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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

- 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 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 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 (MONOLIX¹)
 - 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 concentrations²
 - 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 gene³
 - 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	3	2	1
Patients per group	40	35,25,20	20,80	200
Sampling times	(1,3,6,12)	(1,3),(3,12),(6,12)	(1,3,6,12),(12)	(1,3,6,12)
Number of data sets H_0	1000	1000	1000	1000
simulated 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 5th 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 PFIM⁴ 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 _c	Size	Power	Power _c	Size	Power	Power _c	Size
ANOVA	5.3	71.1	70.9	4.5	94.3	93.5	4.4	79.5	78.3	5.0
Wald	8.9*	81.8	73.0	6.0	96.7	95.8	8.8*	85.7	81.8	5.1
LRT	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

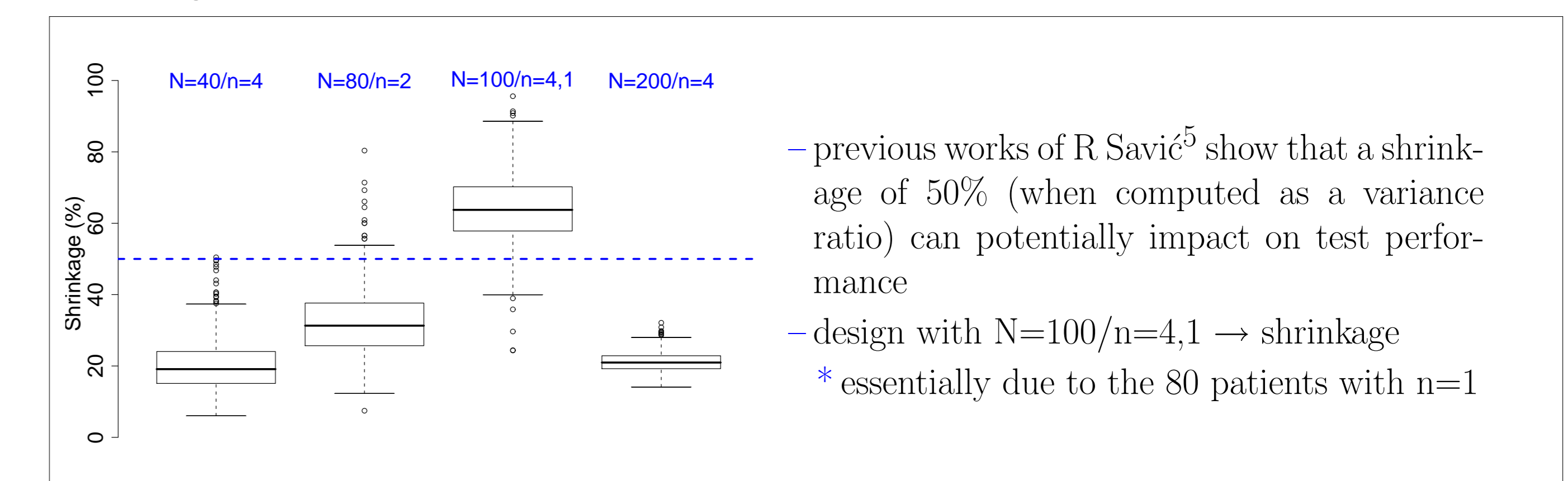


FIGURE 1: Shrinkage on V/F from M_{base} on the 1000 data sets simulated under H_0

- Precision of estimation

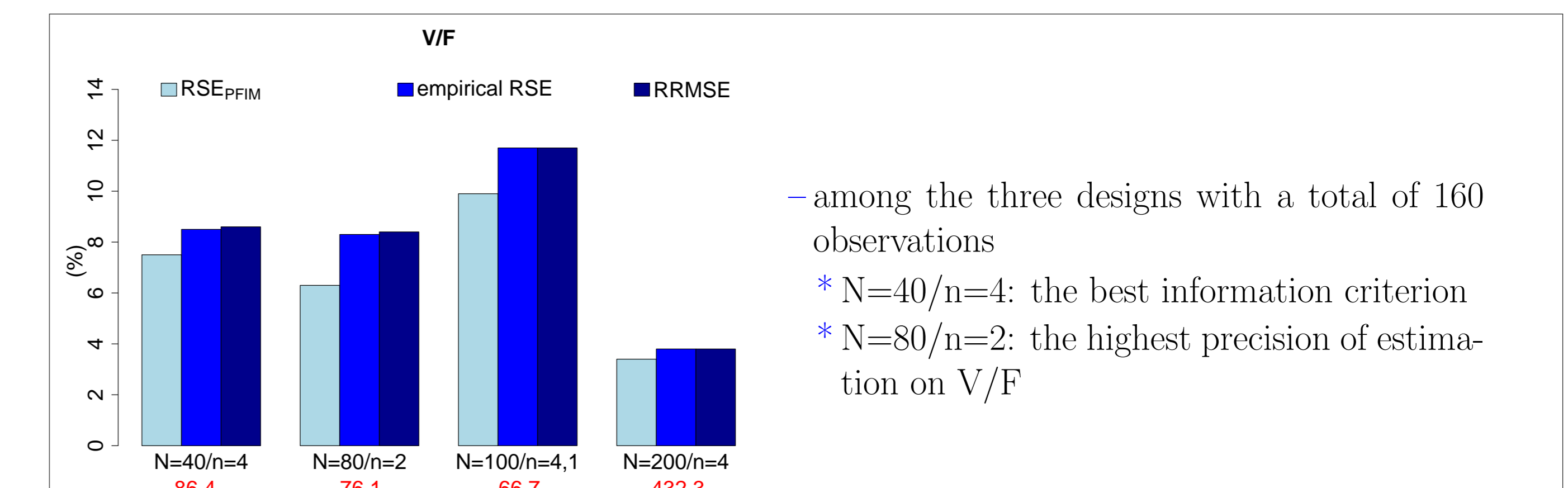


FIGURE 2: Information criterion (in red) and RSE predicted by PFIM, empirical RSE and RRMSE obtained with SAEM for V/F

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 χ^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-I²
 - 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