



## ORIGINAL ARTICLE

# Hierarchical modeling gave plausible estimates of associations between metabolic syndrome and components of antiretroviral therapy

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and the Swiss HIV Cohort Study

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**Abstract**

**Objective:** Hierarchical modeling has been proposed as a solution to the multiple exposure problem. We estimate associations between metabolic syndrome and different components of antiretroviral therapy using both conventional and hierarchical models.

**Study Design and Setting:** We use discrete time survival analysis to estimate the association between metabolic syndrome and cumulative exposure to 16 antiretrovirals from four drug classes. We fit a hierarchical model where the drug class provides a prior model of the association between metabolic syndrome and exposure to each antiretroviral.

**Results:** One thousand two hundred and eighteen patients were followed for a median of 27 months, with 242 cases of metabolic syndrome (20%) at a rate of 7.5 cases per 100 patient years. Metabolic syndrome was more likely to develop in patients exposed to stavudine, but was less likely to develop in those exposed to atazanavir. The estimate for exposure to atazanavir increased from hazard ratio of 0.06 per 6 months' use in the conventional model to 0.37 in the hierarchical model (or from 0.57 to 0.81 when using spline-based covariate adjustment).

**Conclusion:** These results are consistent with trials that show the disadvantage of stavudine and advantage of atazanavir relative to other drugs in their respective classes. The hierarchical model gave more plausible results than the equivalent conventional model. © 2008 Elsevier Inc. All rights reserved.

**Keywords:** Bayesian statistics; Epidemiologic methods; Hierarchical regression; Highly active antiretroviral therapy; HIV; Metabolic syndrome

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## 1. Introduction

The multiple exposure problem complicates many epidemiologic studies. The problem arises when there are few events available for an analysis relative to the number of possible causes (or “exposures”). Conventional modeling typically leads to biased estimates when there are fewer than 10 to 15 events per model parameter [1,2]. In this situation, hierarchical modeling offers advantages and is

likely to give more plausible estimates of associations between the event and each exposure [3]. In classical epidemiology, hierarchical models have been used to estimate associations between breast cancer and nutrition [4]; in genetic epidemiology, these models have been used to estimate associations between diabetes and candidate genes [5].

The problem also arises in clinical epidemiology. We illustrate with an example where efforts to select suitable antiretroviral drugs are complicated owing to the few adverse events available for an analysis relative to the number of different antiretroviral drugs in use. Antiretroviral therapy and its metabolic abnormalities are associated with an increased incidence of myocardial infarction [6,7].

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Hence, there is a need to select drugs that are both effective and have a low risk of metabolic abnormalities [8].

Here we consider the rate at which antiretroviral-naïve patients in the Swiss HIV Cohort Study (SHCS) develop metabolic syndrome when treated, and whether this rate is associated with different components of antiretroviral therapy. We estimate associations using both conventional and hierarchical models. Metabolic syndrome is a constellation of abnormalities associated with an increased risk of both cardiovascular disease and type 2 diabetes, and is, therefore, useful as a surrogate endpoint for these diseases [9–11]. In the SHCS, the prevalence of metabolic syndrome is estimated to be around 15% [12], and is relatively stable over time (Fig. 1).

## 2. Methods

### 2.1. Patients

The SHCS is a prospective cohort with continuing enrollment of HIV-infected adults ([www.shcs.ch](http://www.shcs.ch)). Since April 1, 2000, a cardiovascular risk assessment has been a part of the follow-up visits scheduled every 6 months. Blood is analyzed for cholesterol, triglyceride, and glucose concentrations; weight, waist circumference, and blood pressure are also measured.

Our population of interest consists of all antiretroviral-naïve patients in the SHCS, starting highly active antiretroviral therapy (HAART) after April 1, 2000 with at least one subsequent cardiovascular risk assessment. Our sample from this population is those patients with weight, CD4 cell count, and plasma HIV RNA (viral load) measured within 6 months before to 3 months after starting HAART. We define HAART as the combination of at least three

antiretrovirals, including at least one protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI).

### 2.2. Metabolic syndrome

At each cardiovascular risk assessment, we consider whether a patient has metabolic syndrome under the new International Diabetes Federation (IDF) definition [13]. Essential components in any definition of metabolic syndrome are thought to be central obesity, given that this is a risk factor for cardiovascular disease [9], and impaired fasting glucose, as a precursor to diabetes [10]. This new definition has ethnic-specific cutoffs for central obesity, where the central obesity is measured by waist circumference, so as to improve on earlier definitions. The definition also includes cutoffs (alternatively receiving treatment) for raised fasting glucose, raised triglycerides, raised systolic or diastolic blood pressure, and lowered high-density lipoprotein (HDL) cholesterol. Under this definition, patients with metabolic syndrome must have central obesity plus any two of the other four risk factors.

We are not able to use this definition exactly because of the way information is collected in the SHCS. First, only 20% of cardiovascular risk assessments are made in the fasting state [12]; hence, we assume a raised fasting glucose if casual glucose exceeds 11.1 mmol/L [10]. Second, different Asian ethnicities are not recorded; hence, for all Asian ethnicities, we use the waist circumference cutoff appropriate for Chinese or South Asians (rather than the cutoff appropriate for Japanese).

### 2.3. Survival analyses

A discrete time survival analysis is appropriate for these data, because we can only evaluate metabolic syndrome at each cardiovascular risk assessment. We model the probability  $p_{ij}$  of metabolic syndrome for the  $i$ th patient at the  $j$ th assessment, given that the syndrome was not seen at a previous assessment. The discrete version of the proportional hazards regression model (equation [1]) is similar to a conditional logistic regression model, except that it uses a complementary log log link function instead of the usual logit link function [14–16]:

$$\log(-\log(p_{ij})) = \alpha_j + X_{ij}^T \beta + W_{ij}^T \theta + \log(\Delta t_{ij}). \quad (1)$$

The  $\alpha_j$  parameters allow for possible variation in the baseline hazard between assessments. Because assessments are not always exactly 6-month apart, the model allows for variation in the time between assessments by adding the log difference in time between assessments  $\log(\Delta t_{ij})$  as an offset in the model [14].

We estimate associations ( $\beta$ ) between the rate at which patients develop metabolic syndrome and cumulative exposure to different antiretrovirals, where cumulative exposure is calculated at each assessment in units of 6 months. Here,

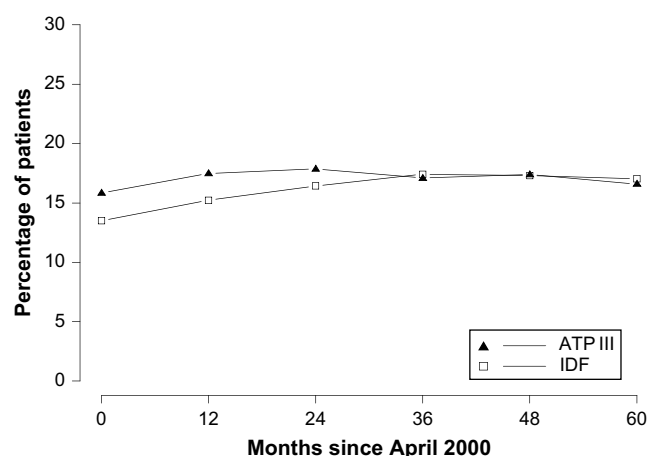


Fig. 1. Prevalence of metabolic syndrome over time in the Swiss HIV Cohort Study. The data are those described by Glass et al. [12]. Prevalence is assessed under two definitions: the National Cholesterol Education Program's Adult Treatment Panel III definition (ATP III) and the International Diabetes Federation definition (IDF). Both definitions were slightly modified to meet the requirements of this cohort's data (see Methods section and the work of Glass et al. [12]).

$\beta$  is a set of 16 parameters estimating the effect of cumulative exposure to antiretrovirals from four classes: seven nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), two NNRTIs, two single PIs, and five PIs boosted with ritonavir. We adjust for other variables that might influence the rate at which patients develop metabolic syndrome. As covariates ( $\theta$ ), we include CD4 cell count, viral load, and body mass index (BMI) at baseline; age, gender, and intravenous (IV) drug use as the likely mode of transmission; and smoking status at each cardiovascular risk assessment.

This model can be fit using conventional software, with model parameters estimated by maximum likelihood. However, this often leads to “illusory significant results” [3]. The estimates of interest ( $\beta$ ) are often biased, because there are too few cases with which to estimate the large number of parameters in the model [1,2,17,18]. Fitting a reduced model, perhaps using data-driven variable selection methods, such as univariate preliminary testing or stepwise selection, only creates other problems. Exposures are likely to be highly correlated, and excluding some may affect the estimate of others even if the excluded exposures are not significant; unimportant exposures may be selected if the selection process involves multiple testing; and standard errors from the final model will be too narrow, because uncertainty about the final model is ignored [3,17]. Because of these problems, observational studies with small sample sizes are unreliable and often fail to replicate [19].

A better alternative is to fit a hierarchical (multilevel) model, with equation (1) as a first level and a model for the 16 parameters in  $\beta$  as a second level [3]:

$$\beta_k = Z_k^T \pi + \partial_k, \quad k \in 1, \dots, 16, \quad (2)$$

where  $Z_k$  is a 4 by 1 vector of drug class indicators, and  $\partial_k$  is distributed normal with mean zero and variance  $\sigma^2$ . This model implies that exposures are correlated when antiretrovirals belong to the same drug class; we assume antiretrovirals from the same class act in a similar fashion. The model also has a random component  $\partial_k$ ; this allows each antiretroviral to have a residual effect beyond the effect of its drug class, and we assume independent residual effects that are normally distributed with variance  $\sigma^2$ .

In the hierarchical model, unreliable estimates of an exposure parameter “shrink” toward its class mean (contained in parameter vector  $\pi$ ). The degree of “shrinkage” depends on the amount of information in the data and on the value of  $\sigma^2$  [20]. As the value of  $\sigma^2$  increases, there is less “shrinkage,” and  $\beta$  parameter estimates approach those of the conventional model. Simulation shows that hierarchical model estimates of  $\beta$  parameters are more accurate—with lower mean squared error—than estimates from the equivalent conventional model [21,22].

We take a “semi-Bayes” approach for fitting this hierarchical model [3]. That is, we assign a plausible value for the residual variance  $\sigma^2$  rather than attempt to estimate it from

the data. Methods for estimating  $\sigma^2$  do not necessarily have good small sample properties [23]. Simulation shows that the “semi-Bayes” approach has good properties, but it is better to overestimate rather than underestimate when assigning a value for  $\sigma^2$  [20].

Hierarchical modeling is not often used in epidemiology but offers considerable advantages over conventional modeling for analyses where multiple exposures are of interest. In the Appendix, we describe how to fit our model in SAS (SAS Institute Inc, Cary, NC, USA). Assigning a plausible value for  $\sigma^2$  might be seen as subjective, but subjectivity is inherent in multivariate modeling. The decision to omit an exposure, for example, is equivalent to assigning it a hazard ratio of exactly 1, a far stronger assumption. Hierarchical modeling allows the analyst to consider a far richer set of models than conventional modeling, and uncertainty about  $\sigma^2$  is best addressed through sensitivity analyses [3].

#### 2.4. Sensitivity analyses

We carry out three different sensitivity analyses. First, we consider the residual effect of each drug beyond its class mean and report results for three values of  $\sigma^2$ :  $\infty$ , 1/8 and 1/24. The first value corresponds to the conventional model; the second reflects a 95% prior probability that hazard ratios for residual effects ( $\delta$ ) will lie within the range 0.50 to 2.0 (i.e.  $[\ln(2)/1.96]^2 = 1/8$ ); the third reflects a 95% prior probability that hazard ratios for residual effects will lie within the narrower range of 0.67 to 1.5 (i.e.  $[\ln(1.5)/1.96]^2 = 1/24$ ).

Second, we alter the composition of our set of covariates ( $\theta$ ). First, we drop viral load as a covariate, because in some patients, this was measured after starting antiretroviral therapy, and therapy should cause viral load to drop rapidly. Second, for both age and BMI in turn, we replace a continuous predictor with a quadratic spline with one knot at the median [24]. Other studies have shown that both covariates are strongly associated with the prevalence of metabolic syndrome [25,26]; using a quadratic spline may provide better covariate adjustment for these important covariates.

Third, we experiment with different ways of applying the IDF definition of metabolic syndrome to our data. Detecting raised triglycerides presents the major difficulty: in general, we use the recommended cutoff irrespective of fasting state, and we lack specific treatment information for this abnormality. As a sensitivity analysis, first, we apply the fasting triglyceride cutoff only if a patient is either known to be in the fasting state or has a casual glucose of less than 5.6 mmol/L; and second, in addition, we take any use of a lipid-lowering drug as evidence of high triglycerides.

### 3. Results

#### 3.1. Patient characteristics

As of December 31, 2006, 2,347 antiretroviral-naïve patients in the SHCS started HAART after April 2000. Of

these, 1,644 (70%) had at least one cardiovascular risk assessment after starting HAART, and had weight, CD4 cell count, and viral load measured within 6 months before to 3 months after starting HAART. At their first assessment, 245 (15%) of these 1,644 patients had metabolic syndrome. A further 181 patients have had just one assessment to date.

Among the remaining 1,218 patients, metabolic syndrome was later seen in 242 patients (20%). From their first assessment until when metabolic syndrome was first seen or their most recent assessment, these 1,218 patients were followed for a median of 27 months, and in total, 3,246 patient years, giving an incidence rate of 7.5 cases per 100 patient years. Patients who develop metabolic syndrome were more likely to be female, older, and starting HAART with a higher BMI, lower CD4 cell count, and an infection of shorter duration (Table 1). At a first cardiovascular risk assessment, patients who develop metabolic syndrome were more likely to have a greater waist circumference and were less likely to smoke (Table 2).

### 3.2. Antiretroviral therapy

Of the 1,218 patients, 521 (43%) used only PI-based therapy at all times, 465 (38%) used only NNRTI-based therapy at all times, and 232 (19%) used both. Common PIs were lopinavir boosted with ritonavir and nelfinavir (Fig. 2), used by 424 (56%) and 244 (32%) patients, respectively, of the 753 using any sort of PI. Most NNRTI use was of efavirenz—this was used by 622 (89%) of the 697 patients using either of the two NNRTIs. Common NRTIs

were lamivudine and zidovudine, used by 1,117 (92%) and 935 (77%) patients, respectively.

### 3.3. Survival analyses

We report the results for three values of the residual variance, but consider  $\sigma^2 = 1/8$  the most plausible value. We wish to shrink unreliable estimates of exposure parameters toward a sensible prior (the class mean). We view hazard ratios for exposure parameters below 0.50 or above 2.0 as unlikely; therefore, residual effects beyond the class mean should also lie well within this range. However, we consider this grouping of antiretrovirals into classes rather a rudimentary model for the differences between antiretrovirals. Therefore, we anticipate considerable variation in the effects of individual antiretrovirals about a class mean, and we are mindful of evidence that it is better to overestimate rather than underestimate when assigning a value for  $\sigma^2$  [20].

With  $\sigma^2 = 1/8$ , metabolic syndrome is more likely to develop in older patients and in those who start antiretroviral therapy with lower CD4 cell counts or higher BMI (with hazard ratios and 95% confidence intervals [CI] 1.30 and 1.14–1.49 per 10 years, respectively; 0.93 and 0.85–1.01 per 100 cells, respectively; and 1.23 and 1.18–1.28 per  $\text{kg}/\text{m}^2$ , respectively). There are no obvious differences between class means (Table 3). This is because each class contains at least one relatively benign antiretroviral. Among NRTIs, metabolic syndrome appears relatively unlikely to develop with the use of didanosine (hazard ratio for  $\sigma^2 = 1/8$ : 0.82; 95% CI: 0.64–1.05 [per

Table 1  
Patient characteristics when starting antiretroviral therapy for those who develop metabolic syndrome compared with those who do not ( $n = 1,644$ )

Characteristic	One assessment only ( $n = 426$ )		Two or more assessments ( $n = 1,218$ )	
	No change ( $n = 181$ )	Metabolic syndrome ( $n = 245$ )	No change ( $n = 976$ )	Metabolic syndrome ( $n = 242$ )
Gender (%)				
Female	26	33	31	35
Transmission (%) <sup>a</sup>				
IV drug	20	12	15	14
Clinical stage (%)				
CDC group C	15	21	20	21
Ethnicity (%)				
Caucasian	76	84	76	76
Age—median (years)	37	41	37	39
BMI—median	22	27	22	24
CD4 count—median (cells/ $\mu\text{L}$ )	220	200	200	190
Log viral load <sup>b</sup> —median	4.8 (14)	4.9 (29)	4.9 (120)	4.9 (29)
Duration of infection <sup>c</sup> —median (months)	24 (22)	9.9 (44)	14 (181)	7.6 (49)
Follow-up <sup>d</sup> —median (months)	3.0	3.7	31	17

Abbreviations: IV, intravenous; BMI, body mass index; CDC, Centers for Disease Control and Prevention ([www.cdc.gov](http://www.cdc.gov)).

<sup>a</sup> Transmission most probably through IV drug use or sexual contact but not clear which one.

<sup>b</sup> Viral load sometimes measured up to 3 months after starting highly active antiretroviral therapy (HAART) (number of patients in parentheses).

<sup>c</sup> From first positive test for HIV until starting HAART—the first positive-test date is usually recorded retrospectively; it is, therefore, of a lesser reliability and is not known for some patients (number of patients in parentheses).

<sup>d</sup> From starting HAART until metabolic syndrome assessed or last assessment to date.

Table 2

First cardiovascular risk assessment for patients who develop metabolic syndrome compared with those who do not ( $n = 1,644$ ).

Cardiovascular Risk Factor	One assessment only ( $n = 426$ )		Two or more assessments ( $n = 1,218$ )	
	No change ( $n = 181$ )	Metabolic syndrome ( $n = 245$ )	No change ( $n = 976$ )	Metabolic syndrome ( $n = 242$ )
Waist circumference—median (cm)	80	98	79	89
Smoking (%)	55	37	47	38
Fasting state (%)	14	21	17	21
Glucose—median (mmol/L)	5.0	5.3	5.0	5.1
Blood lipids—median (mmol/L)				
Triglycerides	1.5	2.5	1.4	1.6
HDL cholesterol	1.2	1.0	1.2	1.2
Blood pressure—median (mm Hg)				
Systolic	120	130	120	120
Diastolic	77	84	76	80
Treatment (%)				
High blood pressure	6	13	3	7
Diabetes mellitus	1	4	0	2
High blood lipids	2	3	1	1

Abbreviation: HDL, high-density lipoprotein.

6 months use]) and relatively likely with the use of stavudine (hazard ratio: 1.07; 95% CI: 0.88–1.31). Among PIs, metabolic syndrome appears relatively unlikely with the use of atazanavir, either as a single PI (hazard ratio: 0.37; 95% CI: 0.18–0.78) or when boosted with ritonavir (hazard ratio: 0.76; 95% CI: 0.48–1.21), and relatively likely with the use of boosted indinavir (hazard ratio: 1.17; 95% CI: 0.83–1.65). Metabolic syndrome appears relatively unlikely with either NNRTI (efavirenz—hazard ratio: 0.90; 95% CI: 0.74–1.10; nevirapine—hazard ratio: 0.82; 95% CI: 0.62–1.10).

For some exposures, parameter estimates are stable across all three values of residual variance  $\sigma^2$  (Table 3). For others, such as emtricitabine, atazanavir, and amprenavir, estimates from the conventional model are almost certainly biased, and estimates from a hierarchical model are more plausible. Removing exposures that are not significant from the conventional model only adds to the illusion of significance. For example, stepwise backward deletion keeping only those exposures with a significance of 0.10 or less leads to four “significant” exposures: didanosine (hazard ratio: 0.77; 95% CI: 0.61–0.97), nelfinavir (hazard

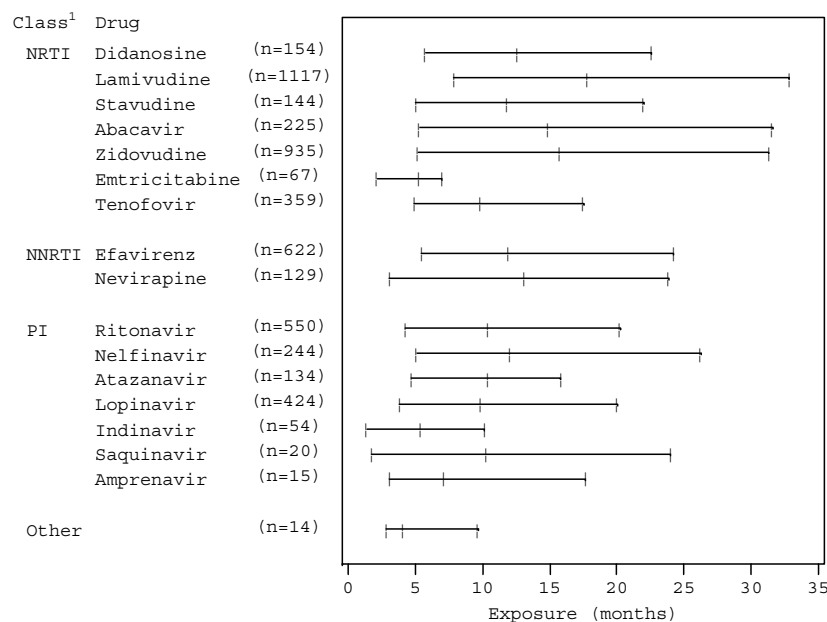


Fig. 2. Exposure (interquartile range and median) to different drugs for 1,218 patients from the Swiss HIV Cohort Study. <sup>1</sup>Class: NRTI, nucleoside or nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; Other: randomized treatment with either a chemokine receptor or efavirenz ( $n = 8$ ), randomized treatment with either a chemokine receptor or placebo ( $n = 2$ ), mycophenolate mofetil ( $n = 3$ ), or tipranavir ( $n = 1$ ).

Table 3

The association between the rate at which patients develop metabolic syndrome and antiretroviral therapy from discrete survival analysis models ( $n = 1,218$ )—hazard ratios (95% confidence interval) for antiretroviral class ( $\pi$ ) and drug ( $\beta$ ) parameters per 6 months' use

Cumulative exposure		Conventional model <sup>a</sup>	Hierarchical model <sup>a</sup>	
Class <sup>2</sup> ( $\pi$ )	Drug ( $\beta$ )	( $\sigma^2 = \infty$ )	$\sigma^2 = 1/8$	$\sigma^2 = 1/24$
NRTI	—	—	0.92 (0.69–1.24)	0.94 (0.78–1.13)
	Didanosine	0.79 (0.60–0.99)	0.82 (0.64–1.05)	0.85 (0.68–1.05)
	Lamivudine	0.93 (0.76–1.14)	0.95 (0.78–1.17)	0.97 (0.81–1.16)
	Stavudine	1.09 (0.89–1.31)	1.07 (0.88–1.31)	1.06 (0.88–1.27)
	Abacavir	0.96 (0.82–1.11)	0.96 (0.82–1.12)	0.95 (0.82–1.10)
	Zidovudine	0.98 (0.83–1.15)	0.97 (0.82–1.14)	0.96 (0.83–1.12)
	Emtricitabine	0.47 (0.05–1.69)	0.83 (0.43–1.59)	0.89 (0.59–1.36)
Tenofovir	0.91 (0.70–1.13)	0.90 (0.70–1.14)	0.90 (0.72–1.14)	
NNRTI	—	—	0.86 (0.51–1.47)	0.86 (0.61–1.22)
	Efavirenz	0.91 (0.76–1.09)	0.90 (0.74–1.10)	0.90 (0.75–1.08)
	Nevirapine	0.82 (0.61–1.06)	0.82 (0.62–1.10)	0.83 (0.63–1.08)
PI	—	—	0.62 (0.33–1.17)	0.75 (0.50–1.14)
	Nelfinavir	1.06 (0.88–1.27)	1.03 (0.84–1.26)	1.01 (0.84–1.23)
	Atazanavir	0.06 (0.01–0.30)	0.37 (0.18–0.78)	0.55 (0.34–0.92)
PI + RTV	—	—	0.92 (0.62–1.37)	0.94 (0.70–1.25)
	Lopinavir	0.98 (0.80–1.17)	0.96 (0.79–1.18)	0.96 (0.79–1.16)
	Atazanavir	0.68 (0.35–1.08)	0.76 (0.48–1.21)	0.82 (0.57–1.18)
	Indinavir	1.25 (0.88–1.65)	1.17 (0.83–1.65)	1.10 (0.80–1.72)
	Saquinavir	0.91 (0.42–1.33)	0.91 (0.57–1.45)	0.92 (0.63–1.34)
	Amprenavir	0.75 (0.11–1.37)	0.85 (0.47–1.56)	0.90 (0.58–1.37)

Abbreviations: NRTI, nucleoside or nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI + RTV, protease inhibitor boosted with ritonavir.

<sup>a</sup> All models include covariates for CD4 cell count, viral load, and body mass index at baseline; age, gender, and IV drug use as the likely mode of transmission; and smoking status at each assessment. Each antiretroviral has a residual effect beyond its class mean with residual variance  $\sigma^2$ ; hence, with smaller values of  $\sigma^2$ , estimates for each antiretroviral shift toward its class mean.

ratio: 1.17; 95% CI: 1.05–1.30), atazanavir (hazard ratio: 0.06; 95% CI: 0.01–0.70), and boosted indinavir (hazard ratio: 1.37, 95% CI: 1.05–1.80).

From a hierarchical modeling perspective, the conventional estimate of  $\beta$  is an estimate of a class mean plus a residual component. Hence, the average squared deviation of all 16 conventional model estimates around their respective class means provides an empirical estimate of the residual variance ( $\sigma^2$ ). However, the implausible conventional estimate for atazanavir makes a large contribution to this empirical estimate ( $\sigma^2 = 0.31$ ), so that the estimate is probably too high. This illustrates the difficulty of estimating the residual variance from the data, which is the reason we take a “semi-Bayes” approach. Empirical estimates of  $\sigma^2$  based solely on the variance of the more reliable classes of conventional estimate ( $\sigma^2 = 0.08$  for the seven NRTIs;  $\sigma^2 = 0.06$  for the five boosted PIs) are intermediate between  $\sigma^2 = 1/8$  and  $\sigma^2 = 1/24$ , and provide some support from the data for our preferred value of  $\sigma^2 = 1/8$ .

Note that in equation (1), the adjustment for variation in the time between assessments is only approximate. In addition, CIs do not reflect this approximation and are only correct if all patients are assessed at exactly the same time. Hence, all CIs in Table 3 are too narrow; however, the underestimate is likely to be immaterial if assessment times are similar for most individuals. Here, the median time between 5,139 assessments on 1,218 patients is 6.3 months,

with interquartile range of 5.8 to 7.0 months. CI inflation can be assessed in the conventional model by calculating robust standard errors using generalized estimating equations [14]. For example, hazard ratios for stavudine and boosted atazanavir in the conventional model are 1.09 (0.89–1.31) and 0.68 (0.35–1.08), respectively, using maximum likelihood (Table 3), and 1.09 (0.89–1.32) and 0.68 (0.40–1.14), respectively, using generalized estimating equations.

### 3.4. Sensitivity analyses

Those exposure parameters that vary with the assumed value of residual variance  $\sigma^2$  (Table 3) also vary in other sensitivity analyses. We illustrate this, focusing on the most and least favorable NRTIs and PIs (Table 4). Replacing the continuous covariate for BMI with a quadratic spline—because this should improve covariate adjustment—causes the estimate for exposure to atazanavir to increase hazard ratio from 0.37 to 0.81 (in the conventional model, the increase is from hazard ratio 0.06 to 0.57). However, this and other estimates are relatively robust to other changes to the set of covariates ( $\theta$ ). Both alternative definitions of raised triglycerides give similar results. With raised triglycerides defined as either above the recommended cutoff (provided the patient is known to be in the fasting state or has a casual glucose of less than 5.6 mmol/L) or after treatment with

Table 4

The association between the rate at which patients develop metabolic syndrome and antiretroviral therapy—hazard ratios (95% confidence interval) from sensitivity analyses for selected antiretroviral drug ( $\beta$ ) parameters per 6 months' use

Cumulative exposure to drug ( $\beta$ )	Model 1 <sup>a</sup>	Model 2 <sup>a</sup>	Model 3 <sup>a</sup>
	Table 3, $\sigma^2 = 1/8$	Model 1 + BMI spline <sup>b</sup>	Model 2 + triglycerides <sup>c</sup>
Didanosine	0.82 (0.64–1.05)	0.80 (0.62–1.04)	0.86 (0.69–1.06)
Stavudine	1.07 (0.88–1.31)	1.06 (0.86–1.30)	1.10 (0.92–1.32)
Atazanavir	0.37 (0.18–0.78)	0.81 (0.42–1.60)	0.84 (0.44–1.61)
Atazanavir + ritonavir <sup>d</sup>	0.76 (0.48–1.21)	0.74 (0.46–1.18)	0.86 (0.60–1.31)
Indinavir + ritonavir <sup>d</sup>	1.17 (0.83–1.65)	1.15 (0.81–1.66)	1.32 (0.97–1.80)

<sup>a</sup> All models include covariates for CD4 cell count, viral load, and body mass index (BMI) at baseline; age, gender, and IV drug use as the likely mode of transmission; and smoking status at each assessment.

<sup>b</sup> Continuous covariate for baseline BMI replaced by a quadratic spline with one knot at the median.

<sup>c</sup> Raised triglycerides: if (1) triglycerides > 1.7 mmol/L either in the fasting state or where casual glucose < 5.6 mmol/L; or (2) after treatment with a lipid-lowering drug.

<sup>d</sup> Boosted with ritonavir.

a lipid-lowering drug, 1,249 patients did not have metabolic syndrome at their first assessment, and of these, 251 patients later developed the syndrome. With these data, the estimate for exposure to boosted indinavir increases from hazard ratio 1.17 to 1.32.

As a general rule, exposure parameter estimates are relatively stable in sensitivity analyses where antiretrovirals are used by a reasonable number of patients (and hence, estimates are supported by a reasonable number of cases). For example, 154 and 144 patients used didanosine and stavudine, respectively (Fig. 2), whereas only 53 patients used indinavir boosted with ritonavir and 24 patients used atazanavir without ritonavir (126 patients used atazanavir boosted with ritonavir).

#### 4. Discussion

These analyses illustrate how conventional modeling leads to Greenland's "illusory significant results." For seldom-used antiretrovirals, such as atazanavir as a single PI, estimates from the conventional model are almost certainly biased. The estimate for exposure to atazanavir increases from hazard ratio 0.06 in the conventional model to 0.37 in the hierarchical model (Table 3), or from 0.57 to 0.81 with better covariate adjustment (Table 4). Here, both spline covariate adjustment and hierarchical modeling are needed to get a plausible estimate for atazanavir.

Removing exposures that are not significant from a conventional model only adds to the illusion of significance. Here, stepwise backward deletion increases the number of "significant" exposures from two to four. With drug combinations as exposures (rather than individual drugs), conventional models suffer from exactly the same problems: too few cases with which to estimate a large number of exposures; exposures that are correlated because combinations have individual drugs in common; and many exposures must be omitted to fit the model (e.g., Table 2 in Jones et al. [27]). Either removing "insignificant" exposures or not including exposures to fit a model is equivalent

to assigning each omitted exposure a hazard ratio of exactly 1—a strong and often unrealistic assumption. Including these exposures in a hierarchical model is likely to lead to more realistic results.

Our approach to analysis is consistent with the view that it is the drug that is important, not the drug class [8]. There are no obvious differences between drug classes, because each class contains at least one relatively benign antiretroviral. However, the drug class provides a useful prior model for the effect of exposure to an antiretroviral before one has evidence from data. This grouping of antiretrovirals into classes is a rudimentary prior model for the differences between antiretrovirals. A more sophisticated model might, for example, take into account the relative mitochondrial toxicity of different NRTIs [28] and of interactions between them [29], or the degree of glucose intolerance caused by different PIs [30]. However, even with a simple prior model at the second level, hierarchical modeling typically outperforms conventional modeling, where some exposures are omitted to reliably estimate others, because this strategy corresponds to an even less plausible prior [31]. Furthermore, although one could "shrink" estimates toward the null rather than toward a prior mean (i.e., fix  $\pi$  at 0 in equation [2]), shrinkage toward a contextually relevant value should lead to estimates with less bias [3].

These results are informative in the context of what is known or suspected about each antiretroviral and are consistent with results from randomized trials and from studies using healthy volunteers. Such results do not necessarily apply to a wider patient population; they represent more of what is possible in an ideal situation, whereas results from observational studies show what happens in practice [32,33]. In HIV-cohort studies, potential confounding variables have been the subject of much discussion, and obvious confounding variables are routinely measured and are included in our models. Although unmeasured confounding can lead to biased estimates in observational studies [34], it is unlikely that unmeasured confounding will cause serious and misleading bias here [35,36].

If one accepts that there are differences between antiretrovirals in their effects on lipid and glucose metabolism, then interest naturally lies in knowing which antiretrovirals are less likely to cause metabolic abnormalities than others. Other studies show that both NNRTIs have relatively favorable lipid profiles [37]. On the other hand, most PIs are associated with metabolic abnormalities; only atazanavir, and to a lesser extent saquinavir, seem relatively benign [8,38]. Atazanavir has a molecular structure that is distinctly different from other PIs [39]. Indinavir was the first PI associated with lipodystrophy [40] and is known to increase glucose levels [41–43], although this does not necessarily mean that it is worse than other PIs. Among NRTIs, both abacavir and tenofovir have been shown to have more favorable lipid profiles than stavudine [44,45]. Our results (Table 3) are largely consistent with this picture. The exception is didanosine, which here appears relatively benign compared with stavudine, but there is little evidence either for or against this from other studies. Early use of didanosine was often in combination with stavudine, making it difficult to estimate the separate effect of each. There is now evidence that didanosine should not be used with either stavudine or tenofovir [46,47] and concern that didanosine causes greater mitochondrial depletion than other NRTIs [28,48,49].

Most CIs for the association between metabolic syndrome and cumulative exposure are wide and include a hazard ratio of 1, implying that cumulative exposure could conceivably have no effect. However, a more considered explanation is that some exposures may have little net effect on the incidence of metabolic syndrome: exposure increases the risk of metabolic abnormalities, but exposure also reduces HIV replication, and this is also a risk factor for metabolic syndrome [50]. In theory, any tendency for patients with a higher risk of cardiovascular disease to switch during follow-up to drugs with a lower risk of metabolic abnormalities could make such drugs appear less advantageous than they really are—that is, the bias will be conservative and toward a hazard ratio of 1. But in practice, any such bias is likely to be trivial, because very strong associations are needed—between cardiovascular risk and both outcome and exposure—before bias becomes appreciable [51].

The wide CIs are hardly surprising, given the large number of antiretrovirals involved and that our data include fewer than 250 cases of metabolic syndrome. This combination of complexity and limited data is typical of single-cohort studies using surrogate endpoints to identify suitable antiretrovirals. It is also typical of multicohort collaborations using clinical endpoints for the same purpose. For example, there were 171 cases of high cholesterol in one single cohort study [27], and 345 cases of myocardial infarction [7] and 435 cases of diabetes mellitus [52] in a multi-cohort collaboration.

Different definitions of metabolic syndrome will give slightly different results. For example, with the syndrome

defined using a BMI-based version of the National Cholesterol Education Program's Adult Treatment Panel III definition [53], 1,182 patients did not have the syndrome at their first assessment, and of these, 294 patients later developed the syndrome. Conventional modeling of these data would then suggest that efavirenz (hazard ratio: 0.78; 95% CI: 0.64–0.93) and atazanavir (hazard ratio: 0.02; 95% CI: 0.00–0.26) are relatively benign, and stavudine (hazard ratio: 1.19; 95% CI: 0.99–1.42) and boosted indinavir (hazard ratio: 1.74; 95% CI: 1.34–2.19) are relatively harmful.

Evidently, it would be better to identify suitable antiretrovirals using clinical rather than surrogate endpoints [11]. Metabolic syndrome appears to be a reasonable surrogate for cardiovascular disease in patients with HIV, but not more predictive than the product of its components [54]. Hierarchical modeling could be extending to multicohort analyses of clinical endpoints by adding an additional level to the model, so that second-level parameters vary between cohorts according to a plausible third-level model.

In HIV infection, observational studies play an increasingly important role in comparing treatment combinations and strategies. The rapid development of new drugs, the variety of drugs now available, and the need to use drugs in combination has meant that many treatment combinations and strategies have never been formally compared in randomized trials or are only compared in trials of short duration [33]. These factors also complicate observational studies, because, often, there are few events available for an analysis relative to the number of different drugs in use. In this situation, hierarchical modeling offers advantages and the results of conventional modeling should be viewed with caution.

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## Appendix

We use SAS version 9.1 for all analyses and fit the hierarchical model specified by equations 1 and 2 using the GLIMMIX macro for SAS version 8 or later (<http://support.sas.com/ctx/samples/index.jsp?sid=536>). Note that GLIMMIX is also available as a procedure and as a macro for version 6.12. None of these methods automatically provide CIs for  $\beta$  parameters; these can be calculated from table MMEqSol (the solution to the mixed model equations) using an additional macro (<http://darwin.cwru.edu/~witte/glimmix.htm>), which processes output from the GLIMMIX macro version 6.12 [55]. Minor changes are needed to make this additional macro work with the GLIMMIX macro version 8 (i.e., “make ‘mmeqsol’ out = mmeqsol” becomes “ods output mmeqsol = mmeqsol”; in the read statement in PROC IML, column variable names changes from `_col` to just `col`). When calling GLIMMIX, the options statement is used to specify a binomial error, a complementary log log link function and an offset (i.e., error = binomial, link = clogl, offset = name).

## References

- [1] Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. *J Clin Epidemiol* 1995;48:1503–10.
- [2] Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 1996;49:1373–9.
- [3] Greenland S. When should epidemiologic regressions use random coefficients? *Biometrics* 2000;56:915–21.
- [4] Witte JS, Greenland S, Haile RW, Bird CL. Hierarchical regression analysis applied to a study of multiple dietary exposures and breast cancer. *Epidemiology* 1994;5:612–21.
- [5] Thomas D, Langholz B, Clayton D, Pitkaniemi J, Tuomilehto-Wolf E, Tuomilehto J. Empirical Bayes methods for testing associations with large numbers of candidate genes in the presence of environmental risk factors, with applications to HLA associations in IDDM. *Ann Med* 1992;24:387–92.
- [6] Friis-Moller N, Sabin CA, Weber R, d’Arminio MA, El Sadr WM, Reiss P, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003;349:1993–2003.
- [7] Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, El Sadr W, et al. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med* 2007;356:1723–35.
- [8] Moyle GJ. Lipid abnormalities during ART: it’s the drug, not the class. *AIDS Read* 2004;14:15–6.
- [9] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415–28.
- [10] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004;27(Suppl 1):S5–S10.
- [11] Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2005;28:2289–304.
- [12] Glass TR, Ungsedhapand C, Wolbers M, Weber R, Vernazza PL, Rickenbach M, et al. Prevalence of risk factors for cardiovascular disease in HIV-infected patients over time: the Swiss HIV Cohort Study. *HIV Med* 2006;7:404–10.
- [13] Alberti KGMM, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. *Lancet* 2005;366:1059–62.
- [14] Carlin JB, Wolfe R, Coffey C, Patton GC. Analysis of binary outcomes in longitudinal studies using weighted estimating equations and discrete-time survival methods: prevalence and incidence of smoking in an adolescent cohort. *Stat Med* 1999;18:2655–79.
- [15] Breslow N. Covariance adjustment of relative-risk estimates in matched studies. *Biometrics* 1982;38:661–72.
- [16] Fahrmeir L, Tutz G. Multivariate statistical modelling based on generalized linear models. New York: Springer-Verlag; 1994: 318–325.
- [17] Steyerberg EW, Eijkemans MJ, Harrell FE Jr, Habbema JD. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. *Stat Med* 2000;19:1059–79.
- [18] Greenland S, Schwartzbaum JA, Finkle WD. Problems due to small samples and sparse data in conditional logistic regression analysis. *Am J Epidemiol* 2000;151:531–9.
- [19] Ioannidis JP. Contradicted and initially stronger effects in highly cited clinical research. *JAMA* 2005;294:218–28.
- [20] Greenland S. Methods for epidemiologic analyses of multiple exposures—a review and comparative-study of maximum-likelihood, preliminary-testing, and empirical-Bayes regression. *Stat Med* 1993;12:717–36.
- [21] Witte JS, Greenland S. Simulation study of hierarchical regression. *Stat Med* 1996;15:1161–70.
- [22] Greenland S. Principles of multilevel modelling. *Int J Epidemiol* 2000;29:158–67.
- [23] Breslow NE, Clayton DG. Approximate inference in generalized linear mixed models. *J Am Stat Assoc* 1993;88:9–25.
- [24] Greenland S. Dose-response and trend analysis in epidemiology—alternatives to categorical analysis. *Epidemiology* 1995;6:356–65.
- [25] Jerico C, Knobel H, Montero M, Ordonez-Llanos J, Guelar A, Gimeno JL, et al. Metabolic syndrome among HIV-infected patients: prevalence, characteristics, and related factors. *Diabetes Care* 2005;28:132–7.
- [26] Bonfanti P, Ricci E, De Socio G, Zeme D, Carradori S, Penco G, et al. Metabolic syndrome: a real threat for HIV-positive patients? Results from the SIMONE study. *J Acquir Immune Defic Syndr* 2006;42:128–31.
- [27] Jones R, Sawleshwarkar S, Michailidis C, Jackson A, Mandalia S, Stebbing J, et al. Impact of antiretroviral choice on hypercholesterolaemia events: the role of the nucleoside reverse transcriptase inhibitor backbone. *HIV Med* 2005;6:396–402.
- [28] Anderson PL, Kakuda TN, Lichtenstein KA. The cellular pharmacology of nucleoside- and nucleotide-analogue reverse-transcriptase inhibitors and its relationship to clinical toxicities. *Clin Infect Dis* 2004;38:743–53.
- [29] Walker UA, Setzer B, Venhoff N. Increased long-term mitochondrial toxicity in combinations of nucleoside analogue reverse-transcriptase inhibitors. *AIDS* 2002;16:2165–73.
- [30] Yan Q, Hruz PW. Direct comparison of the acute in vivo effects of HIV protease inhibitors on peripheral glucose disposal. *J Acquir Immune Defic Syndr* 2005;40:398–403.
- [31] Gustafson P, Greenland S. The performance of random coefficient regression in accounting for residual confounding. *Biometrics* 2006;62:760–8.
- [32] Black N. Why we need observational studies to evaluate the effectiveness of health care. *BMJ* 1996;312:1215–8.
- [33] Sabin CA, Phillips AN. Treatment comparisons in HIV infection: the benefits and limitations of observational cohort studies. *J Antimicrob Chemother* 2001;47:371–5.
- [34] Mamdani M, Sykora K, Li P, Normand SL, Streiner DL, Austin PC, et al. Reader’s guide to critical appraisal of cohort studies: 2. Assessing potential for confounding. *BMJ* 2005;330:960–2.
- [35] Phillips AN, Grabar S, Tassie JM, Costagliola D, Lundgren JD, Egger M. Use of observational databases to evaluate the effectiveness of antiretroviral therapy for HIV infection: comparison of cohort

- studies with randomized trials. EuroSIDA, the French Hospital Database on HIV and the Swiss HIV Cohort Study Groups. *AIDS* 1999;13:2075–82.
- [36] Eg Hansen A-B, Gerstoft J, Kirk O, Mathiesen L, Pedersen C, Nielsen H, et al. Unmeasured confounding caused slightly better response to HAART within than outside a randomized controlled trial. *J Clin Epidemiol*, 2008;61:87–94.
- [37] Nolan D. Do non-nucleoside reverse transcriptase inhibitors contribute to lipodystrophy? *Drug Saf* 2005;28:1069–74.
- [38] Bergersen BM. Cardiovascular risk in patients with HIV infection—impact of antiretroviral therapy. *Drugs* 2006;66:1971–87.
- [39] Hertel J, Struthers H, Horj CB, Hruz PW. A structural basis for the acute effects of HIV protease inhibitors on GLUT4 intrinsic activity. *J Biol Chem* 2004;279:55147–52.
- [40] Miller KD, Jones E, Yanovski JA, Shankar R, Feuerstein I, Falloon J. Visceral abdominal-fat accumulation associated with use of indinavir. *Lancet* 1998;351:871–5.
- [41] Murata H, Hruz PW, Mueckler M. Indinavir inhibits the glucose transporter isoform GLUT4 at physiologic concentrations. *AIDS* 2002;16:859–63.
- [42] Noor MA, Seneviratne T, Aweeka FT, Lo JC, Schwarz JM, Mulligan K, et al. Indinavir acutely inhibits insulin-stimulated glucose disposal in humans: a randomized, placebo-controlled study. *AIDS* 2002;16:F1–8.
- [43] Schwarz JM, Lee GA, Park S, Noor MA, Lee J, Wen M, et al. Indinavir increases glucose production in healthy HIV-negative men. *AIDS* 2004;18:1852–4.
- [44] Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JMAH, Miller MD, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients—a 3-year randomized trial. *JAMA* 2004;292:191–201.
- [45] Podzamczar D, Ferrer E, Sanchez P, Gatell DM, Crespo M, Fisac C, et al. Less lipodystrophy and better lipid profile with abacavir as compared to stavudine—96-week results of a randomized study. *J Acquir Immune Defic Syndr* 2007;44:139–47.
- [46] Ruane PJ, DeJesus E. New nucleoside/nucleotide backbone options: a review of recent studies. *J Acquir Immune Defic Syndr* 2004;37:S21–9.
- [47] Young B. The role of nucleoside and nucleotide reverse transcriptase inhibitor backbones in antiretroviral therapy. *J Acquir Immune Defic Syndr* 2004;37:S13–20.
- [48] White AJ. Mitochondrial toxicity and HIV therapy. *Sex Transm Infect* 2001;77:158–73.
- [49] Cherry CL, Nolan D, James IR, McKinnon EJ, Mallal SA, Gahan ME, et al. Tissue-specific associations between mitochondrial DNA levels and current treatment status in HIV-infected individuals. *J Acquir Immune Defic Syndr* 2006;42:435–40.
- [50] Squillace N, Guaraldi G, Orlando G, Roverato A, Nardini G, Beghetto B, et al. The role of virological and immunological parameters on the diagnosis of metabolic syndrome in HIV-associated lipodystrophy. In: Ninth International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV, 19–21 July 2007, Sydney, Australia [Abstract P38].
- [51] Psaty BM, Koepsell TD, Lin D, Weiss NS, Siscovick DS, Rosendaal FR, et al. Assessment and control for confounding by indication in observational studies. *J Am Geriatr Soc* 1999;47:749–54.
- [52] De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Thiebaut R, et al. Relationship between use of stavudine and diabetes mellitus. In: Eighth International Congress on Drug Therapy in HIV Infection, 12–16 November 2006, Glasgow, UK [Abstract PL9.5].
- [53] Worm SW, Sabin CA, El Sadr WM, Reiss P, D'Arminio Monforte A, Rickenbach M, et al. The metabolic syndrome at baseline in the D:A:D study. In: Eighth International Congress on Drug Therapy in HIV Infection, 12–16 November 2006, Glasgow, UK. [Abstract P124].
- [54] Worm SW, Sabin CA, Reiss P, El-Sadr W, D'Arminio Monforte A, Law M, et al. Presence of the metabolic syndrome is not a better predictor of cardiovascular disease than the sum of its components: data from the D:A:D Study. In: Fourteenth Conference on Retrovirus and Opportunistic Infections, 25–28 February 2007, Los Angeles, USA. A815.
- [55] Witte JS, Greenland S, Kim LL, Arab L. Multilevel modeling in epidemiology with GLIMMIX. *Epidemiology* 2000;11:684–8.