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Accounting for Variability in Individual Hierarchical Clinical Trial Data

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January 26, 2007

Abstract

Meta-analytical approaches have been extensively used to analyze medical data. In most cases the data come from different studies or independent trials with similar characteristics. However, these methods can be applied in a broader sense. In this paper, we show how existing meta-analytic techniques can be used as well when dealing with parameters estimated from individual hierarchical data. Specifically, we propose to apply statistical methods that account for the variances (and possibly covariances) of such measures.

The estimated parameters together with their estimated variances can be incorporated into a general linear mixed model framework. We illustrate the methodology by using data from a first-in-man study and using a simulated dataset. The analysis was implemented with the SAS procedure MIXED and example code is offered.

Some Keywords: Meta-analytical methods; linear mixed models; two-stages models; fractional polynomial; SAS proc MIXED.

1 Introduction

In many areas of research, a topic of interest is how results from studies at previous stages can be used or incorporated into statistical analysis at later stages.

Regression models can be used to estimate measures of interest at a first stage (individual level) to summarize information that will be used next. A typical example is the so-called inverse regression. In this setting, a model is fitted to individual data and a value is estimated together with its standard error based on the inversed regression function. The estimated parameters can then be used for further analysis at a second stage.

The estimated standard errors of the model parameters can easily be used to obtain estimated standard errors of the individual summary measures via, for example, the delta method. The key issue remains how to incorporate this uncertainty in a later modelling step.

The setting considered in this paper bears similarity with meta-analysis (Hedges *et al.*, 1985), where hierarchical structures are also encountered. Here, subjects play the role of trials (studies) in the meta-analytic framework. We discuss existing meta-analytic modeling strategies to deal with this

setting and illustrate them in a case study, introduced in Section 2. In Section 3, we describe the statistical methodology used to estimate the parameters of interest from the first stage. The use of fractional polynomials will be indicated. A description of the statistical models proposed to analyze meta-analytical data will be discussed in Section 4. We then analyze the data of the case study in Section 5 and conclude the paper by some discussion in Section 6.

2 Case Study

We consider a first-in-man single dose cross-over study with three periods. In this dose-escalation trial 9 doses of an investigational compound were tested in 25 healthy volunteers, each subject receiving two active doses and a matched placebo on three separate occasions (periods). One of the objectives of this study was to investigate β_1-adrenoceptor responsiveness by measuring the chronotropic response (i.e., increased heart rate) to rapid bolus intravenous injections of isoproterenol, a β-adrenoceptor agonist. Starting with a dose of 0.25 μg, increasing (twofold) doses of isoproterenol were administered every 5 to 10 minutes and the resulting rises in heart rate measured. The test continued until an increase of 30-35 beats/min was observed or the maximum planned dose of isoproterenol (64 μg) administered. The test was conducted for each studied subject after oral administration of the investigational compound or placebo.

Since, for safety reasons, the isoproterenol dose-response relationship cannot typically be explored to its full extent, the response to isoproterenol is usually assessed by means of the I_{25} (Arnold and McDevitt, 1983). This quantity is defined as the isoproterenol dose required to induce a 25 bpm increase in heart rate. In practice, a model must be chosen to provide an empirical description of the isoproterenol dose-response curve over the range of doses administered. The I_{25} is then determined by deriving the predicted isoproterenol dose level at 25 bpm using an inverse regression approach.

Figure 1 provides an illustration for a particular subject in the study. The observed rises in heart rate corresponding to the three treatment periods are represented by different symbols. Fitted curves are also displayed for each period (see next section for details). The determination of the I_{25} for one of the periods is also represented.

3 First Stage Analysis

The isoproterenol dose required to stimulate a 25 bpm increase in heart rate (I_{25}) for each subject was estimated by fitting fractional polynomials. Fractional polynomials were introduced by Royston and Altman (1994) to extend the class of conventional polynomials as a flexible tool for parsimonious parametric modeling. Fractional polynomials can be written as

$$\delta_0 + \sum_{k=1}^{m} \delta_k X^{(p_k)},$$

where X denotes the isoproterenol dose, the power terms $p_1 \leq \ldots \leq p_m$ can take on real values and

$$X^{(p_k)} = \begin{cases} X^{p_k} & \text{if} \quad p_k \neq 0, \\ \log(X) & \text{if} \quad p_k = 0. \end{cases}$$

Figure 1: *Observed changes in heart rate for a particular subject in the study, in response to increasing isoproterenol doses. Symbols correspond to different occasions where the investigational compound was administered:* ■*=placebo,* ●*=dose 1, and* ♦*=dose 2. Fitted curves based on fractional polynomials are displayed. The reference line corresponds to a 25 bpm increase in heart rate. Determination of the* I_{25} *for one curve is also illustrated.*

Unlike nonlinear regression models, power coefficients are not parameters to be estimated but they are fixed beforehand. To determine the fractional polynomial of degree m that best fits the data, Royston and Altman (1994) propose to restrict the power terms to a small predefined set of (fractional) values. They suggest using $\mathcal{P} = \{-2, -1, -0.5, 0, 0.5, 1, 2, \ldots, \max(3, m)\}$ and to select the power vector $p = (p_1, \ldots, p_m)$ associated to the model with lowest deviance. In this study, the search was done over the set $\mathcal{P} = \{0, 0.5, 1, 1.5, 2, 2.5, 3\}$ for fractional polynomials of degree 1 of the form $\delta X^{(p)}$. The choice of $\mathcal{P}$ guarantees that increasing functions of dose are chosen. The lack of intercept is a natural restriction and it was deemed to provide a reasonable trade-off between goodness-of-fit and robustness for prediction purposes. Second order fractional polynomials were also explored but did not significantly improve the model's fit. The predicted value of the I_{25} from this model is

$$\widehat{I}_{25} = \begin{cases} (25/\hat{\delta})^{1/p} & \text{if} \quad p \neq 0, \\ \exp(25/\hat{\delta}) & \text{if} \quad p = 0. \end{cases}$$

The standard deviation of the $\widehat{I}_{25}$ can be approximated by the delta method:

$$\widehat{\text{sd}}(\widehat{I}_{25}) = \begin{cases} \dfrac{1}{p}\left(\dfrac{25}{\hat{\delta}}\right)^{\frac{1-p}{p}} \dfrac{25}{\hat{\delta}^2}\text{sd}(\hat{\delta}) & \text{if} \quad p \neq 0, \\ \exp(25/\hat{\delta})\dfrac{25}{\hat{\delta}^2}\text{sd}(\hat{\delta}) & \text{if} \quad p = 0. \end{cases}$$

As explained in Section 2, the number of administered isoproterenol doses could differ between test sessions. With placebo or low doses of the investigational compound, a rise of more than 30 bpm in

heart rate was reached very rapidly, typically with 3 or 4 doses of isoproterenol (this is illustrated by the first curve in Figure 1). With higher doses of the compound, β-adrenergic receptors were blocked and higher doses of isoproterenol were needed to induce the required increase in heart rate. This, together with the fact that different subjects react differently under the same dose, makes standard deviations of the estimated I_{25} vary considerably.

The model chosen in this study was based on fractional polynomials of first degree, but higher degrees are possible and they were explored. It should be emphasized that our methodology is not restricted to this kind of models. Other alternatives, possibly involving more complicated models, could be applied in this first step. However, we want to keep this aspect as simple as possible in order to focus on the second part of the strategy where the meta-analytical methodology will be used.

In the next section, we introduce a class of models which allows us to test for dose effects on the estimated I_{25} while incorporating uncertainty on these estimates explicitly into the model.

4 Second Stage Analysis

Let Y_{ij} denote the estimated $\log(I_{25})$ for subject i $(i = 1, \ldots, n)$ within period j $(j = 1, \ldots, q)$. The log transformation is used to better approximate normality. The standard error of $\log(I_{25})$ is approximated using the delta method. In addition, we indicate with n_{ij} the number of points used to estimate I_{25ij} for subject i within period j.

A basic model for Y_{ij} can be written as

$$Y_{ij} = x'_{ij}\beta + \alpha_i + \varepsilon_{ij}, \tag{1}$$

where it is assumed that the random subject effects α_i are $N(0, \sigma^2_\alpha)$ and the residual errors ε_{ij} are $N(0, \sigma^2_\varepsilon)$. The random terms are assumed to be independent of each other. A fixed-effects vector β is introduced into the model to account for covariates (e.g. dose of the investigational compound). The corresponding design vector is x_{ij}.

We have restricted this analysis to a very simple model having only random intercept. However, more elaborated models, including random slopes or random effects with general design, could be used in this setting.

The parameters of interest are the vector β, enabling to explore the dose effects, the variance of the random effects and the residual variance σ^2_ε. We now briefly describe the methods we will use later to analyze the case study.

Model (1) can be fitted using standard linear mixed model methodology, e.g., using maximum likelihood or restricted maximum likelihood estimation. However, this approach suffers from the fact that it completely ignores the uncertainty in estimating the I_{25}'s.

Borrowing ideas from the meta-analytical framework, in a meta analysis, means and standard errors are collected for a set of studies together with covariates such as the dose group at the study level. In our case, we can apply those ideas by considering each subject a study or unit. Therefore, the estimated means and standard errors of the $\log(I_{25})$'s can be considered as coming from a meta-analytic summary. Now, (1) and its distributional assumptions but acknowledge differential variability in the residual error, i.e. replacing $\varepsilon_{ij} \sim N\left(0, \sigma^2_\varepsilon\right)$ by $\varepsilon_{ij} \sim N\left(0, \sigma^2_{\varepsilon_{ij}}\right)$. This is equivalent to a weighted approach, where we use as weights the inverse of the variability of each measurement. In other words, if we know $\widehat{\text{sd}}(\widehat{I}_{25})$, there is no need to estimate the residual variance as long as

we supply this information in the model or if we constrain the variability to be fixed at this value while estimating other parameters. We will show in what follows how this can be effectuated using SAS in the case study.

The weighted approach corrects for some but not all of the heterogeneity. Other approaches, like the method of Van Houwelingen et al. (2002) allow for a more detailed and flexible modelling of the different sources of variability. For example, to exploit these features, this approach was applied in a surrogate marker evaluation context by Tibaldi et al. (2003).

Following the ideas of Houwelingen et al., we introduce a model for the estimated subject-specific effects $(\widehat{\log(I_{25_{i1}})}, \ldots, \widehat{\log(I_{25_{iq}})})'$, given the true subject-specific means $(\log(I_{25_{i1}}), \ldots, \log(I_{25_{iq}}))'$:

$$\begin{pmatrix} \widehat{\log(I_{25_{i1}})} \\ \vdots \\ \widehat{\log(I_{25_{iq}})} \end{pmatrix} \sim N\left(\begin{pmatrix} \log(I_{25_{i1}}) \\ \vdots \\ \log(I_{25_{iq}}) \end{pmatrix}, S_i \right). \tag{2}$$

Here, S_i is the variance-covariance matrix of the estimated treatment effects. In case we assume estimates to be independent, S_i would be diagonal, but the method applies to general covariance structures.

Furthermore, we assume a normal model for the true subject-specific effects around true overall means:

$$\begin{pmatrix} \log(I_{25_{i1}}) \\ \vdots \\ \log(I_{25_{iq}}) \end{pmatrix} \sim N\left(\begin{pmatrix} \log(I_{25_1}) \\ \vdots \\ \log(I_{25_q}) \end{pmatrix}, \Sigma \right). \tag{3}$$

The resulting marginal model, combining (2) and (3), is:

$$\begin{pmatrix} \widehat{\log(I_{25_{i1}})} \\ \vdots \\ \widehat{\log(I_{25_{iq}})} \end{pmatrix} \sim N\left(\begin{pmatrix} \log(I_{25_1}) \\ \vdots \\ \log(I_{25_q}) \end{pmatrix}, \Sigma + S_i \right). \tag{4}$$

Maximum likelihood estimation for this model can be quite easily carried out by using mixed model software, provided the values for S_i can be input and held fixed, as is the case in the SAS procedure MIXED. Let us now apply this methodology to the case study.

5 Analysis of the Case Study

Table 2 contains all numerical results of the estimated fixed effects and variance components. We also display the Akaike Information Criteria (AIC) corresponding to each model in order to have an informal basis for comparison.

The first column displays the estimated values of the standard mixed model. This strategy ignores the estimated standard deviations of the response variable coming from the first stage. However, this model gives an estimation of the residual variance σ_ε^2 and the random intercept variance σ_α^2. The SAS code to fit this model is shown in Appendix 7.1.

Column 2 presents the results for the weighted approach. This was obtained with a WEIGHT statement in the MIXED procedure, using the inversed variance of the $\widehat{\log(I_{25})}$'s as weight. The

SAS code to fit this model is shown in Appendix 7.2. Columns 3, 4, and 5 contain the results of the fully corrected models, using different covariance structure S_i. In column 3 a variance component structure (VC) was used for S_i, that is, $S_i = \sigma_\epsilon^2 I$ (the corresponding SAS code is shown in Appendix 7.3). As expected, this coincides with the weighted approach shown in column 2. In column 4, the within-subject dependence was ignored and S_i was taken to be

$$\begin{pmatrix} \sigma_{11}^2 & 0 & 0 \\ 0 & \sigma_{22}^2 & 0 \\ 0 & 0 & \sigma_{33}^2 \end{pmatrix},$$

while in column 5 the fully parameterized covariance matrix S_i was considered (the corresponding SAS code is shown in Appendix 7.4):

$$\begin{pmatrix} \sigma_{11}^2 & \sigma_{12}^2 & \sigma_{13}^2 \\ \sigma_{21}^2 & \sigma_{22}^2 & \sigma_{23}^2 \\ \sigma_{31}^2 & \sigma_{32}^2 & \sigma_{33}^2 \end{pmatrix}.$$

In practice, this is achieved by considering indicator variables for treatment periods. These effects are included only in the random structure of the model, through the RANDOM statement in the SAS procedure MIXED, to obtain an estimation of the above covariance matrix. In our case, we have used the option '`type=un`' that corresponds to an unstructured variance-covariance matrix.

The model ignoring dependence showed a marked deterioration in model fit as judged by the AIC criterion, while the full model was penalized for the large number of parameters. Therefore, simpler models seem preferable. It can be noted, however, that the AIC criterion does not allow discriminating between the linear mixed model analysis and the corrected method with VC structure in this example.

The comparison of the different models was done through the AIC criterion, which is a popular choice among modelers, but of course other criteria (BIC, AICC, etc.) are also possible.

Table 1, About Here.

We complete this section by adding another example illustrating the advantages of the proposed method. We generated a dataset under the characteristics of the case study but using only placebo and two active doses of the investigational compound. A total number of 27 subjects were considered in the analysis. Each individual had estimated I_{25} values and corresponding standard deviations in three periods. No difference between placebo and dose 1 was assumed, and a difference of 0.25 in $\log(I_{25})$ scale between placebo and dose 2 was used to generate this dataset. Random subjects effects and random errors were added both assuming normal distributions.

The analysis was done in accordance with the methods previously described. A simple linear mixed model was used followed by a weighted analysis. The fully corrected approach using a diagonal matrix and an unstructured version were fitted to the data. To complement this analysis, placebo was compared to each of the two active doses of the compound and the p-values to support the conclusions were obtained as well.

Table 2, About Here.

The results are presented in Table 2. It can be seen that the simpler approaches do not show any statistically significant differences between doses 1 and 2 and placebo. The fully corrected approaches, however, allow us to settle for a difference between dose 2 and placebo, which is consistent with the way the data was generated. In addition, the AIC coefficients indicate that the fully corrected approach with diagonal structure seems the most appropriate.

6 Conclusions

Meta-analytical approaches have been extensively used to analyze medical data. In most cases the data come from different studies or independent trials with similar characteristics. In this paper, we have shown how existing meta-analytic techniques can be used when dealing with parameters estimated from individual hierarchical data. Standard modeling approaches ignoring the uncertainty in the estimates from the first stage might be inappropriate under such circumstances.

The techniques proposed follow the lines of meta-analytical methods where each individual is considered a unit on its own. We take uncertainty into account using a simple weighting procedure, as well as a more elaborate scheme due to Van Houwelingen et al. (2002). In principle, a fully hierarchical analysis may be considered too. However, Tibaldi et al. (2003) have shown that the sometimes prohibitive computational burden can elegantly be avoided using the approaches advocated here. We showed that these approaches are easily implemented with standard software.

In the case study used to illustrate the approach, fractional polynomials were used to estimate the isoproterenol dose required to induce a 25 bpm rise in heart rate. It is important to point out that the methodology we proposed here is not restricted to this parametric case and it can be applied to any broad class of similar settings, including where nonlinear models would be used. Another important issue is the fact that curves within the same subject can be correlated. Therefore, one might denote attention to modelling the variance-covariance matrix in different ways. We used a few for illustration.

Some extensions of the proposed method can easily be entertained such as, for example, models with more elaborate covariance structures, including data stemming from designs with a higher number of stages. For other types of data, such as binary or count data, a generalized linear mixed model approach can be implemented using the SAS procedures GLIMMIX and NLMIXED. In cases where not only the standard deviations are known, but also the covariance between responses within the same subject, such numerical values can be incorporated into the model by keeping the variance-covariance matrix fixed during the iterative fitting algorithm. Finally, these methods can be applied using a hierarchical Bayesian modelling approach. We refer to Van Houwelingen et al. (2002) for further details.

7 Appendix: SAS code

In the example code below, the dataset META contains the following variables: VOL = subject number, ID = observation number, LOGI25 = log I_{25}, DOSE = dose of the investigational compound, PER1-PER3 = indicator variables for treatment periods, EST = variance of LOGI25, and INVVAR = 1/EST.

7.1 Standard Linear Mixed Model

```
proc mixed covtest data=meta;
  class id dose;
  model logI25 = dose / noint s;
  random int / type=un sub=id;
run;
```

7.2 Meta-analytic Weighted Approach

```
proc mixed covtest data=meta ic;
  class id vol dose;
  weight invvar;
  model logI25 = dose / noint s df=1000;
  random int / type=un sub=vol;
  random int / type=un sub=id;
  repeated   / sub=id;
  parms (0.1) (0.1) (1)/ hold=(3);
run;
```

7.3 Meta-analytic Univariate Approach

```
data add;
 est=0; output;
 est=0; output;
run;

data covvar;
 set add meta;
run;

proc mixed data=meta ic;
 class id vol dose;
 model logI25 =  dose / noint s df=1000;
 random int / sub=vol;
 random int / sub=id;
 repeated   / group=id type=vc;
 parms      / parmsdata=covvar eqcons=3 to 81;
run;
```

7.4 Meta-analytic Multivariate Approach

```
data add;
 do j=1 to 6;
    est=0;
    output;
 end;
run;

data covvar;
 set add meta;
```

```
run;

proc mixed data=meta ic;
 class id vol dose;
 model logI25 =  dose  / noint cl df= 1000;
 random per1 per2 per3 / sub=vol type=un;
 repeated / group=id type=un(1);
 parms    / parmsdata=covvar eqcons=7 to 81;
run;
```

Acknowledgments

We greatefully acknowledge support from Inter university Attraction Poles Program P5/24-Belgian State-Federal Office for Scientific, Technical and Cultural Affairs.

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Table 1: *Results of the fit of the different models*

Parameter†	Standard Linear Mixed Model	Weighted by $1/sd^2$	Fully corrected		
			VC	UN(1)	UN
β_0	0.8060 (0.1726)	0.8031 (0.1727)	0.8031 (0.1727)	0.8014 (0.1698)	0.7576 (0.1703)
β_1	1.1241 (0.3144)	1.1345 (0.3165)	1.1345 (0.3165)	1.4473 (0.3286)	1.1913 (0.3050)
β_2	1.8903 (0.3418)	1.8872 (0.3418)	1.8872 (0.3418)	1.9408 (0.3578)	1.8983 (0.3289)
β_3	2.1737 (0.3144)	2.1791 (0.3129)	2.1791 (0.3129)	2.3367 (0.3529)	2.0289 (0.2883)
β_4	2.6000 (0.3418)	2.6161 (0.3405)	2.6161 (0.3405)	2.1825 (0.3871)	2.9506 (0.3119)
β_5	2.2061 (0.3418)	2.1906 (0.3459)	2.1906 (0.3459)	1.9831 (0.3616)	2.1579 (0.3333)
β_6	2.5850 (0.3144)	2.5857 (0.3134)	2.5857 (0.3134)	2.6566 (0.3662)	2.4305 (0.2811)
β_7	2.7654 (0.3159)	2.7706 (0.3150)	2.7706 (0.3150)	2.6406 (0.3662)	2.8549 (0.2843)
β_8	3.4410 (0.3418)	3.4380 (0.3435)	3.4380 (0.3435)	3.4579 (0.3894)	3.4812 (0.3161)
β_9	3.6269 (0.3159)	3.6248 (0.3138)	3.6248 (0.3138)	3.6751 (0.3651)	3.7357 (0.2838)
σ^2_α	0.3512	0.3519	0.3519	-	-
σ^2_{11}	-	-	-	0.6205	0.6337
σ^2_{21}	-	-	-	0*	0.3450
σ^2_{22}	-	-	-	0.7341	0.8438
σ^2_{31}	-	-	-	0*	0.2094
σ^2_{32}	-	-	-	0*	0.6045
σ^2_{33}		-	-	0.7890	0.7740
σ^2_ε	0.3934	0.3742	0.3742		
AIC	175.6	175.6	175.6	188.7	177.8

† β_0-β_9 = estimated effects of doses of the investigational compound (placebo for subscript 0).

* The value was fixed due to the assumption of the covariance matrix.

− The value is not present in the model.

VC : variance component

UN(1) : banded

UN : unstructured

Table 2: *Results of the fit of the different models to the generated data.*

Parameter†	Standard Linear Mixed Model	Weighted by $1/sd^2$	Fully corrected	
			UN(1)	UN
β_0	1.0726 (0.1527)	1.0696 (0.1366)	1.0919 (0.1227)	1.1082 (0.1187)
β_1	1.2693 (0.1527)	1.2540 (0.1413)	1.2493 (0.1239)	1.2427 (0.1196)
β_2	1.2511 (0.1527)	1.3224 (0.1436)	1.4798 (0.1295)	1.5173 (0.1254)
AIC	198.21	191.62	188.29	191.64
$p_{1.0}$	0.333	0.321	0.366	0.414
$p_{2.0}$	0.379	0.177	0.029	0.015

† β_0, β_1 and β_2 are the estimated effects of doses of the investigational compound (β_0 corresponds to placebo).

$p_{1.0}$ p-value for the comparison of dose 1 to placebo.

$p_{2.0}$ p-value for the comparison of dose 2 to placebo.

UN(1) : diagonal

UN : unstructured