UMC Utrecht

Epidemiology and Big Data

Mixed Models part 3: Technical issues in multilevel/longitudinal modelling

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Overview Part 3: technical issues in mixed models

- Missing data & LME’s/CPM’s
- Choosing a model
  - LRT/AIC
  - REML vs ML estimation in mixed models
  - A model building strategy for MM
  - Testing random effects (variances)
  - Testing fixed effects
- Checking assumptions of the model
- Effect of centering explanatory variables
- Polynomials in linear mixed models
- More than 2 levels

Reisby example, revisited

- Design:
  - 66 patients, measured theoretically at 6 points in time
    - weeks 0-5
    - patients treated with imipramine from week 1 on
- Variables in dataset (long version):
  - id: pt identification, level 2
  - hdrs: Hamilton Depression Rating Scale, level 1
  - time: time since start of study in weeks, level 1
  - endo: endogenous (vs. “exogenous”) depression, level 2

- Research Question: is improvement in HDRS over time different for patients with endogenous and non-endogenous depression?

Example: Reisby Data

Descriptive Statistics (R)

```
> reisby.wide <- reshape(reisby.long, v.names="hdrs", idvar="id",
timevar="week", direction="wide")
> by(reisby.wide[,3:8], reisby.wide$endo, describe)
endo: 0
       var  n  mean   sd median
hdrs.0   1 28 22.79 4.12   22.0
hdrs.1   2 29 20.48 3.83   21.0
hdrs.2   3 28 17.00 4.35   16.5
hdrs.3   4 29 15.34 6.17   16.0
hdrs.4   5 29 12.62 6.72   12.0
hdrs.5   6 27 11.22 6.34   11.0
------------------------------------
endo: 1
       var  n  mean   sd median
hdrs.0   1 33 24.00 4.85   24.0
hdrs.1   2 34 23.00 5.10   22.0
hdrs.2   3 37 19.30 6.08   18.0
hdrs.3   4 36 17.28 6.56   16.5
hdrs.4   5 34 14.47 7.17   14.0
hdrs.5   6 31 12.58 7.96   11.0
```

Missing data & LME's/CPM's

Reisby example

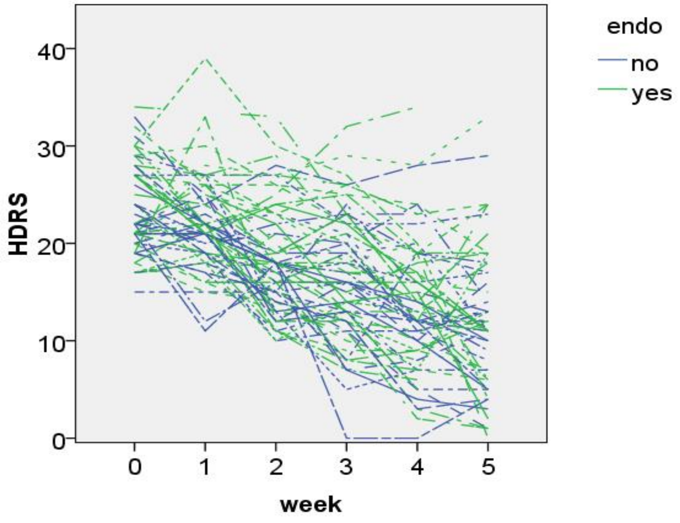
- Some patients have missing *outcomes*
- Nobody has missing *explanatory variables* (here: time & endo)
- LME's & CPM's are maximum likelihood-based methods
  - valid inference in the case of outcomes that are MCAR & MAR
  - MNAR still a problem
- Missing explanatory variables: trouble!
  - Entire level-2 units (patients here) drop out of analysis if level-2 explanatory variables are missing (ex: endo)
  - Individual level-1 observations (repeated measures in this case) drop out if level-1 explanatory variables missing
- Multilevel & longitudinal imputation in MICE is dicey
- JointAI package for imputation of longitudinal data

Reisby example, revisited

Hamilton Depression Rating Score on 66 patients measured at 6 time points, correlations

```
       hdrs.0 hdrs.1 hdrs.2 hdrs.3 hdrs.4 hdrs.5
hdrs.0  1.000  0.493  0.410  0.333  0.227  0.184
hdrs.1  0.493  1.000  0.494  0.412  0.308  0.218
hdrs.2  0.410  0.494  1.000  0.738  0.669  0.461
hdrs.3  0.333  0.412  0.738  1.000  0.817  0.568
hdrs.4  0.227  0.308  0.669  0.817  1.000  0.654
hdrs.5  0.184  0.218  0.461  0.568  0.654  1.000
```

Reisby example, revisited



Reisby example, revisited

- We talked about several logical models to fit the variance-covariance matrix of the HDRS scores over time…
  - CPM with heterogeneous AR(1) correlation structure
  - CPM with unstructured correlation structure
  - LME with random intercept + slope (for time)
- …and several less logical models:
  - CPM with identity correlation structure
  - CPM with compound symmetry/LME with random intercept
  - CPM with homogeneous AR(1) correlation structure

Testing in Linear Mixed Models

- To decide which LMM fits the data best we can use likelihood-based methods:
  - Likelihood Ratio Test (LRT)
    - LRT can be used to test nested models (one is a special case of the other)
    - based on the χ^2-distribution
  - Akaikes Information Criterium (AIC)
    - combination of likelihood and # parameters used in the model (d.f.)
    - model with the lowest AIC (high likelihood with few parameters) is deemed best

(Restricted) Maximum Likelihood Estimation

- Mixed models: maximum likelihood used to estimate fixed regression coefficients and variances of random effects
  - likelihood quite complex, solved by iteration until convergence
- (Empirical Bayes methods used to estimate individual random effects)
- Problem with ML estimation:
  - variance parameters (residual variance, variance(s) of random effect(s)) biased downwards
- Solution: REstricted (or: REsidual) Maximum Likelihood (REML)
  - gives unbiased estimates of variance parameters
  - BUT: adjusts likelihood for number of covariates in model, so cannot be used to compare models that differ w.r.t. fixed parts of model

When to use ML, REML?

- Testing models that differ in variance components:
  - REML will give interpretable LRT, AIC
  - so will ML
- Testing models that differ in fixed effects:
  - only ML will give interpretable LRT, AIC
- Reporting results (esp if you include the random components):
  - use REML!

When to use ML, REML?

- Leading me to suggest the following model-building strategy:
  1. Start with full fixed model and (using ML estimation), select appropriate random part of model
  2. With the random part chosen, (using ML estimation) try to reduce fixed part of model
  3. Once you have your final model: run that model once more using REML; this is the model you present to your audience
- Testing random effect(s):
  - variance parameters are never <0
  - Likelihood ratio test (LRT): REML or ML for random effects: chi-square test, but divide p-value by 2
  - AIC also okay
- Testing fixed effect(s):
  - LRT (ML only!) for fixed effects: chi-square test, usual p-value
  - AIC (ML only!)

Model comparisons

AIC, likelihood ratio test

- AIC
  - Lower is better
  - Can be used to compare any models
- LRT
  - Used for *nested* models
  - Model with more parameters always “better” but…
  - Model with -2LL *significantly* lower is better
  - Model with -2LL not significantly different, but with fewer parameters is better

Reisby example, comparing correlation structures

- Four models with the same fixed structure (endo*week, 12 degrees of freedom) and different random parts.
- Compare these using ML-based methods (LRT and / or AIC):

Model	**# cov par**	**-2*logLike**	**AIC**
Identity	1	2388.027	2414.027
Comp Symm	2	2277.381	2305.381
Unstructured	21	2183.227	2249.227
AR(1) homogen	2	2221.847	2249.847
AR(1) heterogen	7	2207.462	2245.462

Reisby example, comparing correlation structures

- From the comparison of the AIC’s
  - Taking dependence into account greatly improves the model fit
  - Assuming equal variances and equal correlations is not a good option for these data
  - The parsimonious homog. AR(1) not worse than unstructured
  - The AR(1) with heterogeneous variances is best
- We could also have used LRTs (for nested models only), with corrected p-values:
  - Homogeneous vs heterogeneous AR(1) (for instance):
    - LRT = 2221.847 -2207.462 = 14.385 with 7-2= 5 df; p = 0.0133/2 = 0.0067
    - heterogeneous is significantly better than homogeneous AR(1)

Reisby example, comparing correlation structures

- We can also compare LMEs with *linear* time trend with one another:
  - fixed: time, endo, time*endo
  - random: intercept vs. int+slope for time

```
> anova(lme.ris, lme.ril)
        Model df      AIC      BIC    logLik   Test  L.Ratio p-value
lme.ris     1  8 2230.929 2262.345 -1107.465
lme.ril     2  6 2294.137 2317.699 -1141.069 1 vs 2 67.20798  <.0001
```

Model with rand int+slope is better than rand int only (LRT or AIC)

Reisby example, comparing correlation structures

- No LRT to compare LMEs with *linear* time trend to models with CPM
- But we can use AIC to compare these non-nested models

Model	**AIC**
CPM Unstructured	2249.227
CPM AR(1) heterogen	2245.462
LME rand int + slope	2230.929

Model with rand int+slope is best according to AIC

Reisby example, fixed part of the model

- Now we have the random structure, we'll look at the fixed part of the model
- Three possibilities:
  - only time
  - endo + time
  - endo*time (both main effects + interaction)
- We use ML estimation for testing the fixed part of the model

Reisby example, fixed part of the model

```
> lme2.ris<-update(lme.ris, fixed=hdrs ~ time+endo)
> lme3.ris<-update(lme.ris, fixed=hdrs ~ time)
> anova(lme.ris, lme2.ris, lme3.ris)

         Model df      AIC      BIC    logLik   Test  L.Ratio p-value
lme.ris      1  8 2230.929 2262.345 -1107.465
lme2.ris     2  7 2228.933 2256.422 -1107.467 1 vs 2 0.004160  0.9486
lme3.ris     3  6 2231.037 2254.599 -1109.519 2 vs 3 4.104108  0.0428
```

- interaction not significant: no evidence that time effect differs for the groups
- effect of endo (just) significant: evidence for (small) difference between depression scores of people with and without endogenous depression

Reisby example, final model with REML

```
> lme3.ris.reml <- update(lme2.ris.CAR1, method="REML")
> summary(lme2.ris.reml)
Linear mixed-effects model fit by REML
 Data: reisby.long
       AIC      BIC    logLik
  2228.116 2255.548 -1107.058

Random effects:
 Formula: ~time | id
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev   Corr
(Intercept) 3.490342 (Intr)
time        1.457808 -0.287
Residual    3.494719
```

Reisby example, final model (cont.)

```
Fixed effects: hdrs ~ time + endo
               Value Std.Error  DF    t-value p-value
(Intercept) 22.492881 0.7598098 308   29.603306   0.0000
time        -2.380472 0.2103154 308 -11.318581   0.0000
endo         1.956867 0.9658720  64    2.026011   0.0469
 Correlation:
     (Intr) time
time -0.318
endo -0.704 -0.008

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.73520482 -0.49503123  0.03559898  0.49317021  3.62063687

Number of Observations: 375
Number of Groups: 66
```

Note: 66*6 = 396, so we see here that there are missing data

Reisby example, final model (cont.)

```
> intervals(lme2.ris.reml)
Approximate 95% confidence intervals

 Fixed effects:
                 lower       est.      upper
(Intercept) 20.99780674 22.492881 23.987956
time        -2.79430883 -2.380472 -1.966635
endo         0.02731607  1.956867  3.886418
attr(,"label")
[1] "Fixed effects:"

 Random Effects:
  Level: id
                          lower        est.       upper
sd((Intercept))       2.6340279  3.4903416 4.62503996
sd(time)              1.1419605  1.4578084 1.86101482
cor((Intercept),time) -0.5695496 -0.2870567 0.05608577

 Within-group standard error:
   lower     est.    upper
3.194723 3.494719 3.822884
```

“Clinical” conclusions imipramine example

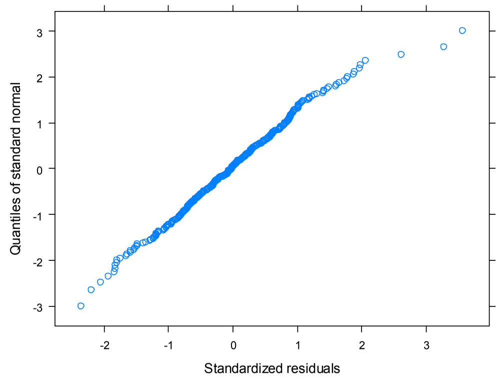
- There was no significant interaction between time and group
  - ~~time trends same for patients with endogenous and exogenous depression: the lines run parallel~~ No evidence for differing time trends between patients with endogenous and exogenous depression
- There was a significant main effect for group
  - at any given point in time, patients with endogenous depression have HDRS scores on average 1.96 (95% CI: 0.03 – 3.89) points higher than those without
- The effect of time is statistically significant
  - For patients with both endogenous and exogenous depression, HDRS scores decrease, on average, by 2.4 (95% CI: 2.0 - 2.8) points per week
  - On average 5*2.33 = 11.9 points in the course of the study
  - Am & Eur guidelines suggest that a 3-point change is clinically relevant
- Before presenting these results, we need to check our model assumptions!

Checking assumptions of the model

- Model assumptions:
  - linearity (if we use time – or other covariates – as linear)
    - check with individual plots, spaghetti plots, residual plots
  - normality of residuals
  - normality of random intercepts (& slopes, if used)
    - these three can be saved and checked using Q-Q plots, boxplots, histograms
    - but: generally not helpful
      1. because deviations from normality probably not a big problem for inference on fixed effects (if your interest is in inference on random effects, there could be a problem)
      2. model ‘inflicts’ normality on the random effects, so normality of the estimated random effects may partly reflect model assumptions
  - independence of residuals (once fixed and random effects are taken into account)
    - as in linear models: keep your fingers crossed!

Checking assumptions of the model in R

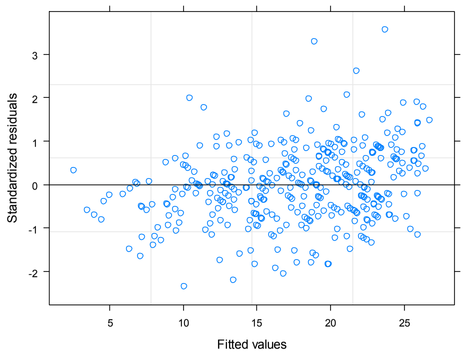
Diagnostic plots for (level-1) residuals: Q-Q plot of residuals



- Aside from three outliers, no departures from normality

Checking assumptions of the model in R

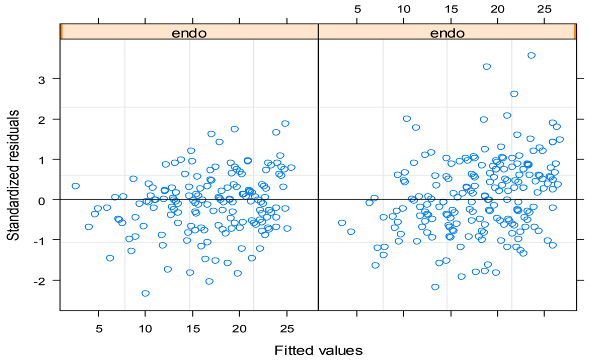
Diagnostic plots for (level-1) residuals: residuals vs. fitted



- (We're hoping for a graph with no patterns)
- This looks a bit problematic: a slight trend towards higher residuals with higher fitted values
- Problems with linearity and/or missing covariates?

Checking assumptions of the model in R

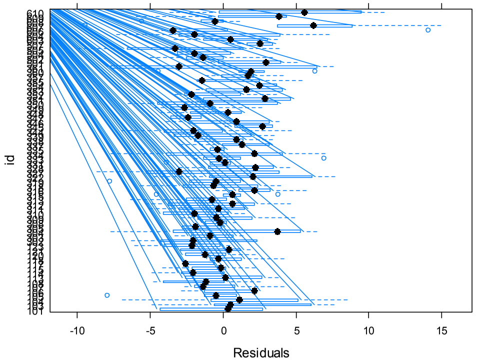
Diagnostic plots for (level-1) residuals: residuals vs. fitted by endo



The problems we saw in the whole sample are present in both groups

Checking assumptions of the model in R

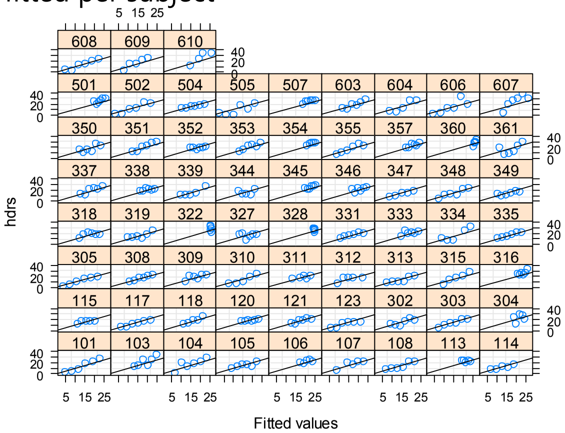
Boxplots residuals per subject



We can use this plot to check for large outliers in residuals per person

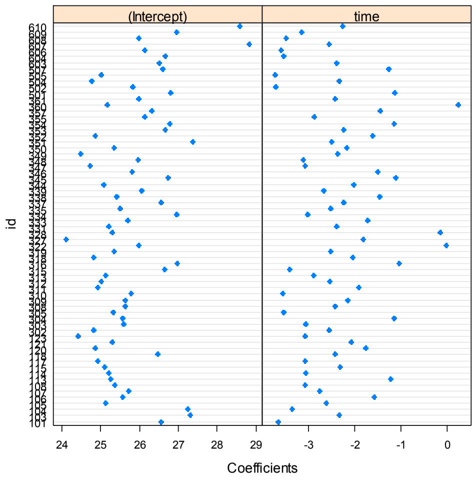
Checking assumptions of the model in R

Observed vs. fitted per subject



This plot can be used to check for individuals with poor agreement between observed and fitted HDRS scores.

Checking assumptions of the model in R



- Quick plot of random intercepts and slopes for time
- (We're not looking for patterns here, just for large outliers)
- No obvious outliers, though a few high-ish slopes

Statistical conclusions imipramine example

- A model with fixed linear time effect and a fixed effect for group, random intercept and random slope for time for the within subject residuals seems to provide the "best" fit for these data
- The assumptions of normality for the level-1 and level-2 random effects seem reasonable
- The assumption of constant variance of residuals (given the random effects) might be violated

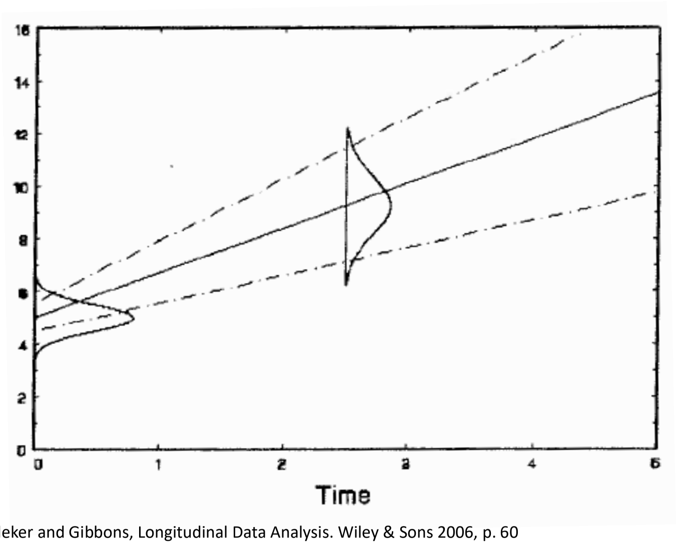
➢ Now we may present our results (with caution)

Centering explanatory variables

- In the London schools dataset, both the outcome and the intake test had been "centered" (actually, both were standardized)
- In the Fox/Weisburg example, age was transformed to age-8

- What is the effect of centering an explanatory variable?
  - changes the interpretation of the fixed intercept
  - can change the variance of the random intercepts, and the correlation of random intercepts with random slopes

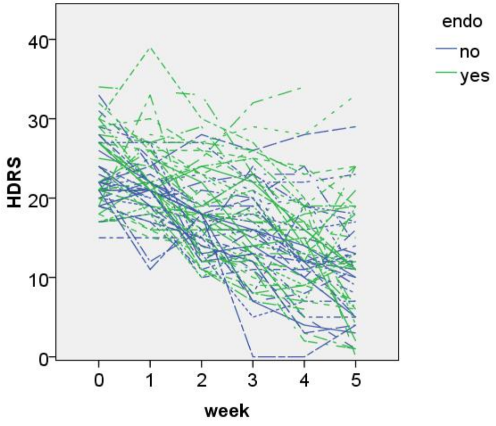
Centering explanatory variables



Source: Hedeker and Gibbons, Longitudinal Data Analysis. Wiley & Sons 2006, p. 60
https://www.wiley.com/en-nl/Longitudinal+Data+Analysis-p-9780471420279
Inclusion is permitted according to the agreement with the publisher.

Centering explanatory variables

- Take Reisby data, center time (week 2.5 becomes 0 point)
  - for sake of simplicity, using model with just fixed effect of time, random effects for intercept and time



Centering explanatory variables

Parameter estimate	Model 1 (time not centered)	Model 2 (time centered)
Fixed: intercept	23.58	17.63
Fixed: time	-2.38	-2.38
Random: intercept (s.d.)	3.60	4.34
Random: slope of time (s.d.)	1.46	1.46
Random: corr (int-slope)	-0.28	0.61
Residual (s.d.)	3.49	3.49

Estimates of intercept, variation of random intercepts and correlation rand int-slope all changed!

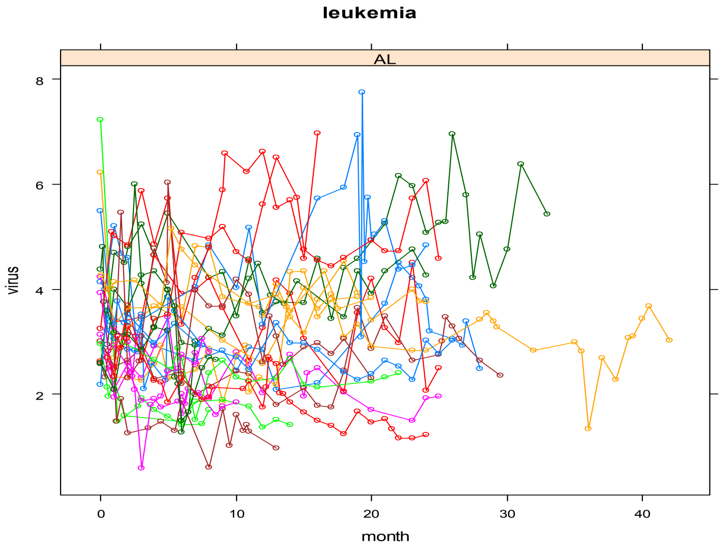
Linear mixed effects models with polynomial terms

- Instead of linear trends over time, it is quite possible to observe non-linear trends (think of children's growth, for instance)
- There are many non-linear models that can be used within mixed models (beyond the scope of this course)
- It is possible to fit polynomials as part of a "linear" mixed model

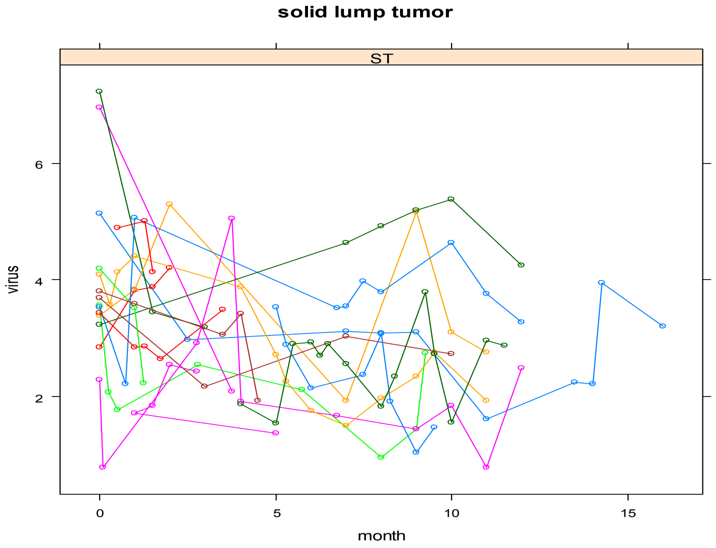
Example: Herpes Antibody Levels

- 45 children suffering from
  - solid lump tumour (N=18)
  - leukemia (N=27)
- Measurements of antibody levels to a herpes virus taken during hospital visits for courses of chemotherapy
- Duration: 1 mo - 3 yrs (median 12 mo)
- Intervals between measurements differed per child
- Questions:
  - are antibody levels affected by chemo?
  - if so, is change related to cancer type?

Linear mixed effects models with polynomial terms



Linear mixed effects models with polynomial terms



Linear mixed effects models with polynomial terms

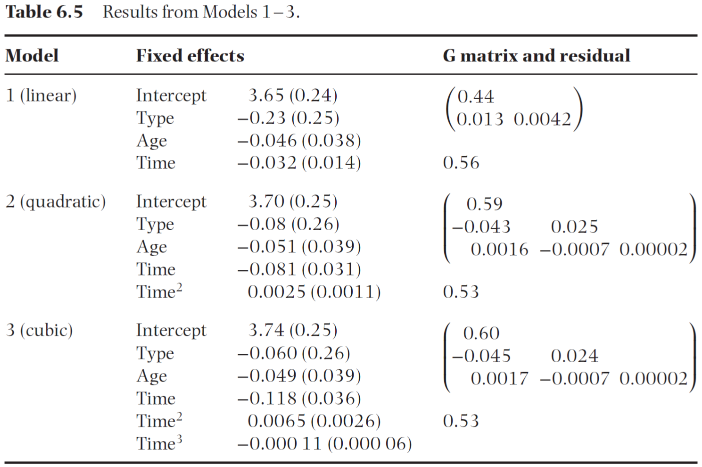


Table 6.5 Results from Models 1 – 3.

Model	Fixed effects		G matrix and residual
1 (linear)	Intercept	3.65 (0.24)	$\begin{pmatrix} 0.44 & \\ 0.013 & 0.0042 \end{pmatrix}$
	Type	−0.23 (0.25)	
	Age	−0.046 (0.038)	
	Time	−0.032 (0.014)	0.56
2 (quadratic)	Intercept	3.70 (0.25)	$\begin{pmatrix} 0.59 & & \\ -0.043 & 0.025 & \\ 0.0016 & -0.0007 & 0.00002 \end{pmatrix}$
	Type	−0.08 (0.26)	
	Age	−0.051 (0.039)	
	Time	−0.081 (0.031)	
	Time^2	0.0025 (0.0011)	0.53
3 (cubic)	Intercept	3.74 (0.25)	$\begin{pmatrix} 0.60 & & \\ -0.045 & 0.024 & \\ 0.0017 & -0.0007 & 0.00002 \end{pmatrix}$
	Type	−0.060 (0.26)	
	Age	−0.049 (0.039)	
	Time	−0.118 (0.036)	
	Time^2	0.0065 (0.0026)	0.53
	Time^3	−0.000 11 (0.000 06)	

Source: Brown & Prescott, Applied Mixed Models in Medicine, 3rd Edition. Wiley, 2015, p. 272

Note: their "G matrix" is our $\boldsymbol{\Sigma}_{\upsilon}$

Other possibilities for nonlinear trends

- Orthogonal polynomials
- Natural cubic splines
- Nonlinear mixed models

Three-level models

- So far: two levels
  - children within schools, patients within hospitals
  - measurements within individuals over time
- What about three levels?
  - children within classrooms within schools
  - longitudinal measurements within patients within hospitals

Analyzing three-level models

- Variance at 3 levels
  - random effects (which??) at 2 levels
- Variables measured at 3 levels?
  - main effects
  - “cross-level” interactions (SES of school * SES of child, gender of teacher * gender of child)
- Think carefully about design
  - possible sources of variation
  - effects at lower level that could possibly differ at higher level
    - teacher-level variables (gender, experience) could have different effects at different schools
    - child-level variables (gender, entrance exam score) could have different effects in different classrooms or at different schools
- Think about research question: simplicity vs generalizability

Example three-level data

- Lab 1: multi-center hypertension trial: 27 centers, 193 patients, 4 post-randomization DBP
- Sources of variation:
  - centers:
    - may serve different populations, with (on average) higher or lower BP
  - patients:
    - patients vary greatly in their blood pressure levels
      - – age, gender, baseline BMI, treatment
    - patients may vary (greatly?) in trend over time
  - measurements in time:
    - BP varies considerably from moment to moment, day to day within individuals
      - – stress level, tx adherence, BMI at the moment

Summary technical issues MM

- Model building better done in protocol
- Otherwise: use LRT or AIC to build random part of model, then to simplify fixed part of model
- Use ML estimation for likelihood-based tests
- Use REML estimation for presenting results
- Some model assumptions (linearity, normality of res) can be checked
- Centering explanatory variables has effect on interpretation of several parameters
- “Linear” mixed models may also include polynomials
- 3+ levels also possible (complicated, but possible)

Example three-level data

- Design:
  - randomized trial, so interest in tx & tx*time
  - hospital-level variables: none provided
  - patient-level variables: treatment
  - measurement (time)-level variables: none provided
- Fixed effects:
  - (intercept,) time (linear or categorical?), tx, tx*time
  - baseline DBP (why?)
- Random effects?
  - differences in avg DBP among centers → random intercept per center
  - difference in tx effect per center? → random tx per center (tricky!!)
  - difference in time trend per center? → random time trend per center
  - differences among patients → random intercept (& time trend) per patient