Quantitative assessment of drug interactions by linear mixed effects modeling

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Non Clinical Statistics Conference 2012, Potsdam

September 26, 2012

Research question

Is interaction effect of two drugs *additive*, *synergistic* or *antagonistic* ?

Interaction index

- **Quantitative** method for **assessment** of **interaction effects** of two drugs
- Definition based on *Loewe Additivity Model:*

$$\tau = \frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} \begin{cases} <1, \text{ synergy} \\ =1, \text{ additivity} \\ >1, \text{ antagonism} \end{cases}$$

- d_1 , d_2 : doses of drugs 1 and 2 that in combination produce effect y (known)
- $D_{y,1}$, $D_{y,2}$: doses of drugs 1 and 2 that produce same effect if applied singly (unknown)
- $D_{y,1}$: inverse of dose-response curve for drug 1
- $D_{y,2}$: inverse of dose-response curve for drug 2

Statistical inference

- Model dose-response curve for drug i (i = 1,2) by fitting **two-parameter log-logistic model** to normalized effect $y \in (0,1)$:

$$y = \frac{1}{1 + \left(\frac{D_{y,i}}{D_{m,i}}\right)^{b_i}}$$

$D_{y,i}$: dose of drug i, producing effect y

$D_{m,i}$: median effective dose of drug i (e.g EC_{50} , LD_{50})

b_i : Hill slope (for drug i)

- Transfer model into **median-effect equation** (Chou and Talalay, *Advances in Enzyme Regulation* ,1984):

$$\frac{y}{1-y} = \left(\frac{D_{y,i}}{D_{m,i}}\right)^{-b_i}$$

- Two analysis approaches (Lee and Kong, *Stat Biopharm Res*, 2009)

Global assessment approach (1)

Assumption: two drugs combined in ‚**fixed dose ratio**':

$$\frac{d_1}{d_2} = \frac{\omega_1}{\omega_2}\,,\; d_1 + d_2 = D_C\,,\; D_C = (d_1, d_2)$$

1. Taking log() of median-effect equation yields **simple linear regression model**:

$$\log\left(\frac{y}{1-y}\right) = -b_i \cdot \left(\log(D_{y,i}) - \log(D_{m,i})\right) =: \beta_{0,i} + \beta_{1,i} \cdot \log(D_{y,i}), \text{ with}$$

$$\beta_{0,i} = b_i \cdot \log(D_{m,i})\;,\quad \beta_{1,i} = -b_i$$

median - effect equation:

$$\frac{y}{1-y} = \left(\frac{D_{y,i}}{D_{m,i}}\right)^{-b_i}$$

2. For drugs i = 1,2: Regress log(y/1-y) on log($D_{y,i}$) to estimate $\beta_{0,i}$ and $\beta_{1,i}$.

3. Estimation of τ:

$$\hat{\tau} = \frac{d_1}{\hat{D}_{y,1}} + \frac{d_2}{\hat{D}_{y,2}} \quad \text{with} \quad \hat{D}_{y,i} = \exp\left(-\frac{\hat{\beta}_{0,i}}{\hat{\beta}_{1,i}}\right) \cdot \left(\frac{y}{1-y}\right)^{1/\hat{\beta}_{1,i}} \quad (i = 1,2)$$

Global assessment approach (2)

4. Make use of ‚fixed dose ratio' assumption to modify equation for τ:

$$d_1 = D_C \frac{\omega_1}{\omega_1 + \omega_2}\,,\; d_2 = D_C \frac{\omega_2}{\omega_1 + \omega_2}$$

'fixed dose ratio' assumption:

$$\frac{d_1}{d_2} = \frac{\omega_1}{\omega_2}\,,\; d_1 + d_2 = D_C\,,\; D_C = (d_1, d_2)$$

5. Estimate combination dose D_C at effect y:

$$\hat{D}_{y,C} = \exp\left(-\frac{\hat{\beta}_{0,C}}{\hat{\beta}_{1,C}}\right) \cdot \left(\frac{y}{1-y}\right)^{1/\hat{\beta}_{1,C}}$$

$$\hat{\tau} = \frac{d_1}{\hat{D}_{y,1}} + \frac{d_2}{\hat{D}_{y,2}}$$

6. *Modified* estimator for τ:

$$\hat{\tau}_{GA} = \frac{\hat{D}_{y,C}\dfrac{\omega_1}{\omega_1 + \omega_2}}{\hat{D}_{y,1}} + \frac{\hat{D}_{y,C}\dfrac{\omega_2}{\omega_1 + \omega_2}}{\hat{D}_{y,2}}$$

Global assessment approach (3)

7. Define grid of effects y.

8. For each effect y:

$$\hat{\tau}_{GA} = \frac{\hat{D}_{y,C}\dfrac{\omega_1}{\omega_1 + \omega_2}}{\hat{D}_{y,1}} + \frac{\hat{D}_{y,C}\dfrac{\omega_2}{\omega_1 + \omega_2}}{\hat{D}_{y,2}}$$

(a) Use *modified* estimator $\hat{\tau}_{GA}$ to estimate interaction index τ.

(b) Apply **delta method** to compute approximative variance for $\hat{\tau}_{GA}$:

$$\begin{aligned} Var(\hat{\tau}_{GA}) = {} & \left(\frac{\omega_1}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,1}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,1})}{\hat{\beta}_{1,1}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,1},\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)}{\hat{\beta}_{1,1}^3} + \frac{Var(\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)^2}{\hat{\beta}_{1,1}^4}\right] \\ & + \left(\frac{\omega_2}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,2}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,2})}{\hat{\beta}_{1,2}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,2},\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)}{\hat{\beta}_{1,2}^3} + \frac{Var(\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)^2}{\hat{\beta}_{1,2}^4}\right] \\ & + \left(\frac{\hat{D}_{y,C}}{\omega_1+\omega_2}\cdot\left(\frac{\omega_1}{\hat{D}_{y,1}}+\frac{\omega_2}{\hat{D}_{y,2}}\right)\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,C})}{\hat{\beta}_{1,C}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,C},\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)}{\hat{\beta}_{1,C}^3} + \frac{Var(\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)^2}{\hat{\beta}_{1,C}^4}\right] \end{aligned}$$

Global assessment approach (4)

(c) Calculate approximative (1-α) confidence interval for τ :

$$\hat{\tau}_{GA} \cdot \exp\left(\pm \frac{t_{n+n_c-6;\,\alpha/2}}{\hat{\tau}_{GA}} \cdot \sqrt{Var(\hat{\tau})}\right)$$

$n = \sum_{i=1}^{2} n_i$; n_i : # of observations when drug i is applied alone

n_c: # of observations when combination of two drugs is applied

9. Plot: effects y vs. estimated interaction indices $\hat{\tau}_{GA}$

10. Computation of pointwise (1-α) confidence bound for curve of effects vs. interaction indices.

R code provided by Lee and Kong at:

http://biostatistics.mdanderson.org/SoftwareDownload/.

Drawback

Global assessment approach:

Assumes that all data were collected from a single dose-response experiment.

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Naive solution

(1) Merge data of all dose-response experiments.

(2) Apply global assessment approach to merged data set.

Problem

Recall: Approximative variance of estimated interaction index:

$$Var(\hat{\tau}_{GA}) = \left(\frac{\omega_1}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,1}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,1})}{\hat{\beta}_{1,1}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,1},\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)}{\hat{\beta}_{1,1}^3} + \frac{Var(\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)^2}{\hat{\beta}_{1,1}^4}\right]$$

$$+\left(\frac{\omega_2}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,2}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,2})}{\hat{\beta}_{1,2}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,2},\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)}{\hat{\beta}_{1,2}^3} + \frac{Var(\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)^2}{\hat{\beta}_{1,2}^4}\right]$$

$$+\left(\frac{\hat{D}_{y,C}}{\omega_1+\omega_2}\cdot\left(\frac{\omega_1}{\hat{D}_{y,1}}+\frac{\omega_2}{\hat{D}_{y,2}}\right)\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,C})}{\hat{\beta}_{1,C}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,C},\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)}{\hat{\beta}_{1,C}^3} + \frac{Var(\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)^2}{\hat{\beta}_{1,C}^4}\right]$$

Problem

Recall: Approximative variance of estimated interaction index:

$Var(\hat{D}_{y,1})$

$Var(\hat{D}_{y,2})$

$Var(\hat{D}_{y,C})$

- Accounts for within-experiment variability (by variance terms $Var(\hat{D}_{y,i})$, i = 1, 2, C)

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- Does not account for between-experiment variability !!!

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$$+\left(\frac{\omega_2}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,2}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,2})}{\hat{\beta}_{1,2}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,2},\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)}{\hat{\beta}_{1,2}^3} + \frac{Var(\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)^2}{\hat{\beta}_{1,2}^4}\right] \quad Var(\hat{D}_{y,2})$$

$$+\left(\frac{\hat{D}_{y,C}}{\omega_1+\omega_2}\cdot\left(\frac{\omega_1}{\hat{D}_{y,1}}+\frac{\omega_2}{\hat{D}_{y,2}}\right)\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,C})}{\hat{\beta}_{1,C}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,C},\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)}{\hat{\beta}_{1,C}^3} + \frac{Var(\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)^2}{\hat{\beta}_{1,C}^4}\right] \quad Var(\hat{D}_{y,C})$$

- Accounts for within-experiment variability (by variance terms $Var(\hat{D}_{y,i})$, i = 1, 2, C)
- Does not account for between-experiment variability !!!
- Large between-experiment variability can have great impact on estimation of interaction index

Proposal (1)

(1) Merge data of all dose-response experiments.

(2) Global assessment approach: For each drug (combination), replace (fixed effects) simple linear regression model

$$z_j = \beta_0 + \beta_1 \cdot d_j + \varepsilon_j\,,\ \ \varepsilon_j \sim N(0,\sigma^2)$$

d_j : log(dose) for observation $j = 1,\ldots,N = \sum_{k=1}^{K} n_k$, n_k : # of observations in experiment k = 1,…,K

$$z_j = \log\left(\frac{y_j}{1-y_j}\right),\ \ y_j \in (0,1)$$

by **linear mixed effects model**

$$z_{kl} = \beta_0 + \beta_1 \cdot d_{kl} + b_{0,k} + b_{1,k}\cdot d_{kl} + \varepsilon_{kl}\,,\ \ \varepsilon_{kl} \sim N(0,\sigma^2)\,,\ \begin{pmatrix} b_{0,k} \\ b_{1,k}\end{pmatrix} \sim N(0,\Sigma)\,,\ k=1,\ldots,K\,,\ l=1,\ldots,n_k$$

slope (random effect)
intercept (random effect)
intercept (fixed effect)
slope (fixed effect)

Proposal (2)

(3) Plug in mixed effects model estimates of $\beta_{0,i}$, $\beta_{1,i}$, $Var(\beta_{0,i})$, $Var(\beta_{1,i})$ and $Cov(\beta_{0,1},\beta_{1,i})$ (i = 1, 2, C) into formulas for $\hat{\tau}_{GA}$ and $Var(\hat{\tau}_{GA})$, to yield reliable estimates of τ :

$$\hat{\tau}_{GA} = \frac{\hat{D}_{y,C}\frac{\omega_1}{\omega_1+\omega_2}}{\hat{D}_{y,1}} + \frac{\hat{D}_{y,C}\frac{\omega_2}{\omega_1+\omega_2}}{\hat{D}_{y,2}}\ ,\quad \hat{D}_{y,i} = \exp\left(-\frac{\hat{\beta}_{0,i}}{\hat{\beta}_{1,i}}\right)\cdot\left(\frac{y}{1-y}\right)^{1/\hat{\beta}_{1,i}}$$

$$Var(\hat{\tau}_{GA}) = \left(\frac{\omega_1}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,1}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,1})}{\hat{\beta}_{1,1}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,1},\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)}{\hat{\beta}_{1,1}^3} + \frac{Var(\hat{\beta}_{1,1})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,1}\right)^2}{\hat{\beta}_{1,1}^4}\right]$$

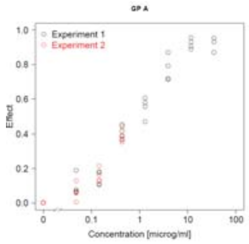
$$+\left(\frac{\omega_2}{\omega_1+\omega_2}\cdot\frac{\hat{D}_{y,C}}{\hat{D}_{y,2}}\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,2})}{\hat{\beta}_{1,2}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,2},\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)}{\hat{\beta}_{1,2}^3} + \frac{Var(\hat{\beta}_{1,2})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,2}\right)^2}{\hat{\beta}_{1,2}^4}\right]$$

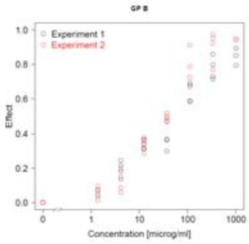
$$+\left(\frac{\hat{D}_{y,C}}{\omega_1+\omega_2}\cdot\left(\frac{\omega_1}{\hat{D}_{y,1}}+\frac{\omega_2}{\hat{D}_{y,2}}\right)\right)^2 \cdot \left[\frac{Var(\hat{\beta}_{0,C})}{\hat{\beta}_{1,C}^2} + \frac{2\cdot Cov(\hat{\beta}_{0,C},\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)}{\hat{\beta}_{1,C}^3} + \frac{Var(\hat{\beta}_{1,C})\cdot\left(\log\left(\frac{y}{1-y}\right)-\hat{\beta}_{0,C}\right)^2}{\hat{\beta}_{1,C}^4}\right]$$

(4) Modify R code provided by Lee and Kong accordingly.

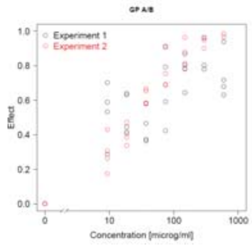
Application to cancer research study

Data





GP: glycoprotein



- Large <u>within-experiment</u> variability of replicate measurements per concentration
  (especially for experiment 1)

- Large <u>between-experiment</u> variability
  (especially for GP B and the combination GP A/B)

Parameter estimates

Situation	$\hat{\beta}_0$		$\hat{\beta}_1$	
	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*
GP A	0.0812	0.0804	0.8876	0.8864
GP B	- 2.9581	- 2.9330	0.8042	0.8050
GP A/B	- 2.1628	- 2.3112	0.7290	0.7732

- **Small difference** in **parameter estimates** between fixed effects and mixed effects modeling approach

* random intercept + random slope

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GP A/B	- 2.1628	- 2.3112	0.7290	0.7732

* random intercept + random slope

Parameter estimates/variances

Situation	$\hat{\beta}_0$ (Var)		$\hat{\beta}_1$ (Var)	
	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*
GP A	0.0812 (0.0055)	0.0804 (0.0053)	0.8876 (0.0012)	0.8864 (0.0044)
GP B	- 2.9581 (0.0324)	- 2.9330 (0.1875)	0.8042 (0.0019)	0.8050 (0.0285)
GP A/B	- 2.1628 (0.1528)	- 2.3112 (1.5555)	0.7290 (0.0079)	0.7732 (0.1200)

- **Small difference** in **parameter estimates** between fixed effects and mixed effects modeling approach

* random intercept + random slope

Parameter estimates/variances/covariances

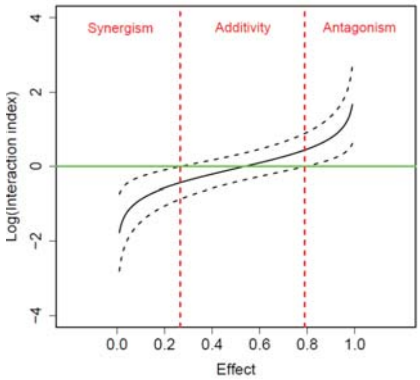
Situation	$\hat{\beta}_0$ (Var)		$\hat{\beta}_1$ (Var)		$Cov(\hat{\beta}_0, \hat{\beta}_1)$	
	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*
GP A	0.0812 (0.0055)	0.0804 (0.0053)	0.8876 (0.0012)	0.8864 (0.0044)	- 0.0002	- 0.0003
GP B	- 2.9581 (0.0324)	- 2.9330 (0.1875)	0.8042 (0.0019)	0.8050 (0.0285)	- 0.0068	- 0.0717
GP A/B	- 2.1628 (0.1528)	2.3112 (1.5555)	0.7290 (0.0079)	0.7732 (0.1200)	- 0.0331	- 0.4303

- **Small difference** in **parameter estimates** between fixed effects and mixed effects modeling approach

* random intercept + random slope

Plot of effects vs. log(interaction indices): artificial data

Grid of effects: y ∈ [0.01;0.99] , stepsize : Δ = 0.005



— estimated interaction indices (on log scale)

- - 95% pointwise confidence bound

— additivity line

Parameter estimates/variances/covariances

Situation	$\hat{\beta}_0$ (Var)		$\hat{\beta}_1$ (Var)		$Cov(\hat{\beta}_0, \hat{\beta}_1)$	
	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*	Fixed Effects Model	Mixed Effects Model*
GP A	0.0812 (0.0055)	0.0804 (0.0053)	0.8876 (0.0012)	0.8864 (0.0044)	- 0.0002	- 0.0003
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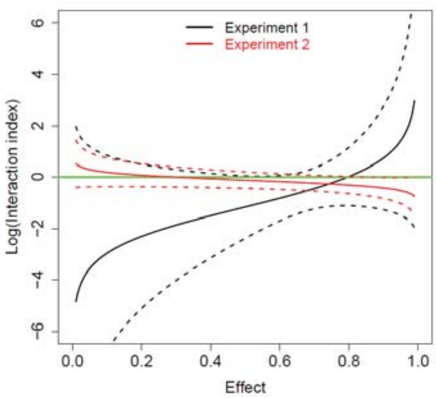
GP A/B	- 2.1628 (0.1528)	- 2.3112 (1.5555)	0.7290 (0.0079)	0.7732 (0.1200)	- 0.0331	- 0.4303

- **Small difference** in **parameter estimates** between fixed effects and mixed effects modeling approach
- **Fixed effects modeling** approach **underestimates variances/covariances** of parameter estimates

* random intercept + random slope

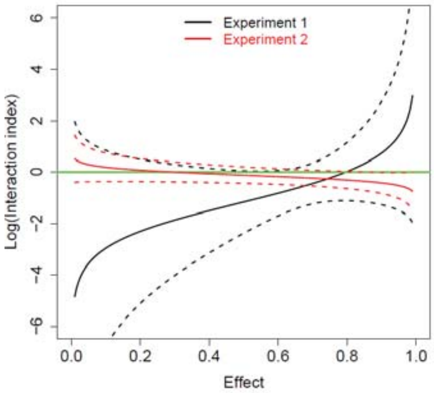
Plot of effects vs. log(interaction indices): study data

Grid of effects: y ∈ [0.01;0.99] , stepsize : Δ = 0.005



Plot of effects vs. log(interaction indices): study data

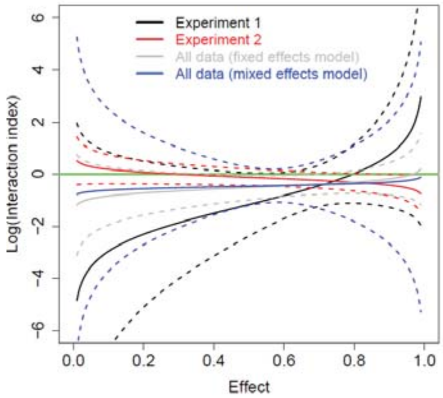
Grid of effects: $y \in [0.01;0.99]$, stepsize : $\Delta = 0.005$



- Large difference between experiments

Plot of effects vs. log(interaction indices): study data

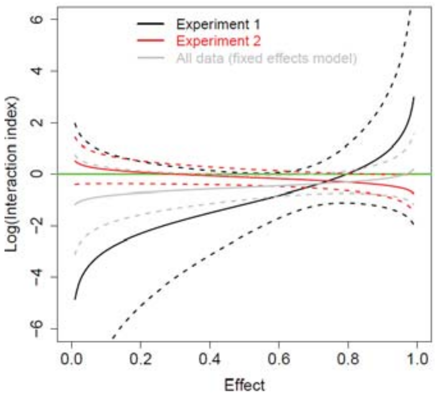
Grid of effects: $y \in [0.01;0.99]$, stepsize : $\Delta = 0.005$



- Large difference between experiments
- Fixed/mixed effects modeling approach result in similar estimates of interaction index
- 95% confidence bound wider for mixed effects modeling approach

Plot of effects vs. log(interaction indices): study data

Grid of effects: $y \in [0.01;0.99]$, stepsize : $\Delta = 0.005$



- Large difference between experiments

Plot of effects vs. log(interaction indices): study data

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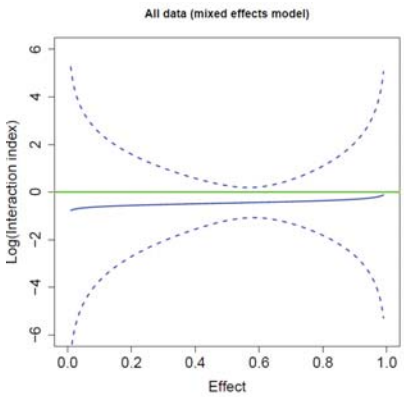

- Concentration-independent interaction effect of two glycoproteins
- Slight synergy tendency at all concentrations
- No statistically significant deviation from additivity assumption (α=5%)

Discussion

- Global assessment approach (Lee and Kong, 2009) allows quantitative assessment of drug interactions for complete dose range.
- Drawback: approach assumes that all data were collected from a single dose-response experiment
- If more than one experiment:

  (1) Merge data of all dose-response experiments.

  (2) Lee and Kong procedure: replace simple linear regression model by linear mixed effects model.

  - Accounts for variability between experiments.
  - Yields reliable estimates of the interaction index.
  - Confidence bounds for curve of estimated interaction indices will be wide in case of few experiments with large between-experiment variability.

References

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