Influence of the design on testing the effect of a genetic covariate on pharmacokinetic parameters, with the SAEM algorithm

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Context

- Increasing number of investigations on the role of genetic covariates in pharmacokinetics (PK) and/or pharmacodynamics (PD)
- High diversity in analysis methods with no consensus
  - mainly non-compartmental approach followed by one-way analysis of variance (ANOVA) on the individual parameters
  - more sophisticated approaches using nonlinear mixed effects models (NLMEM)
    - concentrations $y_{i,j}$ of the individual $i = 1, ..., N$ at times $j = 1, ..., n_i$ are described as

$$y_{i,j} = f\left(t_{i,j}, \theta_i\right) + \epsilon_{i,j}$$

      with $\epsilon_{i,j}$ the residual error
    - θ_i is the vector of the subject specific parameters of the nonlinear function f

$$\theta_i = \mu \cdot e^{\eta_i}$$

      where η_i follow a gaussian distribution with zero mean and variance matrix Ω
    - accommodation of different designs (sparse or rich data)
    - larger population providing information on genes with rare genotype or multiple alleles

Objective

- We consider the effect of a diploid single nucleotide polymorphism (SNP) on the p^{th} PK parameter
  - C the wild type replaced with T the mutant allele
  - k=3 possible genotypes (G): wild homozygote CC, heterozygote CT , mutant homozygote TT

$$\theta_{p,i} = \mu_p \cdot \beta_{G_i} \cdot e^{\eta_{p,i}}$$

  with $\beta_{G_i} = \{1, \beta_1, \beta_2\}$ for $G_i = \{CC, CT, TT\}$
- We want to evaluate by means of simulation:
  - three methods to test for a gene effect based on NLMEM
  - the influence of the study design on the performance of these three tests

Methods to test for a gene effect

- Definition of the models used in the three tests
  - M_{base}: the model without the gene effect $\{\beta_1 = \beta_2 = 1\}$ i.e. $\{CC = CT = TT\}$
  - M_{mult}: the model including the gene effect $\{\beta_1 \neq \beta_2 \neq 1\}$ i.e. $\{CC \neq CT \neq TT\}$
- ANOVA
  - data analysed with M_{base}
  - comparison of the empirical Bayes estimates (EBE) of the parameter of interest between the k groups of genotypes
  - statistic following a Fisher with (k-1, N-k) df
- Wald global test
  - data analysed with M_{mult}
  - computation of the statistic $W = \begin{pmatrix}\beta_1 - 1\\ \beta_2 - 1\end{pmatrix}^T \cdot \Sigma^{-1} \cdot \begin{pmatrix}\beta_1 - 1\\ \beta_2 - 1\end{pmatrix}$ with Σ the block for β_1 and β_2 of the estimation variance matrix
  - statistic following a χ^2 with (k-1) df
- Likelihood ratio test (LRT)
  - comparison of the likelihood of M_{base} and M_{mult}
  - computation of the statistic $LRT = -2 \times (L_{base} - L_{mult})$ with L_{base} and L_{mult} the log-likelihood of M_{base} and M_{mult}, respectively
  - statistic following a χ^2 with (k-1) df
- Parameter estimation using the exact algorithm SAEM (MONOLIX[1]version 2.1)
  - use of Monte Carlo Markov Chain methods and a stochastic version of the EM algorithm
  - estimation of the model likelihood using importance sampling
  - estimation of the standard errors using a linearisation from individual conditional estimates

The simulation study

- Simulation settings
  - pharmacokinetic framework
    - one compartment model with first order absorption and elimination at steady state
    - parameters: absorption rate k_a, elimination rate k and apparent volume of distribution V/F
    - simulated values set based on preliminary analysis of indinavir concentrations[2]
  - genetic framework
    - two biallelic single nucleotide polymorphisms SNP_1 (24% CC, 48% CT and 28% TT) and SNP_2 (29% GG, 44% GT and 27% TT) inspired from exon 26 and 21 of the ABCB1 gene[3]
    - genotypes drawn from these distributions for each individual of the dataset
    - effect on the drug bioavailability through the parameter V/F
- Designs

	N=40/n=4	N=80/n=2*	N=100/n=4,1	N=200/n=4**
Total of observations	160	160	160	800
Number of groups	1	4	2	1
Patients per group /Sampling times	40/(1,3,6,12)	30/(1,3) 10/(3,12) 30/(6,12) 10/(1,12)	20/(1,3,6,12) 80/(12)	200/(1,3,6,12)
Number of data sets simulated H_0	1000	1000	1000	1000
H_1	1000	1000	1000	-

*Design optimized using PFIM Interface 2.1[4]

**Design with more samples to be closer to asymptotic conditions, for evaluation of type I error

- Evaluation of tests
  - tests
    - type I error (size)
    - power accross designs with the same total number of samples
    - corrected power (power$_c$) with as threshold the 5^{th} percentile of the P value distribution under H_0

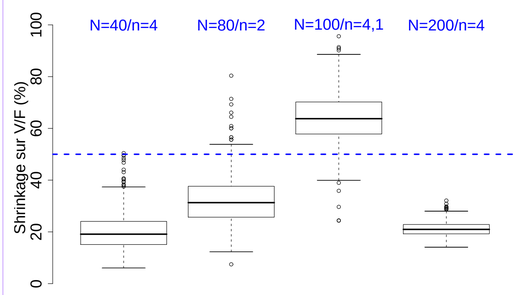
Results

- Type I error and power with SAEM

	N=40/n=4			N=80/n=2			N=100/n=4,1			N=200/n=4
	Size	Power	Power$_c$	Size	Power	Power$_c$	Size	Power	Power$_c$	Size
ANOVA	5.3	71.1	70.9	6.4	93.4	91.5	4.4	79.5	78.3	5.0
Wald	8.9*	81.8	73.0	8.7*	95.5	92.5	8.8*	85.7	81.8	5.1
LRT	7.6*	78.6	73.3	7.8*	94.6	92.2	7.4*	82.9	79.7	5.9

* Prediction interval for a value of 5% = [3.7 – 6.3]

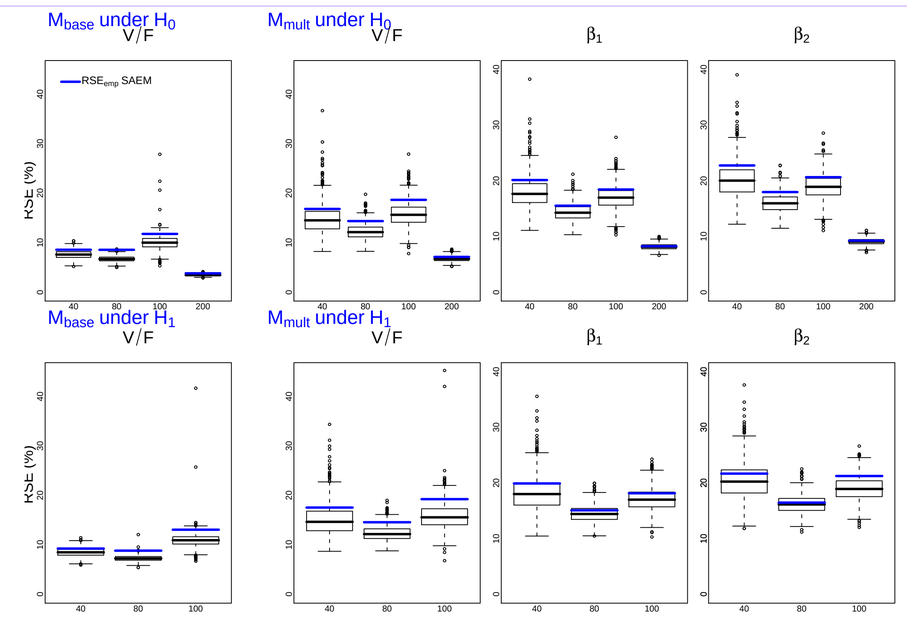
  - ANOVA: correct type I error estimate regardless of the design
  - Wald and LRT
    - correct type I error estimate for the N=200/n=4 design
    - similar type I error inflation for the N=40/n=4, N=80/n=2 and N=100/n=4,1 designs
  - power
    - analogous powers accross tests for each design
    - different powers accross designs with a total of 160 observations
    - highest power achieved for the sparse design, N=80/n=2
- Shrinkage on V/F
  - $Sh_{\eta_{V/F}} = 1 - \frac{var(\eta_{V/F,i})}{\omega^2_{V/F}}$



  - previous works of Savić[5] show that a shrinkage of 50% (when computed as a variance ratio) can impact test performance
  - design with N=100/n=4,1 → shrinkage
    - essentially due to the 80 patients with n=1

Figure 1: Shrinkage on V/F from M_{base} on the 1000 data sets simulated under H_0

- Precision of estimation



  - among the three designs with a total of 160 observations
    - N=80/n=2≥N=100/n=4,1≥N=40/n=4 for precision of estimation on β_1 and β_2

Figure 2: Boxplots of estimated RSE and empirical RSE (blue strokes) for V/F using M_{base} and V/F, β_1 and β_2 using M_{mult} under H_0 and H_1 with SAEM

Discussion

- ANOVA on EBE from the model without gene effect
  - best performance in terms of type I error: no effect of the shrinkage
  - less sensitive to unbalanced design
  - our simulation setting (considering an effect on V/F) may not have really approached the limits of ANOVA
- Wald test and LRT
  - slight inflation on designs not yielding asymptotic conditions resulting from a trade off N against n
  - degrees of freedom for the χ^2 statistic do not account for N and n
- Precision of estimation
  - power of tests is linked to precision of estimation for β[6]

Conclusion

⇒ Inference on genetic effect does not necessarily require a conventional design with extensive sampling

- asymptotic issues on type I error can be handled
  - empirical correction by simulation or permutation
  - investigation of t and F-approximate statistics for the Wald test
- large power for optimized study with only 2 samples per patients

- Further studies are required to provide recommendations on which test to use depending on the design

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