Multiple Treatments on the Same Experimental Unit

Lukas Meier (most material based on lecture notes and slides from H.R. Roth)

Introduction

- We learned that **blocking** is a very helpful technique to reduce variance.
- In the intro lecture we had a look at the example of **blood plasma**

Patient ID	Treatment			
	A	***B***	***C***	***D***
1	8.4	9.4	9.8	12.2
2	12.8	15.2	12.9	14.4
3	9.6	9.1	11.2	9.8
4	9.8	8.8	9.9	12.0
5	8.4	8.2	8.5	8.5
6	8.6	9.9	9.8	10.9
7	8.9	9.0	9.2	10.4
8	7.9	8.1	8.2	10.0

- There, we **blocked on patient** and we were able to apply **all** the 4 treatments in **parallel** (as a blood sample can be split up into 4 parts).

Blood Plasma: Analysis

- The analysis of the blood plasma is straightforward.
- We **block** on patient by using a (random) block factor.
- Residual analysis: Transform response (see the corresponding R-file).
- Output

```
> fit <- lmer(1 / time ~ group + (1 | person), data = blood)
> anova(fit)
Analysis of Variance Table of type III  with  Satterthwaite
approximation for degrees of freedom
         Sum Sq    Mean Sq NumDF  DenDF F.value    Pr(>F)
group 0.0013019 0.00043397     3 21.006   7.961 0.0009841 ***
```

- A parallel application of treatments is of course **not** always possible: think for example of an experiment with 4 different pills.

Example: Mathematical Test

Another example was the time to solve 4 mathematical problems, where we had a control and a treatment group.

Control Group				
	Problem			
Person	*1*	*2*	*3*	*4*
C1	43	90	51	67
C2	87	36	12	14
C3	18	56	22	68
C4	34	73	34	87
C5	81	55	29	54
C6	45	58	62	44
C7	16	35	71	37
C8	43	47	87	27
C9	22	91	37	78

Extra Training				
	Problem			
Person	*1*	*2*	*3*	*4*
E1	10	81	43	33
E2	58	84	35	43
E3	26	49	55	84
E4	18	30	49	44
E5	13	14	25	45
E6	12	8	40	48
E7	9	55	10	30
E8	31	45	9	66

Example: Mathematical Test

- As we do **not** have the ordering in which the tests were performed we analyze this data with an ordinary **split-plot model**.
- Persons are **whole-plots**, time-slots are **split-plots**.
- Again, analysis can be performed with `lmer`.

```
> fit <- lmer(log(time) ~ group * problem + (1 | group:subject), data = math.long)
> anova(fit)
Analysis of Variance Table of type III  with  Satterthwaite
approximation for degrees of freedom
              Sum Sq Mean Sq NumDF DenDF F.value  Pr(>F)
group         2.0013 2.00128     1    15  5.7428 0.03004 *
problem       3.9907 1.33022     3    45  3.8171 0.01609 *
group:problem 1.1160 0.37202     3    45  1.0675 0.37237
```

- Conclusions?
- Ideally, we would also use the **sequence** in which problems were solved in our model as this would increase efficiency (remove variance).

Crossover Trials

- If we cannot apply the treatments in parallel we can use a **sequence** of **treatments** for each patient.
- Typically, we allow some time between the different treatments (= **washout-period**).
- This is called a **crossover trial** (D: “Wechselversuch”)
- When planning such a study we have to be (very) careful because of (new) effects.
  - **period effects** (learning, fatigue, …): very common.
  - **carry-over effects** = effect of the treatment of **previous** treatment that we see in the current time-period (can be reduced by using a long enough washout period).
- Assume we only have two treatments A and B: Why is it a bad idea to give everyone first treatment A and then treatment B?

Short Comparison of Designs

Treatment application	*Grouping of subjects*	*Design*
Parallel[1)]	no yes	Block design Split-plot design (person = whole-plot)
Sequential	yes or no	Crossover trial

[1)] or sequential but without considering the sequence

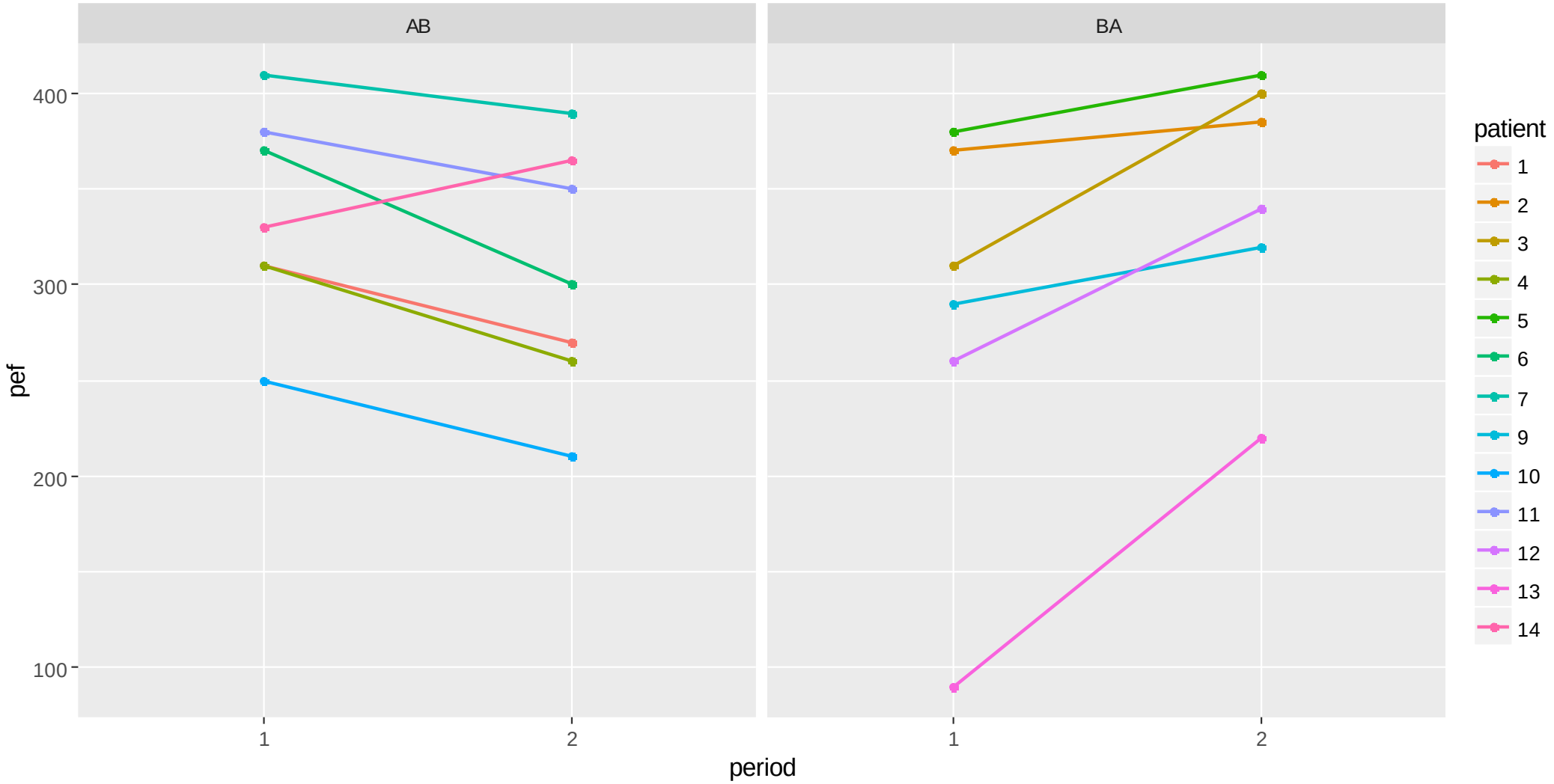
Example: Peak Expiratory Flow

- Measure **peak expiratory flow** (PEF) [l/min] 8 hours after treatment with
  - Formoterol (A)
  - Falbutamol (B)
- 7 patients get treatment sequence $A \rightarrow B$,
- 6 patients get treatment sequence $B \rightarrow A$.
- Hence, we have two possible sequences: AB and BA

	Period 1	***Period 2***
Sequence AB	A	B
Sequence BA	B	A

- Patients got allocated **randomly** to one of the two sequences. 
- This is a so called ***AB / BA* crossover trial**.

Example: Peak Expiratory Flow



AB / BA Crossover Trial

- We want a model that includes (see also lecture notes)
  - Treatment effects
  - Period effects
  - Carry-over effects
  - Effects for individual patient levels
  - Error terms
- Expected cell means according to terms above:

	Period 1	***Period 2***
Sequence AB	$\mu + \pi_1 + \alpha_A$	$\mu + \pi_2 + \alpha_B + \lambda_A$
Sequence BA	$\mu + \pi_1 + \alpha_B$	$\mu + \pi_2 + \alpha_A + \lambda_B$

where

- α_A, α_B are **treatment effects**,
- λ_A, λ_B are **carry-over effects** and
- π_1, π_2 are **period effects**

with the usual **sum-to-zero side constraints**.

Example: Peak Expiratory Flow

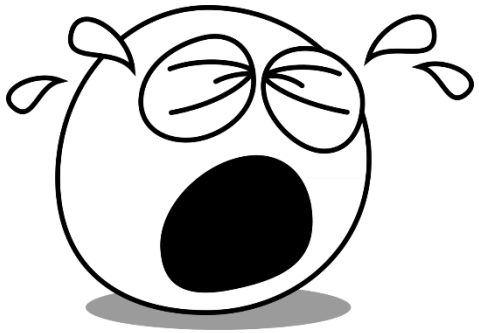
- Both **subjects** and **periods** act as block factors.
- For every subject (period) we have a **complete block design** for the treatment effects.
- Typically, we just want to control for these block factors (but not do tests etc.).
- For every sequence we have an **incomplete block design** for the carry-over effects.

AB / BA Crossover Trial: Parameter Estimation

- **Treatment effect $\alpha_B - \alpha_A$**
  - Per patient in AB: Calculate difference $B - A$ and take average over all patients in this group.
  - Per patient in BA: Calculate difference $B - A$ and take average over all patients in this group.
  - Take average of these two numbers.
  - Expected value of estimator : $\alpha_B - \alpha_A + 0.5 \cdot (\lambda_A - \lambda_B)$
- **Period effect $\pi_2 - \pi_1$**
  - As above but with difference (period 2) – (period 1) per patient
  - Expected value of estimator: $\pi_2 - \pi_1$
- **Carryover effect $\lambda_A - \lambda_B$**
  - As above but with sum per patient
  - Expected value of estimator: $\lambda_A - \lambda_B$
- Note: **Treatment effect contains part of carry-over effect** in this design. **This is a problem if the carry-over effect is non-zero**.
- We could do appropriate **two sample *t*-tests** using the reasoning above to perform statistical tests.

AB / BA Crossover Trial

- According to Jones & K. (2015)
- Notation: τ instead of α.
- Carry-over, interaction and sequence effects **cannot be distinguished** in this design!



sequence effects

To illustrate this aliasing, consider writing the fixed effects in the full model as given below:

Group	Period 1	Period 2
1 (AB)	$\mu+\pi_1+\tau_1+(\tau\pi)_{11}$	$\mu+\pi_2+\tau_2+(\tau\pi)_{22}$
2 (BA)	$\mu+\pi_1+\tau_2+(\tau\pi)_{21}$	$\mu+\pi_2+\tau_1+(\tau\pi)_{12}$

Here $(\tau\pi)_{ij}$ is the interaction parameter associated with treatment i and period j and allows the model to account for a treatment effect that is not the same in each of the two periods. If the usual constraints $\pi_1+\pi_2=0$ and $\tau_1+\tau_2=0$ are applied to the parameters, and we set $\pi_1=-\pi$ and $\tau_1=-\tau$, the model can be written as

Group	Period 1	Period 2
1 (AB)	$\mu-\pi-\tau+(\tau\pi)_{11}$	$\mu+\pi+\tau+(\tau\pi)_{22}$
2 (BA)	$\mu-\pi+\tau+(\tau\pi)_{21}$	$\mu+\pi-\tau+(\tau\pi)_{12}$

If the usual constraints $(\tau\pi)_{11}+(\tau\pi)_{12}=0$, $(\tau\pi)_{21}+(\tau\pi)_{22}=0$, $(\tau\pi)_{11}+(\tau\pi)_{21}=0$ and $(\tau\pi)_{12}+(\tau\pi)_{22}=0$ are applied to the interaction parameters, and we set $(\tau\pi)_{11}=(\tau\pi)$, the model becomes

Group	Period 1	Period 2
1 (AB)	$\mu-\pi-\tau+(\tau\pi)$	$\mu+\pi+\tau+(\tau\pi)$
2 (BA)	$\mu-\pi+\tau-(\tau\pi)$	$\mu+\pi-\tau-(\tau\pi)$

Using these constraints therefore reveals the aliasing of the interaction and the group effects. If, however, we use the less familiar constraints $(\tau\pi)_{11}=0$, $(\tau\pi)_{21}=0$, $(\tau\pi)_{12}+(\tau\pi)_{22}=0$ and set $(\tau\pi)_{22}=-(\tau\pi)$, the model becomes

Group	Period 1	Period 2
1 (AB)	$\mu-\pi-\tau$	$\mu+\pi+\tau-(\tau\pi)$
2 (BA)	$\mu-\pi+\tau$	$\mu+\pi-\tau+(\tau\pi)$

That is, the interaction effects are now associated with the carry-over effects. (See Cox (1984) for further, related discussion.)

Example: Peak Expiratory Flow

- Hence, in the *AB / BA* crossover trial, the carry-over effect can be translated as a **sequence effect** or as a period-specific treatment effect (= **interaction**!).
- Model as a sequence effect:

```
> fit <- lmer(pef ~ treatment + period + sequence + (1 | patient), data = lung)
> anova(fit)
Analysis of Variance Table of type III  with  Satterthwaite
approximation for degrees of freedom
          Sum Sq Mean Sq NumDF  DenDF F.value   Pr(>F)
treatment 14035.9 14035.9     1 11.001 18.7044 0.001205 **
period     1632.1  1632.1     1 11.001  2.1749 0.168312
sequence     24.1    24.1     1 11.000  0.0321 0.861076
```

identical results!

- Model as an interaction effect:

```
> fit <- lmer(pef ~ treatment * period + (1 | patient), data = lung)
> anova(fit)
Analysis of Variance Table of type III  with  Satterthwaite
approximation for degrees of freedom
                 Sum Sq Mean Sq NumDF  DenDF F.value   Pr(>F)
treatment        14035.9 14035.9     1 10.999 18.7044 0.001205 **
period            1632.1  1632.1     1 10.999  2.1749 0.168316
treatment:period    24.1    24.1     1 11.000  0.0321 0.861076
```

AB / BA Crossover Trial: Conclusions

Although the design looks “nice” at first sight, the *AB / BA* crossover trial has severe drawbacks.

Statistical Methods in Medical Research 1994; **3**: 303—324

The AB/BA crossover: past, present and future?

Stephen Senn Ciba, Basle, Switzerland

The AB/BA design is reviewed from a historical perspective. Particular attention is paid to the problem of carry-over and various attempts to deal with it. The two-stage procedure, an approach which was popular for many years, is shown to be unsafe. The analysis of AB/BA designs with baseline data is also considered. It is shown that such baselines do not provide a cure for the problem of carry-over; and it is concluded that any rational analysis of such trials will always be dependent on assumptions regarding carry-over, and that it is necessary to pay particular attention to washout periods. Under such circumstances analysis of covariance may be useful. In conclusion, some speculative comments about future lines of research are offered.

ABB / BAA Crossover Trial

- A **better design for two treatments** is the following design based on **three periods**.
- Two possible sequences: ABB and BAA

	Period 1	Period 2	Period 3
Sequence ABB	A	B	B
Sequence BAA	B	A	A

- Patients get **randomly** allocated to one of the **two** sequences.
- The design is called **strongly balanced** (with respect to first-order carryover effects) because every treatment precedes every other treatment and itself **equally often**
- This results in some nice statistical properties.

ABB / BAA Crossover Trial

- Expected cell means for such a design are

	Period 1	*Period 2*	*Period 3*
Sequence ABB	$\mu + \pi_1 + \alpha_A$	$\mu + \pi_2 + \alpha_B + \lambda_A$	$\mu + \pi_3 + \alpha_B + \lambda_B$
Sequence BAA	$\mu + \pi_1 + \alpha_B$	$\mu + \pi_2 + \alpha_A + \lambda_B$	$\mu + \pi_3 + \alpha_A + \lambda_A$

- This design has the nice property that we can “untangle” the different treatment effects and the carry-over effects (without derivation).

ABB / BAA Crossover Trial: Analysis

- We use the “standard” ANOVA or mixed effects model approach to fit such models.
- Model formula typically looks as follows

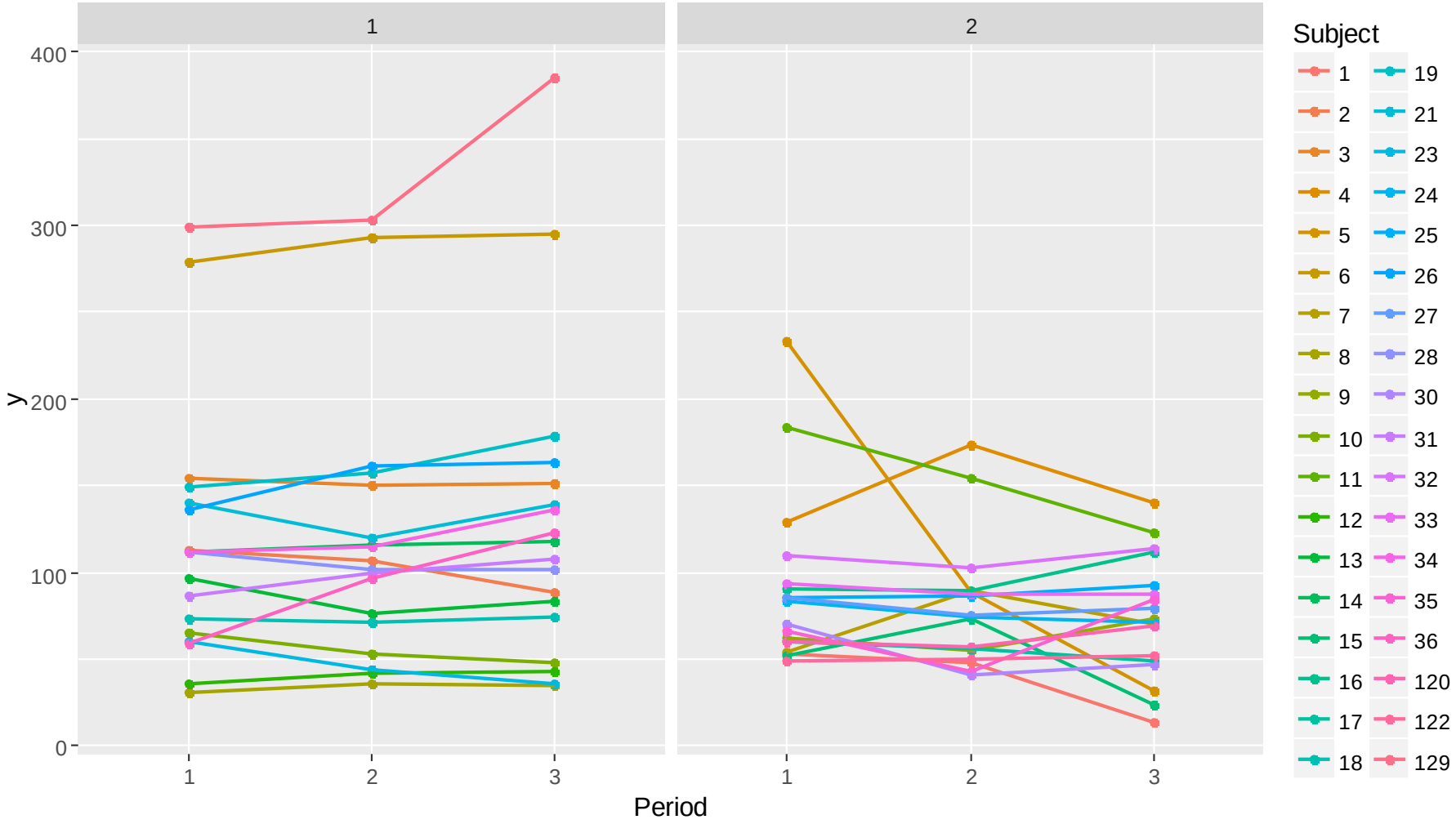
$$Y \sim \text{Period} + \text{Treatment} + \text{Carryover} + (1 \mid \text{Subject})$$

- This approach can of course also be used for **other designs with more than two periods**.
- The important “take-home message” is:
  - Adjust for period effects.
  - Use carry-over effect if needed.

Example Bioequivalence (Chi, 1994)

- Analyze the area under the concentration-time profile (AUC) of a bioequivalence study.
- Study design: **three-period crossover design** *ABB, BAA* with two different formulations A and B of a compound.
- The following plot shows that there are some special subjects which look like outliers (we will ignore this for the moment).

Example Bioequivalence



Example Bioequivalence

- We fit a suitable model with the following R-Code

```
> fit <- lmer(y ~ Period + Treat + Carry + (1 | Subject), data = bioequiv)
> anova(fit)
Analysis of Variance Table of type III  with  Satterthwaite
approximation for degrees of freedom
        Sum Sq Mean Sq NumDF  DenDF F.value  Pr(>F)
Period  929.54  464.77     2 67.972  0.8833 0.41810
Treat  2518.73 2518.73     1 68.739  4.7870 0.03208 *
Carry  1050.58 1050.58     1 67.972  1.9967 0.16221
```

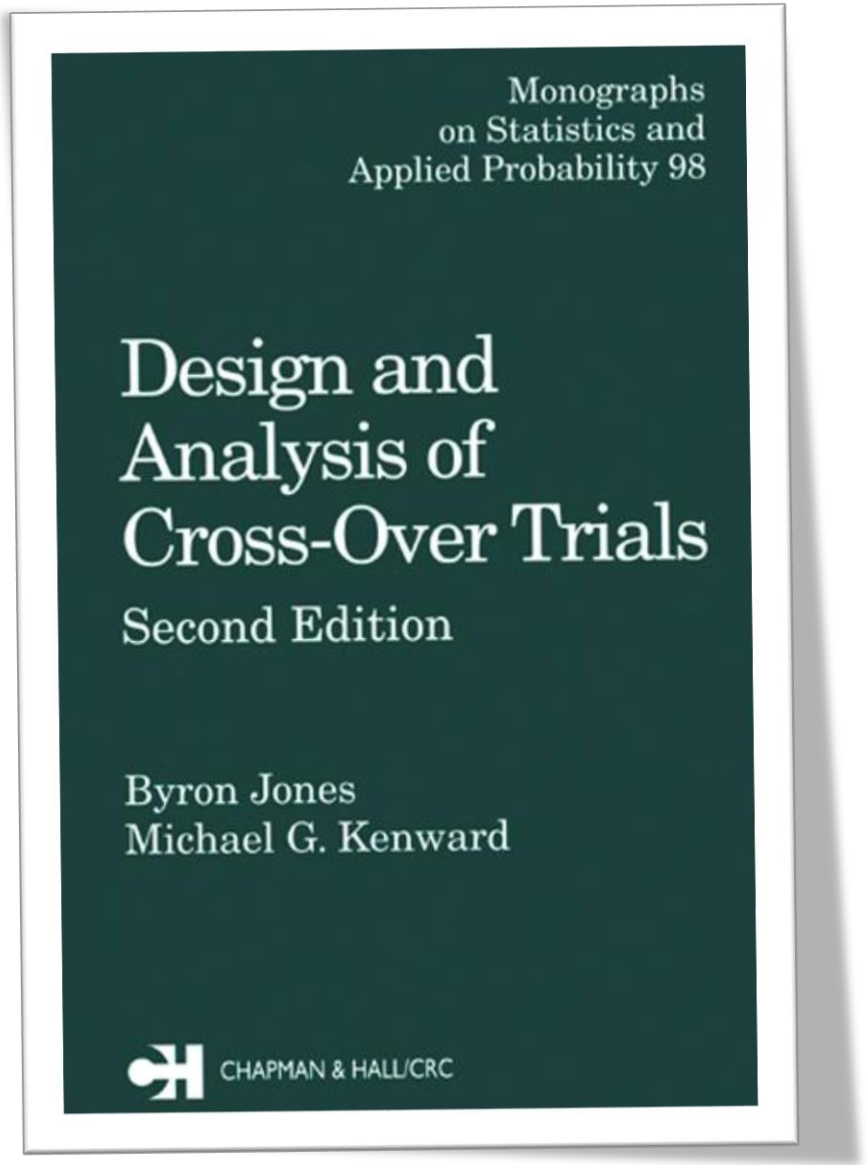
- The first period (without carry-over effect) needs some special initialization (see R-Code).

Disadvantages of Crossover Trials

Besides having many benefits, crossover trials also have disadvantages:

- Carry-over and/or period by treatment interaction.
- Increased burden on individual patient.
- Only possible in certain indications.
- Drop-outs and missing values may be more of a problem than for parallel group studies.
- Analysis is more complex.
- Unsuitable for drugs with non-reversible effects or very long half-lives.
- Interpretation of side-effects may be complex.

Further Reading



- Overview of different designs.
- R-package `Crossover` to design efficient experiments (incl. GUI).