs, *NAT2* polymorphisms, gender, and body mass index. The effect of combined treatment on CD4 recovery rate was also explored and suggested that initial CD4 count, older age, the female gender, and lower weight hindered normal recovery. These findings identify groups of patients that should be targeted for effective and safe therapeutic dosing and management when these drugs are co-administered.

[1] *CoMPLEX, University College London, London, United Kingdom*
***Primary Email**: dionna.jacobson.17@ucl.ac.uk
Supervisors: Joseph Standing and Julie Bertrand

Contents

1. Introduction

Tuberculosis is the most common infection as well as the most prevalent cause of death in individuals with advanced human immunodeficiency virus (HIV), largely in resource-limited settings [1]. According to the World Health Organization, people infected with HIV are 16-27 times more likely to develop tuberculosis as compared to those without HIV [1]. Studies investigating timing of antiretroviral treatment (ART) with respect to tuberculosis therapy have recommended early rather than late initiation of ART to decrease mortality in adults who are severely immunocompromised [2]. However, hepatotoxicity is a well-recognized, overlapping complication of ART and anti-tuberculosis drugs, and should be considered when these therapies are given in combination, especially when delay between treatment types is reduced. Providing safe and effective therapies to combat co-infection of HIV and tuberculosis is crucial to reduce mortality in these populations. Therefore, in this study, the hepatotoxicity and efficacy of concomitant treatment was investigated in patients with HIV-tuberculosis-coinfection from the Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA) trial.

1.1 Hepatotoxicy of ART and anti-tuberculosis drugs

Hepatotoxicity is characterized as drug-induced liver damage and usually detected by changes in normal liver function as well as alternations in tissue and cellular integrity. Serum concentrations of cell-specific components that leak out of damaged hepatic cells, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are measured and compared to a threshold value of three times the biomarker's upper limit of normal range [3]. High AST or ALT measurements will prompt clinicians to discontinue standard tuberculosis treatment [4]. Combined with concomitant symptom assessment and measurements of serum total bilirubin for evaluation of liver function, drug-induced liver injury is diagnosed [3].

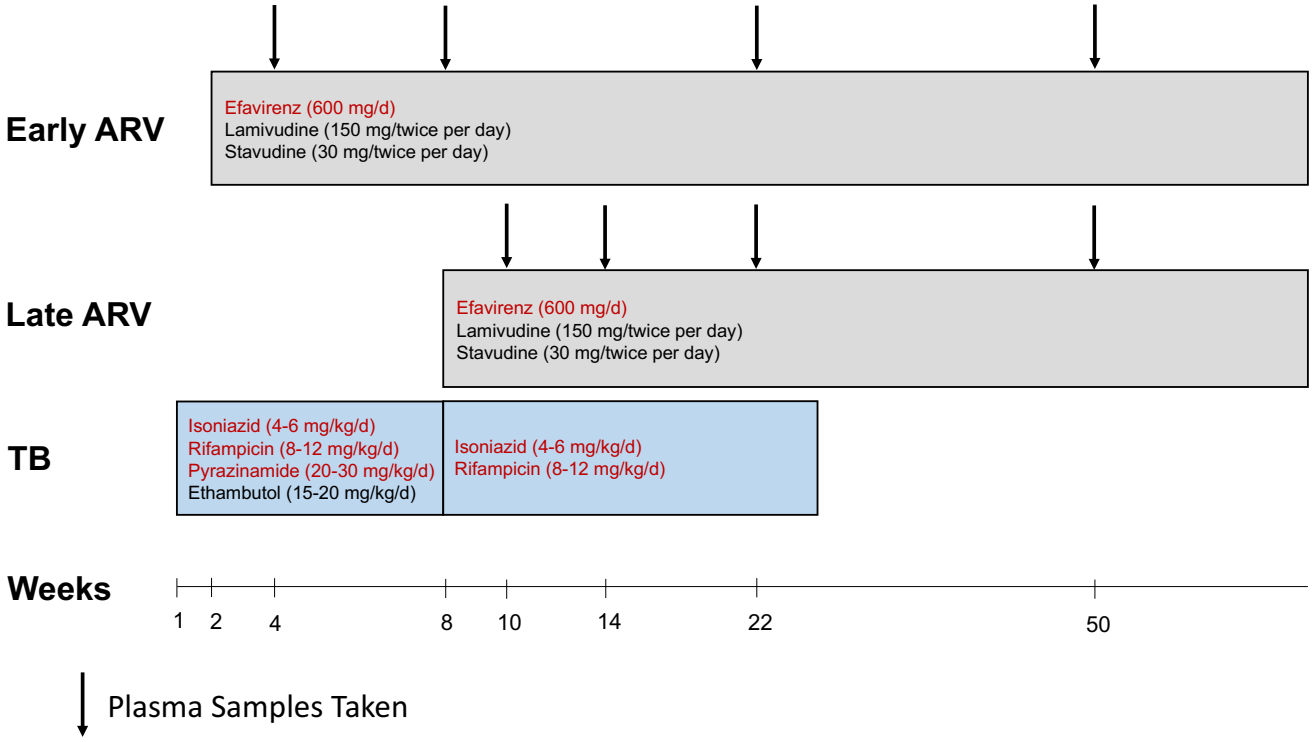

Figure 1. CAMELIA study design. Drugs related to hepatotoxicity are highlighted in red. Drug concentrations per day indicated in parenthesis. ARV, antiretroviral; TB, tuberculosis.

The nonnucleoside reverse-transcriptase inhibtor, efavirenz (EFV), is recommended by the World Health Organization as a preferred first-line therapy for ART [5], and was administered in the CAMELIA trial. Efavirenz has been associated with hepatic toxicity, and the drug's long-term effects on liver injury are of concern, as a common cause of death among patients with HIV is liver-related complications [6]. It has been suggested that EFV interaction with mitochondria may induce hepatotoxicity by triggering a stress response in the entoplasmic reticulum [7].

Hepatotoxicity is the most common adverse event associated with standard tuberculosis drugs isoniazid, rifampicin, and pyrazinamide, which are usually co-administered [8]. Demographic features that make certain individuals more susceptible to developing liver toxicity by tuburculosis drugs include the female sex, malnutrition, high alcohol consumption, co-infection with HIV, and age 60 or above [8]. Similarly, genetic susceptibility has been well characterized, with particular attention focused on polymorphisms in the *NAT2* gene, which encodes for an enzyme responsible for isoniazid metabolism. Individuals with the slow *NAT2* metabolizer phenotype process isoniazid at a slower rate and have a five fold risk of developing isoniazid-induced hepatotoxicity when compared to individuals with the fast *NAT2* metabolizer phenotype [9].

While liver toxicity of antiretroviral and anti-tuburculosis drugs have been characterized separately, little is known about their joint toxicity. Certain predictors have been associated with increased risk of developing hepatotoxicity including tuberculosis-HIV-co-infection, concomitant treatment, lower CD4, hemoglobin and platelet counts, and higher baseline AST and bilirubin levels [10]. Previous analysis on data from the CAMELIA trial revealed that patients who maintained high plasma concentrations of efavirenz (above 4,000 ng/mL) had a greater risk of developing hepatotoxicity of any grade than patients with concentrations either lower than or intermittently above 4,000 ng/mL [11]. Furthermore, genetic polymorphisms in cytochromes *NAT2* and *CYP2B6* (associated with increased plasma EFV concentrations) made certain individuals more susceptible to developing high efavirenz concentrations in the presence of anti-tuburculosis drugs [12].

Here, predictors of hepatotoxicity induced by co- administration of anti-tuberculosis and ART treatment were identified using generalized linear-mixed models. A pharmacokinetic-pharmacogenetic model analyzing plasma concentrations of efavirenz over time, which included demographic and genetic covariates, was previously created based on clinical data from CAMELIA [12]. As pharmacokinetic approaches predict population and individual parameters, values describing efavirenz drug properties within individual patients were extracted from the output of this model. Since efavirenz concentration has been linked to enhanced risk of hepatotoxic events, the extracted parameters were included in the predictor analysis.

1.2 Influence of ART on CD4 recovery

It is standard to first assess the toxicity associated with a drug before investigating its ability to carry out a desired function

in the body. Therefore, following evaluation of hepatotoxicity, efficacy of ART in the presence of anti-tuberculosis drugs was investigated by modeling CD4 count recovery in the CAMELIA trial.

HIV replication is suppressed by ART, reducing HIV viral loads that infect and kill CD4 cells. Restoration of adult CD4 cells via ART have shown to follow an asymptotic recovery over time with an initial steep climb that gradually slows until it reaches a homeostatic set point [13]. Positive predictors of CD4 count recovery include a greater initial CD4 count, the female gender, and lower age [14]. While patient demographic and clinical predictors of CD4 recovery have previously been explored in adults, the association between efavirenz pharmacokinetic parameters and CD4 count have not been described.

In this study, a nonlinear mixed-effect model was developed to predict the evolution of CD4 count over time following onset of ART. Initial CD4 count, final CD4 count, and the rate at which CD4 counts increased were estimated from the data and influence of demographic and pharmacokinetic covariates on these parameters were explored.

2. Materials and Methods

2.1 Patient Population and Trial Design

Patient data was collected from CAMELIA, which was carried out under the Cambodian National Ethics Committee for Health Research guidelines. Summary of enrolled patients and clinical design have been described elsewhere [2]. Patients were randomly assigned to begin ART either two weeks (early) or eight weeks (late) after onset of anti-tuberculosis therapy to determine if earlier initiation of ART would improve survival in patients with advanced HIV (CD4+ T-cell count $\leq 200\ cells/\mu L$ at enrollment) [2].

Tuberculosis treatment followed the WHO-recommended 6 month regimen: isoniazid (4 - 6 mg/kg), rifampicin (8 - 12 mg/kg), ethambutol (15 - 20 mg/kg), and pyrazinamide (20 - 30 mg/kg) taken daily for two months proceeded by isoniazid and rifampicin taken daily for four months. ART treatment complied with Cambodian national guidelines and consisted of daily efavirenz (600 mg in the evening), and twice daily lamivudine (150 mg) and stavudine (30 mg). Blood samples from all patients were drawn two weeks and six weeks after ART initiation, corresponding to weeks four and eight in the early-ART group and weeks ten and fourteen in the late-ART group. Blood samples were also obtained from all patients at weeks 22 and 50 following inclusion, accounting for ART with and without concomitant tuberculosis therapy. Laboratory measurements from additional samples at weeks 18, 26, 78, 102, 126, 150, 174, and 189 following onset of tuberculosis therapy were taken. Study design and timeline is represented in Figure 1.

2.2 Hepatotoxic Events

Aspartate aminotransferase (AST) levels were measured from all whole blood samples kept frozen at -80°C until analysis via automate COBAS MIRA (Roche Diagnostics Co, Indianapolis, USA). Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events (2014) was used to classify grade of event based on aminotransferase levels. In this study, patients were recorded as experiencing an event if AST measurements were greater than a threshold of two times upper limit of normal range corresponding to a grade one event (mild). Number of AST events associated with three times upper limit normal range, corresponding with a more severe hepatotoxicity diagnosis, were too few and therefore AST threshold had to be lowered. AST normal range is defined as 1-40 IU/L in females, and 8-34 IU/L in males [15]. While Alanine aminotransferase (ALT) levels were also recorded, only a small subset of measurements were characterized as toxic events, limiting further statistical analysis associating ALT with liver toxicity.

2.3 Efavirenz body drug exposure

In pharmacokinetics, clearance represents the volume of fluid removing drug per unit time. Body drug exposure (also known as area under the curve, AUC, of drug concentration versus time) simply indicates the total amount of exposure a system receives from a drug, and it can be calculated by taking the difference between a fixed drug dose and the drug's clearance. In this study, patient efavirenz (EFV) AUC values when antiretrovirals were given with and without tuberculosis treatment were used to assess hepatotoxicity. Additionally, EFV AUC was incorporated into the CD4 model as a covariate to examine the effect of efavirenz exposure on CD4 count.

A pharmacokinetic- pharmacogenetic model analyzing efavirenz plasma concentrations over time was previously built [12]. In this study, estimated individual patient clearance (CL) values were extracted from the output of this final efavirenz pharmacokinetic model to derive efavirenz drug body exposure values. The model was re-created in the nonlinear mixed effects software, NONMEM (ICON Development Solutions).

2.4 Genotyping Analysis

Puregene Blood Kits (Gentra systems, MN, USA) were used to extract DNA according to the Quiagen protocol. Genetic polymorphisms associated with transport and metabolism of EFV and TB drugs were genotyped using the TaqMan allelic discrimination assay [16]. The single nucleotide polymorphisms in two metabolizers – *NAT2* and *CYP2B6* – were evaluated based on their previously described effect on efavirenz clearance in the presence of isoniazid (INH)and rifampicin [12]. *NAT2* is the main enzyme involved in INH metabolism and is highly polymoprhic. Individuals can be categorized as "rapid" or "slow" metabolizers of INH based on the relative number of functional and loss-of-function *NAT2* alleles. Presence of one or two wild-type alleles (*NAT2*4*) phenotyped individuals as fast metabolizers, while presence of two loss-of-function (NAT2*5, textitNAT2*6, or textitNAT2*7) alleles phenotyped individuals as slow metabolizers [17]. The *CYP2B6 G516T* polymorphism was examined in this study,

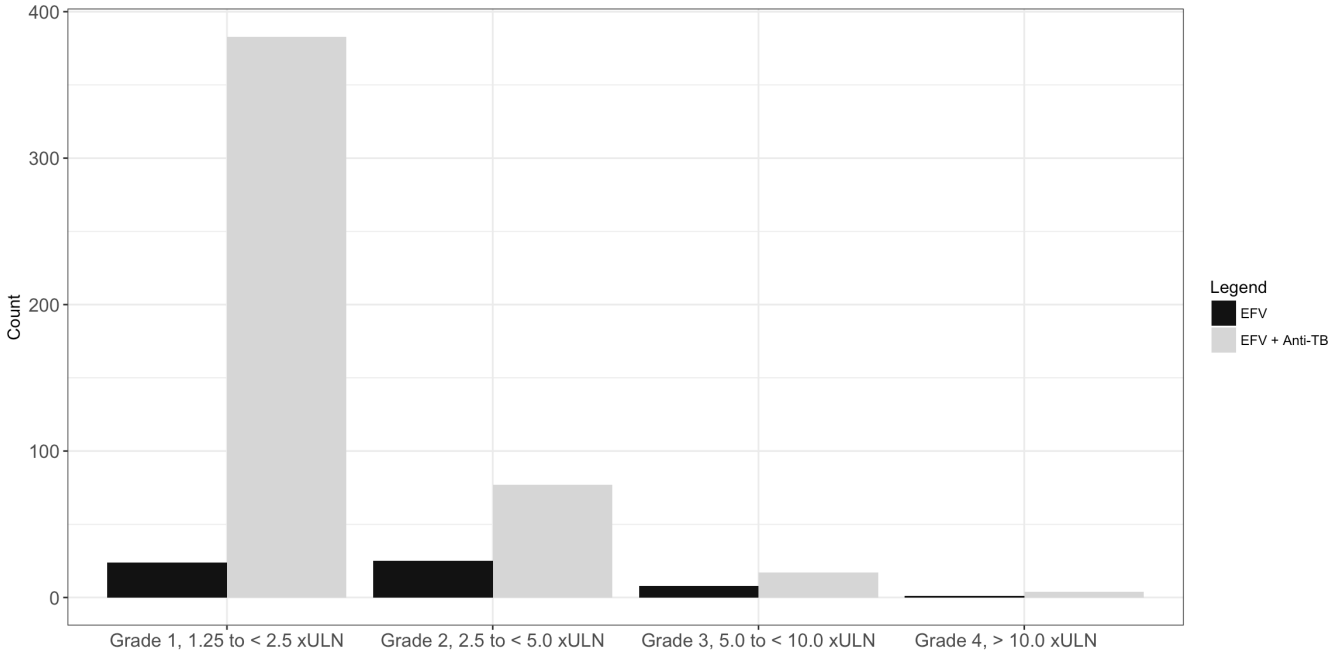

Figure 2. Number of events (AST $>$ 2x ULN) in EFV versus EFV + TB treatment groups, separated by grade of event.

and has been associated with greater efavirenz concentrations and half-life [18]. The relative distribution and Hardy-Weinburg equilibrium values of these alleles in the CAMELIA population are described in Bertrand, *et al* (2013).

2.5 Hepatotoxicity prediction model

In this analysis, significant predictors associated with a binary outcome - patient AST levels above two times upper limit normal during ART-based therapy - were identified. Generalized linear models (GLMs) measure response variables from a binary distribution (logistic regression), but ignore correlation between groups in longitudinal, multilevel data by only estimating fixed effects and assuming all observations are independent. Mixed-effect models incorporate both fixed and random effects into their framework to account for group-level variation. Therefore, variability across repeated patient measurements was controlled using generalized linear mixed-models (GLMMs), a subset of mixed-effect modeling that can predict binary responses.

Predictor inclusion was justified by forward stepwise selection using a likelihood ratio test with a significance threshold of 5%. Influence of predictors on AST event likelihood were assessed using a Wald Chi-Square test with a significance threshold of 5%. Age, efavirenz body drug exposure (AUC), and body mass index were considered after normalization to their median values, and were imputed to corresponding subject median values when missing. Time since ART onset (weeks), concomitant tuberculosis treatment status, arm of study, and sex were also tested. Genetic predictors - *CY2B6 and NAT2* - were assessed with and without interaction of TB status. Additionally, between-subject random effects of intercept and AUC were considered. This analysis was done using the lme4 package in R 3.4.3 [19].

2.6 CD4 recovery model

CD4 cell count was measured in the blood at screening weeks 1, 8, 10, 14, 18, 22, 26, 50, 78, 102, 126, and 150 following tuberculosis treatment onset using the BD FACSCount^{TM} system (BD Biosciences, San Jose, USA). Like GLMMs, nonlinear mixed-effect models account for group-level variation with the addition of patient-level random effects that

Table 1. Patient characteristics at ART initiation.

No. of Patients	210
Gender	
No. of Females	60
No. of Males	120
CAMELIA Randomization Arm	
No. of Patients in Early Arm	112
No. of Patients in Late Arm	98
Weight, kg, median (range)	45 (30 - 74)
Height, cm, median (range)	160 (140 - 180)
Age, median (range)	36 (20 - 63)
CD4 Count, cells/μL, median (range)	25 (1 - 384)
AST, IU/L, median (range)	43 (14 - 391)
ALT, IU/L, median (range)	30 (5 - 225)
NAT2 metabolizer phenotype	
No. of Rapid Metabolizers	133
No. of Slow Metabolizers	77

* AST, aspartate aminotransferase; ALT, alanine aminotransferase.

describe deviation of individual responses from the universal fixed effect mean response. Furthermore, they allow for models to make both population and group level prediction of parameters, which can be useful when characterizing how certain parameters in the model correlate with patient-specific features. As adult CD4 count increases with time following an asymptotic, nonlinear function, evolution of CD4 count was fit against time using nonlinear mixed-effect (nlme) software, saemix in R 3.4.3 [20], which utilizes the stochastic approximation estimation algorithm for parameter estimation.

Previous studies have investigated the dynamics of CD4 T-cell recovery due to ART [21], from which the biological model in this study was informed on and is outlined as follows:

$$\frac{dT}{dt} = \gamma - \mu T \tag{1}$$

Where change in CD4 count over change in time is represented by CD4 thymic output (γ) and CD4 death rate (μ). Integration of the above equation provides a structural framework for predicting CD4 concentration from time. The statistical model is outlined:

$$T_{ij} = \frac{\gamma_i}{\mu_i} + (T(0)_i - \frac{\gamma_i}{\mu_i})e^{-\mu_i t_{ij}} + \varepsilon_{ij} \tag{2}$$

Parameters γ, μ, and $T(0)$ were used to predict T_{ij}, CD4 count for patient i at time t_{ij} days after ART initiation. $T(0)$ and $\frac{\gamma}{\mu}$ represent initial CD4 count and long-term, asymptotic CD4 count after treatment, respectively. Rate of CD4 count increase is determined by μ, with $ln(2)/\mu$ being the amount of time taken for half the CD4 count to rise since ART onset. ε_{ij} characterizes prediction error after patient and time variability are accounted for.

Influence of covariates on fixed effects of γ, μ, and $T(0)$ were assessed with a backwards selection approach using a likelihood ratio test and a p-value cutoff of 5%. Sex, age, weight, efavirenz body drug exposure, and arm of study were considered. Additionally, between-subject random effects of all three parameters were explored. The most conservative model was taken forward.

3. Results

The clinical and demographic characteristics of adults co-infected with HIV and tuberculosis from the CAMELIA trial included in this study are described in Table 1. Patients with baseline AST measurements above two times upper limit normal (ULN) were removed unless they demonstrated an AST level increase above 3.5 times ULN to account for abnormal baseline levels. In the CAMELIA trial, patients with AST or ALT levels above five times ULN discontinued tuberculosis treatment, so observations from these patients taken after high aminotransferase level recordings were discarded.

3.1 Co-administration of ART and tuberculosis treatment reveals greater risk of hepatotoxicity in slow *NAT2* metabolizers

This analysis used data from 210 patients, from which 2,036 AST measurements were taken, to identify predictors associated with abnormal AST concentration. AST levels were recorded at seven occasions with concomitant ART and tuberculosis treatment (EFV + TB), and seven occasions when therapy consisted of only ART (EFV). Most patient were observed at least eight times (n = 193) throughout treatment. There were 1,161 measurements taken during EFV + TB treatment, and 875 measurements recorded during EFV only treatment.

AST measurements were transformed into a binary category indicating whether or not levels reached above a threshold of two times upper limit normal (event). A total of 172 observations from 101 patients were identified as events in EFV + TB treatment, while 58 observations from 38 patients corresponded to events during EFV only treatment. Number of events categorized by severity (DAIDS grading of adverse events) in EFV + TB vs. EFV therapy groups are outlined in Figure 2.

GLMMs were used to identify significant predictors associated with patients experiencing an AST event. Intercept between-subject random effects were incorporated to account for variation between patients. Information about predictors that improved fit of the mixed-effects model are shown in Table 2. Intercept, *NAT2* metabolizer status during tuberculosis and antiretroviral treatment, EFV body drug exposure, and body mass index were identified as significant features associated with a higher likelihood of experiencing an AST event. A one unit increase in EFV exposure resulted in a 0.0038 ± 0.0018 (Estimate $\pm$ SE) increase in likelihood of developing AST levels above two times ULN. A one unit increase in body

Table 2. Final population estimates for predicting likelihood of developing AST levels greater than two times ULN.

Parameter	Estimate	SE	p-value	OR
Intercept	-2.3	0.84	0.0070	0.11
EFV body drug exposure (mg*h/L)	0.0038	0.0018	0.043	1.003
NAT2 rapid metabolizer, (EFV + TB vs EFV only)	0.63	0.22	0.0070	1.9
NAT2 slow metabolizer, (EFV + TB vs EFV only)	1.3	0.23	6e-08	3.5
Body Mass Index, $\frac{kg}{m^2}$	-2.2	0.85	0.008	0.11
Sex, (females vs males)	-0.53	0.26	0.044	0.59
Between-subject variability, intercept (ω^2)	1.5	0.0057	...	...

* OR, odds ratio; SE, standard error; EFV, efavirenz; TB, tuberculosis. Wald Chi-square test used to estimate p-value.

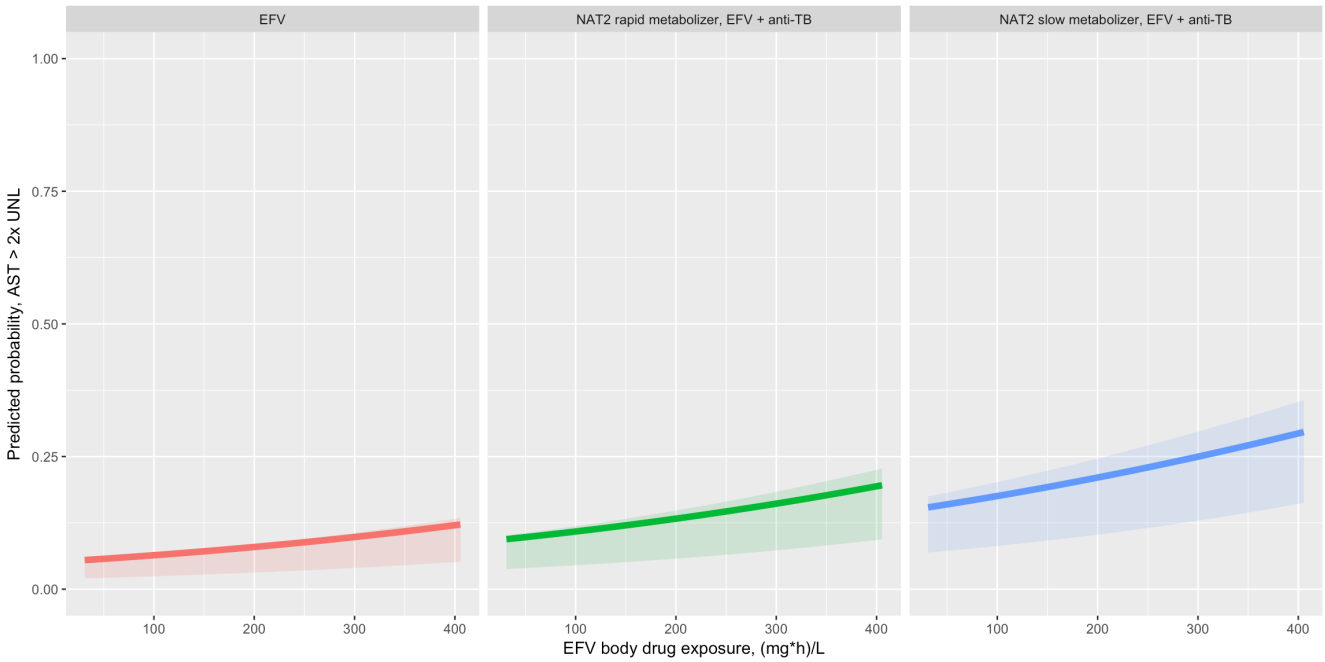

Figure 3. Predicted probability of experiencing an event (AST $>$ 2x ULN) over efavirenz body drug exposure vaues in the EFV treatment group and the EFV + TB treatment group, which is further seperated by *NAT2* metabolizer status. 95% confidence intervals are represented around the predicted values.

mass index resulted in a 2.2 ± 0.85 decrease in AST event likelihood and females were less likely than males to experience an event (-0.53 ± 0.26). *NAT2* slow and fast metabolizers on EFV + TB status yielded positive estimates (1.3 ± 0.23 and 0.63 ± 0.22, respectively), indicating that co-administration of ART and anti-tuberculosis drugs compared to only ART was associated with a greater likelihood of developing an AST event. Furthermore, likelihood of experiencing an event in slow metabolizers was greater than likelihood in fast metabolizers (OR = 1.9) when both groups maintained EFV + TB status (Figure 3). There was no association between event likelihood and *CYP2B6* phenotype, arm of study, or age.

3.2 Evaluating predictors of CD4 recovery

Figure 4 shows mean values of CD4 count across the trial, with time corresponding to patient visit number (roughly the

Table 3. Population parameter estimates in final CD4 recovery model.

Parameter		Estimate (SE)
Initial CD4 count, cells/μL	Population Mean	26.7 (3.2)
	BSV (ω^2)	1.2 (0.14)
	Arm (early vs late)	1.2 (0.15)
	Sex (female vs male)	-0.5 (0.16)
Thymic output, cells/μL/day	Population Mean	2.4 (0.83)
	BSV (ω^2)	0.19 (0.03)
	Age	-0.42 (0.15)
	Sex (female vs male)	0.39 (0.11)
CD4 death rate, 1/day	Population Mean	0.0066 (0.001)
	BSV (ω^2)	0.0043 (0.051)
	Age	0.00016 (0.00004)
	Sex, (female vs male)	0.0017 (0.001)
	Weight, kg	-0.00012 (0.00006)
Residual Variability	additive error	5.9 (0.92)
	proportional error	0.33 (0.0004)

* SE, standard error; BSV, Between-subject variability.

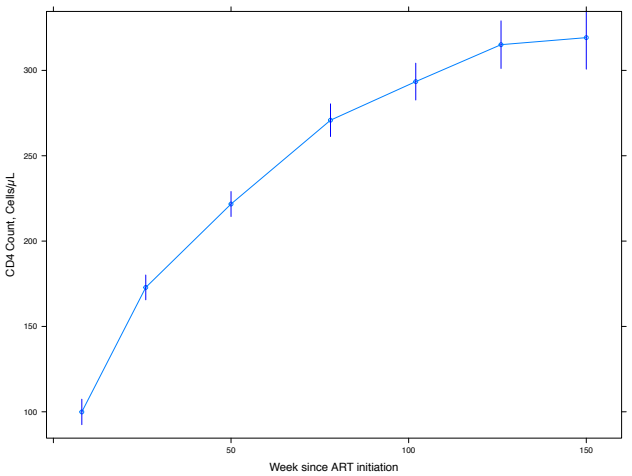

Figure 4. Population CD4 recovery mean at several time points since ART initiation. Error bars are standard error.

same as week since ART initiation), providing an overview of changes in CD4 count during recovery. There is an initial steep increase in the CD4 count that gradually slows until population numbers reach a steady state.

In this analysis, 1,006 measurements of adult CD4 counts from 257 patients were fit against time in days after initiation of ART treatment (final measurement taken 1,041 days following inclusion). The fixed and random effects, as well as covariate effects on parameters estimates in the final model are outlined in Table 3. Figure 5 demonstrates the model's predicted population and individual fits for CD4 count recovery in ten patients.

Rate of CD4 count increase was 0.0066 ± 0.001 days^{-1}, with the amount of time taken for half of the CD4 population to recover being $ln(2)/\mu \sim 105$ days or roughly 15 weeks. Evaluation of covariate effects reveal that the population mean of initial CD4 count was 26.6 ± 3.2 cells/μL, which was 0.5 ± 0.15 lower if patients were from the late arm group and 1.2 ± 0.16 higher if patients were females. CD4 thymic output had a population mean of 2.4 ± 0.83 cells/μL/day. Patient's expected thymic output decreased 0.42 ± 0.15 for each year older, and was 0.39 ± 0.11 greater if patients were female. CD4 death rate, with a predicted population mean of 0.0066 ± 0.001 1/day, was 0.00016 ± 0.0004 higher for each year older, 0.0017 ± 0.001 greater in female patients, and 0.00012 ± 0.00006 lower for each kg of weight added (Table 3). There was no association between EFV body drug exposure and CD4 death rate (data not shown).

4. Discussion

Here, predictors associated with increased risk of developing liver damage, as indicated by an abnormal rise in AST levels, were identified. The positive association between EFV body drug exposure and AST event likelihood indicates that either longer exposure to EFV at normal plasma concentrations, the accumulation of EFV resulting in higher plasma concentrations, or a combination of the two may biochemically induce toxicity. Previous studies have shown concentrations of EFV above a threshold in the cell may interfere with mitochondrial functions that activate deleterious stress responses in the ER, partially explaining the mechanism behind efvairenz-induced toxicity [7]. This finding implies that higher concentrations of EFV more so than time exposed to EFV describes the correlation between body drug exposure and liver toxicity. However, the increase in AST event likelihood relative to a one unit increase in body drug exposure is only slight (.0038), with an odds ratio approximately equal to 1.003. Therefore, greater EFV body drug exposure values may not strongly correlate with higher EFV concentrations.

When comparing EFV versus EFV + TB treatment groups, both rapid and slow *NAT2* metabolizers have a greater likelihood of experiencing an adverse AST event when the drugs are given in combination versus when ART drugs given alone (OR = 1.9 and 3.5, respectively). Therefore, hepatotoxicity may be exacerbated when antiretrovirals and tuberculosis drugs are given in combination. Interestingly, arm of treatment (early versus late initiation of ART) was not indicated as a significant predictor in this study. Therefore, toxicity from the combined drug regimen was not dependent on timing of ART relative to tuberculosis treatment.

Individuals with *NAT2* slow metabolizer status are 1.9 times more likely than individuals with rapid *NAT2* metabolizer phenotypes to develop an AST event when tuberculosis and ART drugs are given in combination, and this finding is consistent across all efavirenz exposure values (Figure 3). The enzyme NAT2 metabolizes isoniazid (INH) and is primarily responsible for INH acetylation into nontoxic metabolites. The breakdown of some metabolite intermediates into their nontoxic form is also facilitated by NAT2. However, when NAT2 metabolism is stalled, both INH and its daughter compounds accumulate, and are chemically driven to take a minor pathway that results in the formation of toxic metabolites involved in INH-related hepatotoxicity [17]. This effect is enhanced in the presence of rifampicin [9]. Therefore, INH-induced toxicity with the association of rifamipicin may explain enhanced hepatic toxicity of ART and tuberculosis drugs in combination, especially in individuals with the *NAT2* acetylator phenotype. As risk in *NAT2* fast acetylators were also heightened when the drugs were co-administered, ART therapy indirectly could be affecting the *NAT2* acetylator pathway or be interacting with other anti-tuberculosis drugs to heighten AST levels. Further investigation of interaction mechanisms between EFV and specific tuberculosis drugs are needed to decipher how hepatotoxic risk is enhanced.

Following assessment of hepatotoxicity related to co-administration of tuberculosis and ART therapy, it was important to determine if efficacy of ART treatment was maintained during combined therapy and how timing of ART initiation affected CD4 evolution over time. Mean patient CD4 counts measured at scheduled visits throughout the trial (weeks) since inclusion demonstrated that adult CD4 counts from the CAMELIA

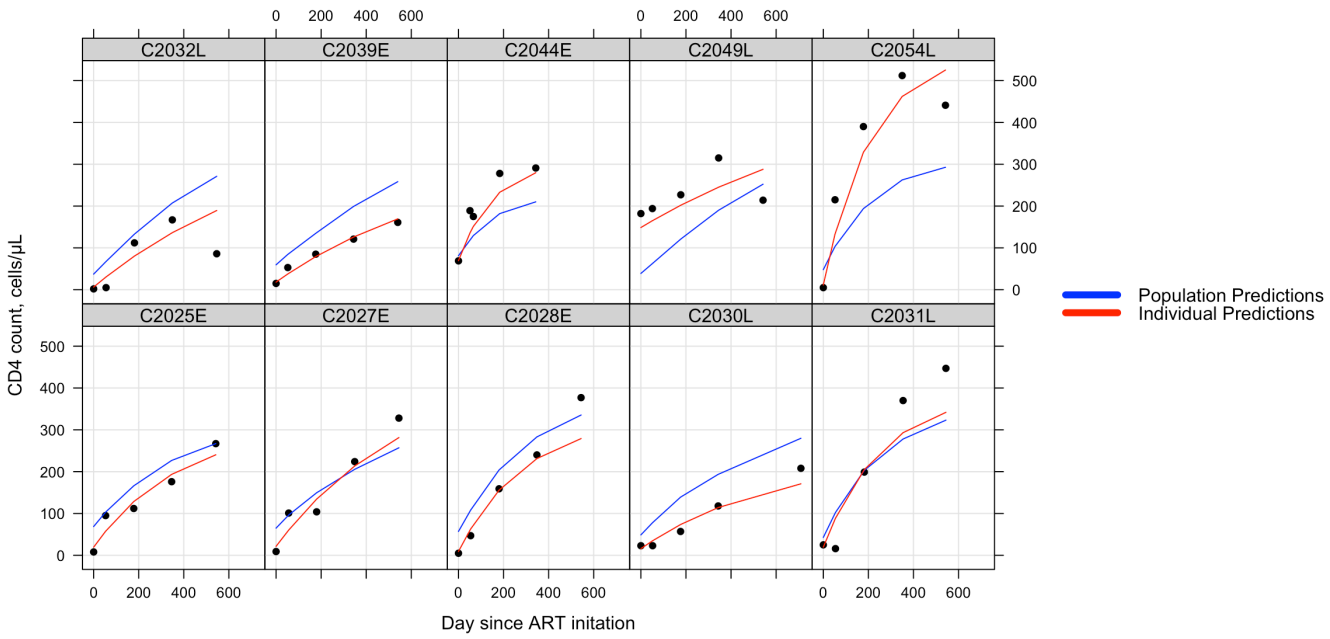

Figure 5. Population predicted fit (blue) and individual predicted fit (red) of CD4 count recovery for ten patients in the CAMELIA trial

study followed a similar asymptotic function described previously [13] (Figure 4). Therefore, a nonlinear mixed-effects model was used to fit CD4 count over time, estimating mean population values, individual values, and effect of covariates on three model parameters: initial CD4 cell count, CD4 thymic output, and CD4 death rate.

The population mean time at which half the CD4 count recovered was predicted to be about 105 days, which is similar to values found in previous studies (approximately 99 days) [13]. The slight discrepancy in values may be explained by adults initiating ART in this study were severely immunocompromised (CD4 count < 200 cells/µL). The value $\frac{\lambda}{\mu}$ represents a long-term CD4 recovery population value, which was estimated to be 363.6 $cells/\mu L$. A normal range for CD4 count is about 500 - 1500 $cells/\mu L$ in healthy persons. Previous work has emphasized that long-term immune recovery is highly dependent on CD4 baseline levels in Cambodian populations [22], which verifies the suboptimal long-term population recovery estimate in this study.

Initial CD4 count is predicted to be slightly lower in individuals initiating ART later rather than earlier relative to onset of tuberculosis treatment. Therefore delaying treatment causes a slight decline in the initial CD4 population, which can harm CD4 ART-based recovery [22] and may partially justify enhanced mortality rates in the late-arm group [2]. Decrease in thymic CD4 T-cell output is expected with older age and corresponds to the estimate found in this study (-0.42 $\pm$ 0.15). The structural integrity of the thymus diminishes with age, resulting in a declined production of naïve CD4 cells [23]. As CD4 death rate characterizes rate of CD4 recovery in this model, the covariate effects on this parameter are of particular interest. Increase in the population estimate of CD4 death rate with older ages indicates that the CD4 counts of older populations are recovering at a slower rate, corresponding to findings from a study assessing CD4 recovery predictors in HIV-tuberculosis co-infected patients [**?**].

Efavirenz drug body exposure was not identified as a significant covariate influencing CD4 death rate in this analysis. When plotting patient exposure values against CD4 death rate calculated for each patient, no linear correlation was identified. It was expected that CD4 death rate would decline with greater efavirenz body exposure. As efavirenz is an inhibitor of HIV virus replication, greater exposure to the drug would prevent further infection and eventual death of CD4 cells. This phenomenon may be explained by the Emax model frequently used in dose-response analysis:

$$E = \frac{EmaxAUC}{EC50 + AUC} \tag{3}$$

Where E is the drug effect of a certain EFV body drug exposure (AUC), emax is maximum possible effect, and EC50 is the AUC that produces half the maximum effect. If AUC is very large relative to the EC50 value, then the drug effect is at or close to maximum, flattening the relationship between CD4 death rate and efavirenz body drug exposure. Therefore, efavirenz drug effect in the body may always be close to maximum, explaining lack of correlation with this covariate.

This study has a number of limitations that must be addressed. First, an AST threshold of two times ULN was used. While this threshold does represent abnormal AST levels corresponding with mild hepatotoxicity, AST and ALT levels are known to vary excessively between measurements [24]. Therefore, hepatotoxicity is not clinically diagnosed without

AST and/or ALT levels above a threshold of three times ULN with presence of symptoms or above a threshold of five times ULN without symptoms present [8]. Furthermore ALT more so than AST is directly associated with hepatotoxicity [24]. As diagnosis of hepatotoxic symptoms were not included in the dataset and it was not possible to examine ALT-related hepatotoxicity, the results from this analysis should be verified by studies with more restrictions on classification of hepatotoxic events.

Another assumption made when implementing GLMM was that observations at each level of the data assumed a normal distribution. This assumption may not hold true and in the future, a bootsrapping method should be implemented to resample the data and generate a mean estimate value from the resampled population. Bootstrap mean estimates can be used to verify individual estimates from the original data.

Addressing limitations in the CD4 model, patient viral load was missing in this dataset and could be used to characterize the conversion of uninfected CD4 cells to infected CD4 cells, improving the model fit. In the future, parameterization of the $\frac{\gamma}{\mu}$ value into a single value that represents long-term CD4 count, while also transforming the death rate into a constant, fixed effect, may enhance stability of the model and provide more reliable estimates. Additionally, the saemix software does not allow for analysis of time-varying covariates, so predictors were static at time of each measurement. Therefore, as the model does not account for variation between occasions, associations found with time-varying covariates, such as weight and age, may be misleading. This restriction in the software also prevented the evaluation of concomitant tuberculosis treatment on CD4 recovery.

In conclusion, predictors of hepatotoxicity and CD4 recovery in adult patients receiving concomitant antiretroviral and anti-tuberculosis treatment were characterized and should be considered when attempting to optimize efavirenz dosing relative to tuberculosis drugs. Increased risk of hepatotoxicity was shown to be associated with enhanced efavirenz body drug exposure, the female gender, a lower body mass index, and co-administration of efavirenz and tuberculosis drugs, especially in individuals with the slow *NAT2* phenotype. While modeling the evolution of CD4 counts over time could not distinguish the effect of tuberculosis treatment, it revealed that a lower CD4 baseline, older age, the female gender, and lower weights affected the rate of CD4 cell restoration.

References

[1] Andrea Low, Georgios Gavriilidis, Natasha Larke, Marie-Renee B-Lajoie, Olivier Drouin, John Stover, Lulu Muhe, and Philippa Easterbrook. Incidence of opportunistic infections and the impact of antiretroviral therapy among hiv-infected adults in low- and middle-income countries: A systematic review and meta-analysis. *Clinical Infectious Diseases*, 62(12):1595–1603, 2016.

[2] François-Xavier Blanc, Thim Sok, Didier Laureillard, et al. Earlier versus later start of antiretroviral therapy in hiv-infected adults with tuberculosis. *New England Journal of Medicine*, 365(16):1471–1481, 2011. PMID: 22010913.

[3] J. Aubrecht, S. J. Schomaker, and D. E. Amacher. Emerging hepatotoxicity biomarkers and their potential to improve understanding and management of drug-induced liver injury. *Genome Med*, 5(9):85, 2013.

[4] T Schaberg, K Rebhan, and H Lode. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *European Respiratory Journal*, 9(10):2026–2030, 1996.

[5] Consolidated guidelines on the use of anti- retroviral drugs for treating and preventing hiv infection: recommendations for a public health approach. *World Health Organization*, 2013.

[6] Michelle Jones and Marina Núñez. Liver Toxicity of Antiretroviral Drugs. *Semin Liver Dis*, 32(02):167–176, 2012.

[7] M. Polo, F. Alegre, H. A. Funes, A. Blas-Garcia, V. M. Victor, J. V. Esplugues, and N. Apostolova. Mitochondrial (dys)function - a factor underlying the variability of efavirenz-induced hepatotoxicity? *Br. J. Pharmacol.*, 172(7):1713–1727, Apr 2015.

[8] V. Ramappa and G. P. Aithal. Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management. *J Clin Exp Hepatol*, 3(1):37–49, Mar 2013.

[9] P. Y. Wang, S. Y. Xie, Q. Hao, C. Zhang, and B. F. Jiang. NAT2 polymorphisms and susceptibility to anti-tuberculosis drug-induced liver injury: a meta-analysis [Review article]. *Int. J. Tuberc. Lung Dis.*, 16(5):589–595, May 2012.

[10] G. Yimer, M. Gry, W. Amogne, E. Makonnen, A. Habtewold, Z. Petros, G. Aderaye, I. Schuppe-Koistinen, L. Lindquist, and E. Aklillu. Evaluation of patterns of liver toxicity in patients on antiretroviral and anti-tuberculosis drugs: a prospective four arm observational study in ethiopian patients. *PLoS ONE*, 9(4):e94271, 2014.

[11] L. Borand, Y. Madec, D. Laureillard, M. Chou, O. Marcy, P. Pheng, N. Prak, C. Kim, K. K. Lak, C. Hak, B. Dim, E. Nerrienet, A. Fontanet, T. Sok, A. E. Goldfeld, F. X. Blanc, and A. M. Taburet. Plasma concentrations, efficacy

and safety of efavirenz in HIV-infected adults treated for tuberculosis in Cambodia (ANRS 1295-CIPRA KH001 CAMELIA trial). *PLoS ONE*, 9(3):e90350, 2014.

[12] Julie Bertrand, Céline Verstuyft, and Monidarin Chou. Dependence of efavirenz- and rifampicin-isoniazid–based antituberculosis treatment drug-drug interaction on cyp2b6 and nat2 genetic polymorphisms: Anrs 12154 study in cambodia. *The Journal of Infectious Diseases*, 209(3):399–408, 2014.

[13] N.G. Pakker, Notermans D.W., and othes. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nat Med*, 4(2):208–14, 1998.

[14] Lemma Derseh Gezie, Kassahun Alemu Gelaye, Abebaw Gebeyehu Worku, Tadesse Awoke Ayele, and Destaw Fetene Teshome. Time to immunologic recovery and determinant factors among adults who initiated art in felege hiwot referral hospital, northwest ethiopia. *BMC Research Notes*, 10(1):277, Jul 2017.

[15] Carl A Burtis, Edward R Ashwood, and David E Burns. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. St. Louis, Mo: Elsevier Saunders, 4 edition, 2006.

[16] Ma Chou, Julie Bertrand, Ola Segeral, et al. Population pharmacokinetic-pharmacogenetic study of nevirapine in hiv-infected cambodian patients. *Antimicrob. Agents Chemother.*, 54(10):4432–4439, 2010.

[17] M. Blum, D. M. Grant, W. McBride, M. Heim, and U. A. Meyer. Human arylamine N-acetyltransferase genes: isolation, chromosomal localization, and functional expression. *DNA Cell Biol.*, 9(3):193–203, Apr 1990.

[18] Heather J. Ribaudo, David W. Haas, Camlin Tierney, et al. Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: An adult aids clinical trials group study. *Clinical Infectious Diseases*, 42(3):401–407, 2006.

[19] Douglas Bates, Martin Mächler, Ben Bolker, and Steve Walker. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1):1–48, 2015.

[20] Emmanuelle Comets, Audrey Lavenu, and Marc Lavielle. Parameter estimation in nonlinear mixed effect models using saemix, an R implementation of the saem algorithm. *Journal of Statistical Software*, 80(3):1–41, 2017.

[21] Alan S. Perelson, Avidan U. Neumann, Martin Markowitz, John M. Leonard, and David D. Ho. Hiv-1 dynamics in vivo: Virion clearance rate, infected cell lifespan, and viral generation time. *Science*, 271(5255):1582–1586, 1996.

[22] J. van Griensven and S. Thai. Predictors of immune recovery and the association with late mortality while on antiretroviral treatment in Cambodia. *Trans. R. Soc. Trop. Med. Hyg.*, 105(12):694–703, Dec 2011.

[23] N. Salam, S. Rane, R. Das, and others. T cell ageing: effects of age on development, survival function. *Indian J Med Res.*, 138(5):595–608, Nov 2013.

[24] Josef Ozer, Marcia Ratner, Martin Shaw, Wendy Bailey, and Shelli Schomaker. The current state of serum biomarkers of hepatotoxicity. *Toxicology*, 245(3):194 – 205, 2008. Biomarkers of Toxicity.