

**Title:** Model-based tests of the effect of a genetic covariate on pharmacokinetic parameters using the SAEM algorithm

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**Background:** Despite the increasing number of investigations on the role of genetic covariates in the pharmacokinetics (PK) and/or pharmacodynamics (PD) of drug, a high diversity in analysis methods remains. Pharmacogenetic data are mainly analyzed using non-compartmental methods ensued by a one-way analysis of variance (ANOVA) on the individual parameters [1]. More sophisticated approaches using nonlinear mixed effects models (NLMEM) have also been applied to analyze genetic data in PK studies with various testing strategies based on ANOVA [2], Wald test [3] or likelihood ratio test (LRT) [4]. The advantages of NLMEM is that they can accommodate different designs (sparse or rich data), and a larger population can be studied to investigate the influence of genes with rare genotype or multiple alleles.

**Objectives:** In the present work, we analyze through a simulation study the statistical properties of three different tests used for the selection of genetic covariate following analysis with NLMEM. Different designs are studied in order to address the cases of asymptotic conditions (high number of subjects) and sparse data (few observations per subject).

**Methods:** We use for the analysis of the simulated data sets the stochastic EM algorithm (SAEM), implemented in the MONOLIX software version 2.1 [5]. SAEM avoids the linearization of the likelihood function and computes exact maximum likelihood estimates of the model parameters using a stochastic version of the EM algorithm including a MCMC procedure. The likelihood is computed by importance sampling.

In this work, we assess three methods to test the genetic effect: i) data are analysed with a model not including the genetic covariate ( $M_0$ ), then an ANOVA is performed to test the relationship between the empirical Bayes estimates of the model parameter of interest and the genetic covariate, ii) data are analysed with a model including the genetic covariate ( $M_{\text{mult}}$ ), and a global Wald test is used to assess whether estimates for the genetic effect are significant by comparing the statistic to a  $\chi^2$  with two degrees of freedom and iii) data are analysed with  $M_{\text{mult}}$  and  $M_0$ , and the likelihood of the two models are compared through a LRT where the statistic is compared to a  $\chi^2$  with two degrees of freedom.

We designed a simulation study to evaluate these three tests. The setting is based on a real PK substudy on indinavir performed during the COPHAR2-ANRS 111 trial [6] in HIV patients. The PK model for indinavir is a one compartment model at steady-state with first order absorption and elimination parameterized in absorption constant ( $k_a$ ), elimination constant ( $k$ ) and apparent volume of distribution ( $V$ ). Simulated values for the fixed and random effects are set based on a preliminary analysis of the observed data in 40 patients. We simulate a multiplicative effect of two biallelic single nucleotide polymorphisms (SNP) on the drug bioavailability through parameter  $V$ . The extent and the distribution of the simulated genetic effect are inspired from the exons 26 and 21 of the ABCB1 gene coding for the P-glycoprotein known to be involved in drug transport in the organism [7]. In the following, the tests focus on the effect of one SNP and as all human beings have two versions of each gene, three genotypes are possible so that the covariate model contains two coefficients for the genetic effect.

We investigate several designs under the null hypothesis of no relationship with the genetic covariate ( $H_0$ ): i) 40 patients with 4 plasma samples measured at 1, 3, 6 and 12h after the drug intake (similar to the original study), ii) 200 patients with the same design to reach asymptotic conditions, iii) 80 patients with 2 samples (35 subjects sampled at 1 and 3h, 35 sampled at 3 and 12h, and 20 sampled at 6 and 12h) which reflects sparse conditions and has been optimized using the PFIM software [8]. The designs with 40 and 80 patients have the same total number of samples.

For each design, we simulate 1000 data sets and apply the three tests after parameter estimation using SAEM. We then compute for each test and each algorithm the type I error as the percentage of data sets for which the test is significant. For the design with 40 and 80 patients, we perform a simulation under an alternative hypothesis ( $H_1$ ) to assess the power of each test along with the corrected power using as correction threshold the 5th percentile of the distribution of the p-values of the test under  $H_0$ .

**Results:** SAEM achieves convergence on all data sets. ANOVA has a correct type I error estimate whatever the design. The Wald test and the LRT have significantly increased type I error in the design with 40 patients but give correct values for the designs with 200 or 80 patients. For each design, the corrected power is analogous for the three tests. For the three tests, the power is greater for the design with more patients and less sample per patients. The shrinkage for the apparent volume of distribution is lower than 20% (median value on the 1000 data sets) for all designs under both hypotheses.

	N=40, n= 4			N=200, n=4		N=80, n=2	
	Size	Power	Power <sub>corr</sub>	Size	Size	Power	Power <sub>corr</sub>
ANOVA	5.3	71.1	70.9	5	4.5	94.3	93.5
Wald	8.9*	81.8	73.0	5.1	6	96.7	95.8
LRT	7.6*	78.6	73.3	5.9	5.2	95.8	95.4

\*values significantly higher than 5%

**Discussion:** In this simulation we find that 80 subjects with 2 samples are provided both adequate type I error and high power for the three tests studied. ANOVA is the only test to keep a type I error close to 5% for all designs tested. This good performance could be ascribed partly to the simulation setting where we consider a model with three PK parameters, with an effect on the apparent volume of distribution which might be in this case the less sensitive parameter to changes in sampling design. Furthermore under sparse sampling conditions the design was optimized using PFIM which may have improved the results. The Wald and the LRT tests require greater sample sizes to maintain an adequate type I error.

We can compare these results obtained with SAEM, to those obtained with linearization-based algorithms, the FO and FOCE-I implemented in NONMEM [9] for designs with 40 and 200 patients. These results were reported previously [10]. FO showed very poor performances in term of type I errors with the exception of ANOVA on all designs. The FOCE-I algorithm obtained equivalent results to those obtained with SAEM on the design with 40 patients but failed to correct the type I error inflation of the LRT for 200 patients. FOCE-I provided corrected powers in the same range as SAEM with the exception of the Wald test for which the power fell to 25%.

This work enlightened SAEM's correct performance in testing and that inference on genetic effect does not necessarily require a conventional design with extensive sampling.

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