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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  $i = 1, \dots, N$  at times  $j = 1, \dots, n_i$  are described as

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

with  $\epsilon_{i,j}$  the residual error

- $\theta_i$  is the vector of the subject specific parameters of the nonlinear function  $f$

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

where  $\eta_i$  follow a gaussian distribution with zero mean and variance matrix  $\Omega$

- 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 group 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  $\Sigma$  the block for  $\beta_1$  and  $\beta_2$  of the estimation variance matrix
  - statistic following a  $\chi^2$  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  $\chi^2$  with (k-1) df
- Parameter estimation using the exact algorithm: SAEM (MONOLIX<sup>1</sup>)
  - 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<sup>2</sup>
  - genetic framework
    - two biallelic single nucleotide polymorphisms  $SNP_1$  (C>T) and  $SNP_2$  (G>T) inspired from exon 26 and 21 of the ABCB1 gene<sup>3</sup>
    - 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*
<b>Total of observations</b>	160	160	160	800
<b>Number of groups</b>	1	3	2	1
<b>Patients per group</b>	40	35,25,20	20,80	200
<b>Sampling times</b>	(1,3,6,12)	(1,3),(3,12),(6,12)	(1,3,6,12),(12)	(1,3,6,12)
<b>Number of data sets</b> $H_0$	1000	1000	1000	1000
<b>simulated</b> $H_1$	1000	1000	1000	-

\*Design with more samples to be closer to asymptotic conditions

- Evaluation

- tests
  - type I error (Size)
  - power across designs with the same total number of samples
  - corrected power ( $Power_c$ ) with as threshold the 5<sup>th</sup> percentile of the P value distribution obtained under  $H_0$
- impact of the study design
  - shrinkage on  $V/F$ :  $Sh_{N/V/F} = 1 - \frac{var(\eta_{N/V/F})}{\omega_{V/F}^2}$
  - information criterion and relative standard error (RSE) predicted by PFIM<sup>4</sup> for  $V/F$
  - empirical RSE and relative root mean square error (RRMSE) obtained for  $V/F$  from  $M_{base}$  on the 1000 simulations 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 <sub>c</sub>	Size	Power	Power <sub>c</sub>	Size	Power	Power <sub>c</sub>	Size
<b>ANOVA</b>	5.3	71.1	70.9	4.5	94.3	93.5	4.4	79.5	78.3	5.0
<b>Wald</b>	8.9*	81.8	73.0	6.0	96.7	95.8	8.8*	85.7	81.8	5.1
<b>LRT</b>	7.6*	78.6	73.3	5.2	95.8	95.4	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 whatever the design
- Wald and LRT
  - correct type I error estimate for the N=200/n=4 and N=80/n=2 designs
  - type I error inflation for the N=40/n=4 and N=100/n=4,1 designs
- Power
  - analogous powers across tests for each design
  - different powers across designs with a total of 160 observations
  - highest power achieved for the sparse design, N=80/n=2

- Shrinkage

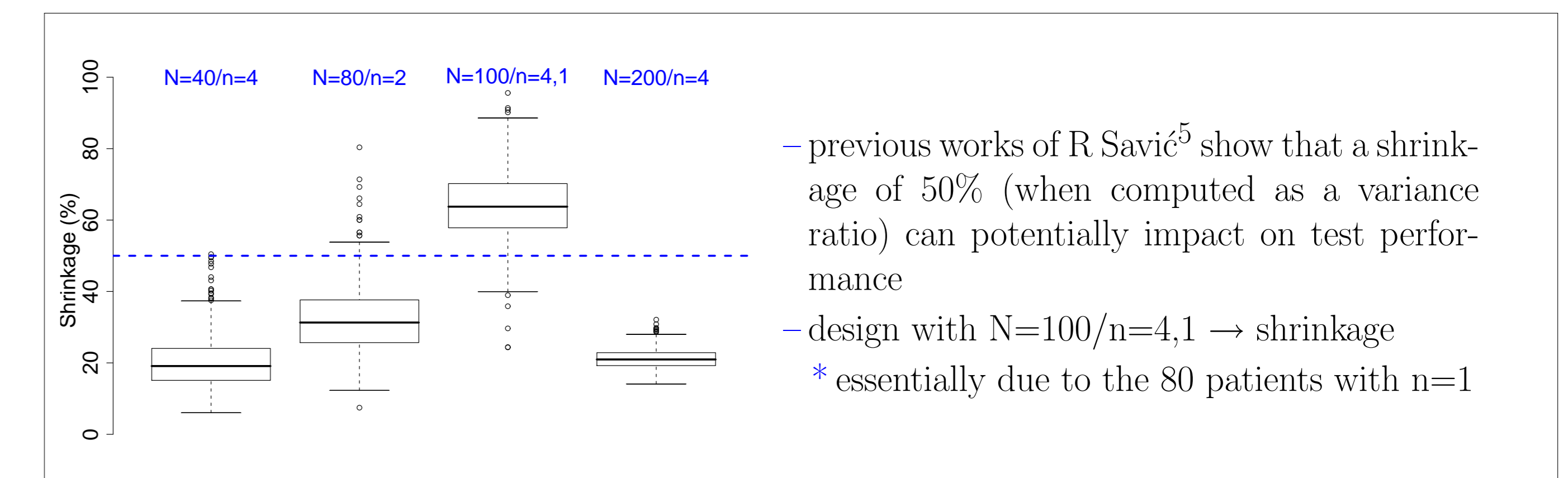


FIGURE 1: Shrinkage on  $V/F$  from  $M_{base}$  on the 1000 data sets simulated under  $H_0$

- Precision of estimation

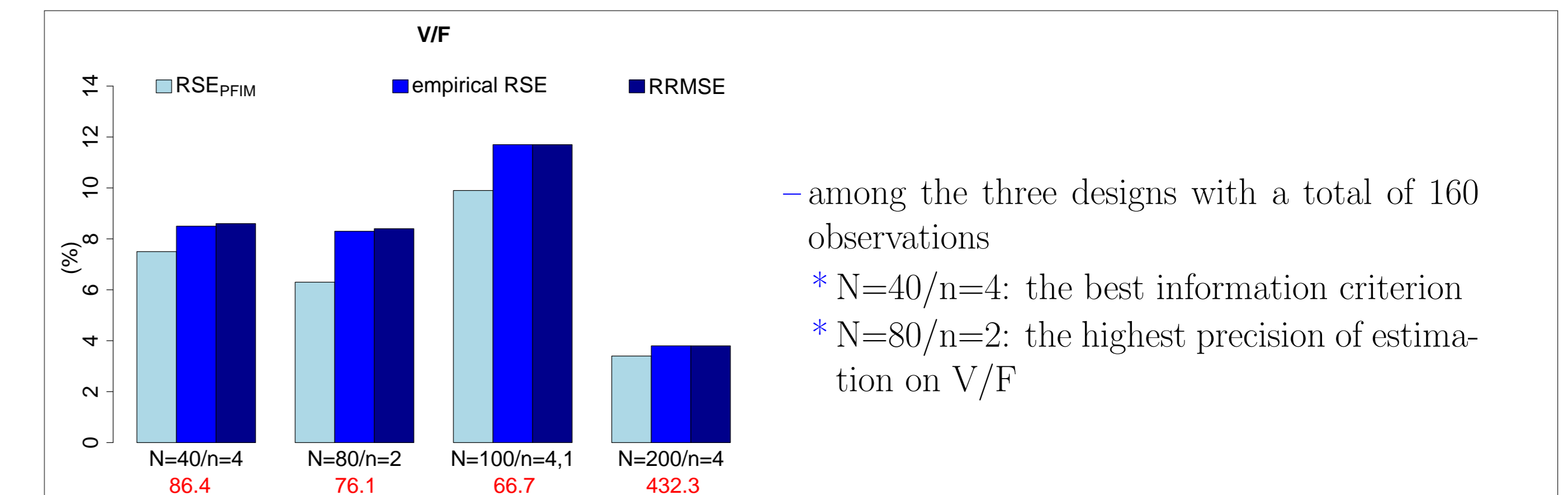


FIGURE 2: Information criterion (in red) and RSE predicted by PFIM, empirical RSE and RRMSE obtained with SAEM for  $V/F$

## DISCUSSION

- ANOVA on EBE from the model without gene effect
  - best performance in terms of type I error: no effect of the shrinkage
  - ANOVA less sensitive to difference in sample size
  - our simulation setting (considering an effect on  $V/F$ ) may not have really approached the limits of ANOVA
- Wald test and LRT
  - need correction on design yielding shrinkage or small number of patients
  - the degrees of freedom for the  $\chi^2$  statistic do not account for N and n
  - we plan to investigate t and F-approximate statistics for the Wald test
- Precision of estimation
  - PFIM predicts well the precision of estimation observed with SAEM
  - performance of tests is linked to precision of estimation for  $V/F$  rather than to the global matrix of information
- Comparison with previous results on designs N=40/n=4 and N=200/n=4 using FO and FOCE-I<sup>2</sup>
  - FO: poor performances in terms of type I errors with the exception of ANOVA on all designs
  - FOCE-I
    - equivalent results to SAEM on the design N=40/n=4
    - no correction of the type I error inflation for N=200/n=4 design
    - powers in the same range as SAEM with the exception of the Wald test (25%)

## CONCLUSION

- SAEM shows better performance in estimation and testing than the linearisation-based methods
  - Test methods in NLMEM show suitable statistical properties
    - with correct type I error and large power for study with only 2 samples per patients
    - asymptotic issues are easily handled (empirical correction)
- ⇒ NLMEM are a powerful tool to study pharmacogenetics in specific populations such as patients with acute diseases or children, for whom extensive sampling is obviously impractical