Design and Analysis of 2x2 Cross-Over Trials with Continuous Data

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Introduction to Cross-Over Trials

The Cross-Over Design

Definition

A cross-over trial is a "trial in which subjects are given sequences of treatments with the object of studying differences between individual treatments" [1].

Randomisation

Only the *order* of treatments is randomised:

- Validity of treatment comparison does not depend on randomisation.
- Randomisation does not guarantee unbiased comparison of treatments.
- Treatment groups differ with respect to their recent exposure to potentially effective treatments.

Fundamental Issue of Cross-Over Design

The comparability of treatments is not guaranteed by the structure of the trial alone, but instead depends on the treatments themselves [2].

Advantages

- More observations per treatment [1]
- Data in terms of *difference to control*
- Improved recruitment rates
- Reduced spill-over rates [2]

Disadvantages

- Longer/inconvenient for subjects
- Complex analysis
- Cannot be used for infectious diseases
- Risk of drop-out
- Period-by-treatment interactions

Carryover

Definition

Carryover is the persistence of a treatment applied in one period in a subsequent period of treatment [1].

- Introduces bias to direct treatment effect estimates.
- Difficult to test and adjust for.
- Best solution is to introduce a wash-out period [1].

Summary and Visualisation of Cross-Over Trial Data

COPD Trial

- 2x2 cross-over design.
- Comparing effectiveness of an inhaled drug A against a placebo (B).
- Treatment administered twice daily to patients with chronic obstructive pulmonary disease (COPD).
- 66 patients randomised into either AB or BA sequence (complete data obtained on 56).
- Outcome measurement is *peak expiratory flow rate* (PEFR), measured 3 times per day (recording highest value).

Sample Data

Table 1: Subsample of COPD Trial Data (PEFR in L/min)

Sequence	Subject	Subject Label	Period 1	Period 2
AB	1	7	121.90	116.67
AB	2	8	218.50	200.50
AB	3	9	235.00	217.14
AB	4	13	250.00	196.43
AB	5	14	186.19	185.50

Summary Table

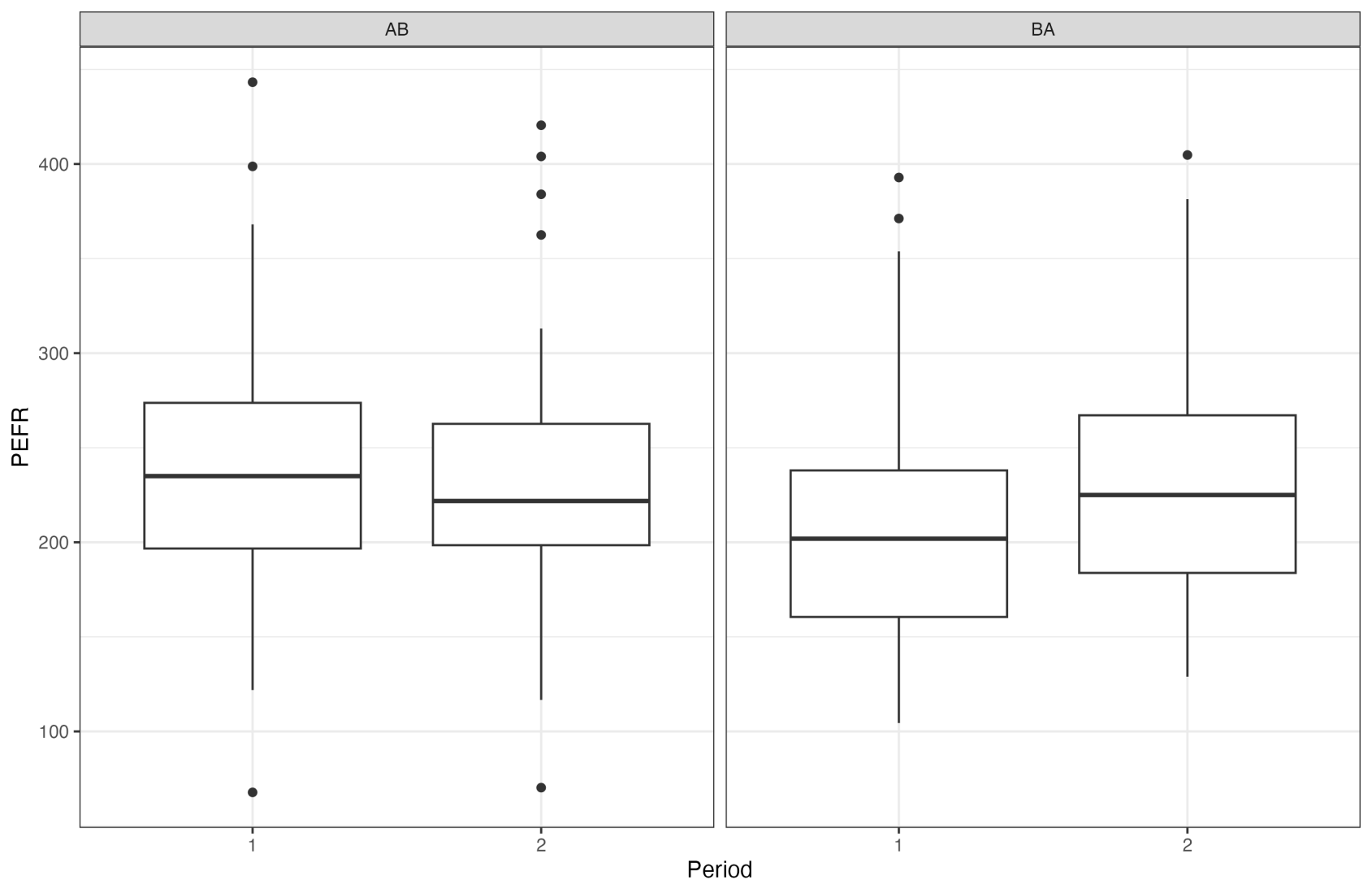
Table 2: Summary Table for COPD Trial Data

		Overall		Period 1		Period 2	
	Subjects	Mean	SD	Mean	SD	Mean	SD
AB	27	242.52	81.53	245.84	82.78	239.20	81.70
BA	29	223.08	72.99	215.99	72.63	230.16	73.94
Total	56	232.45	77.49	230.38	78.43	234.52	77.20

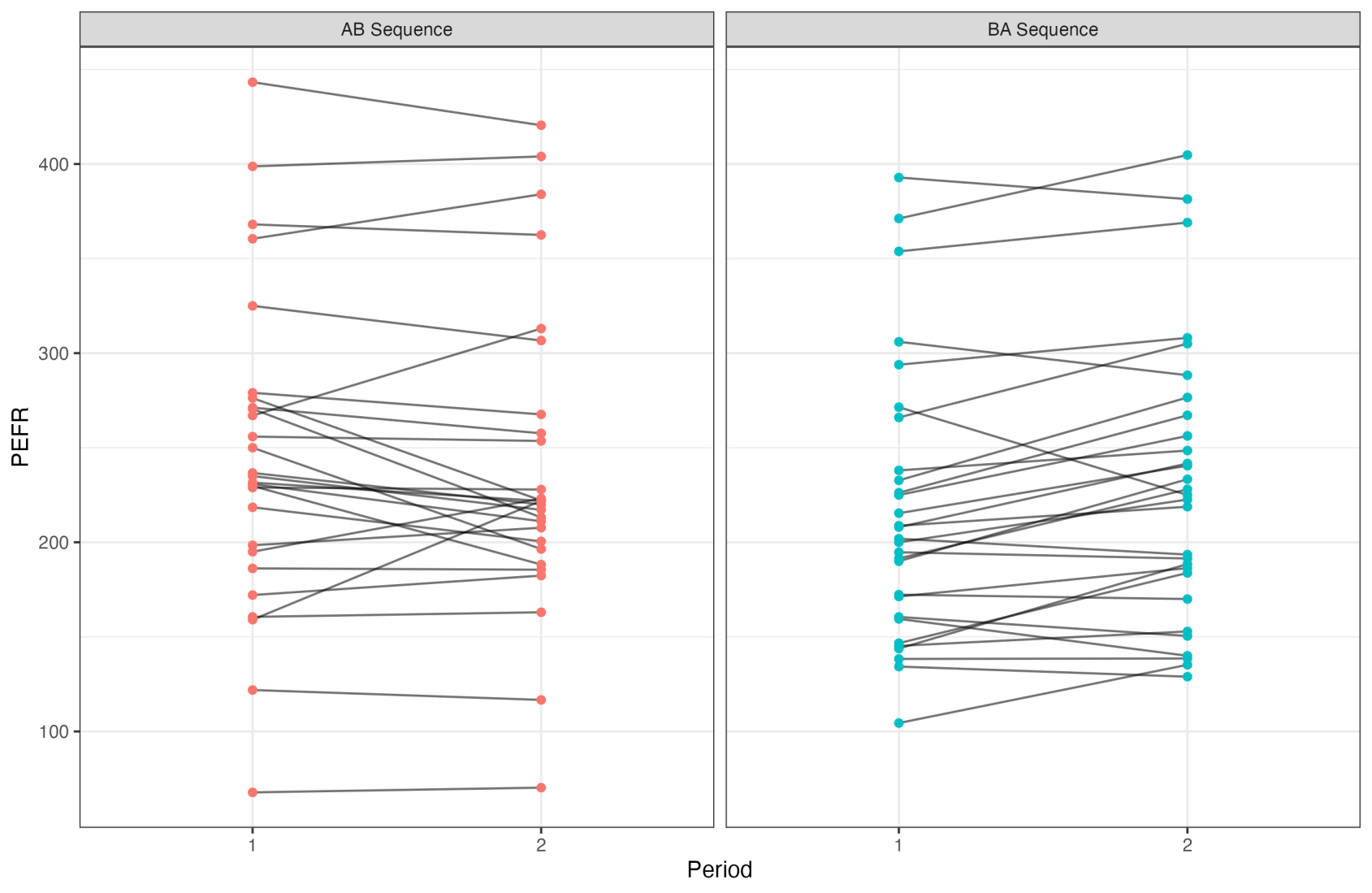
Summary and Visualisation of Cross-Over Trial Data

Summary Plots

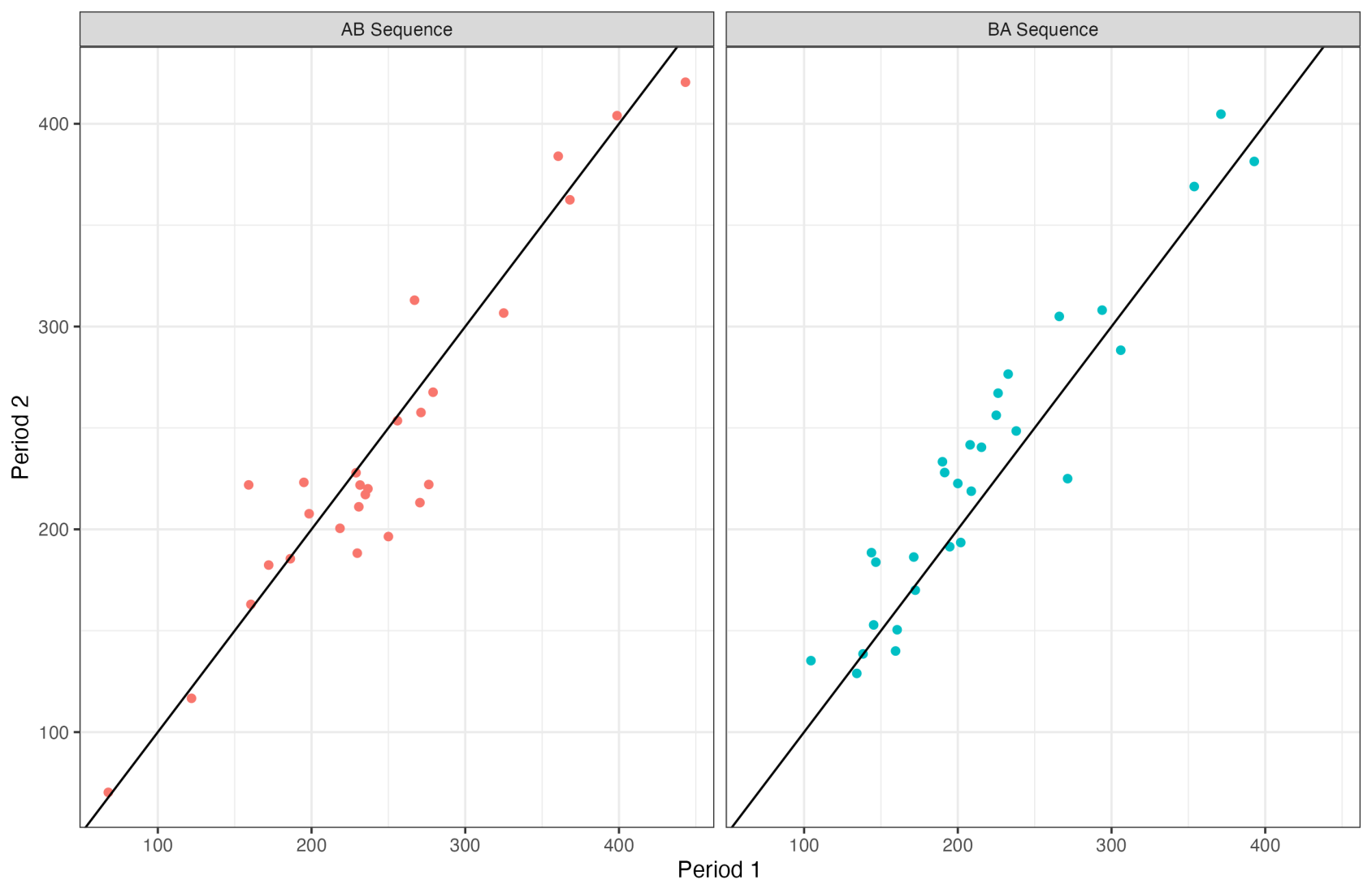
Boxplot



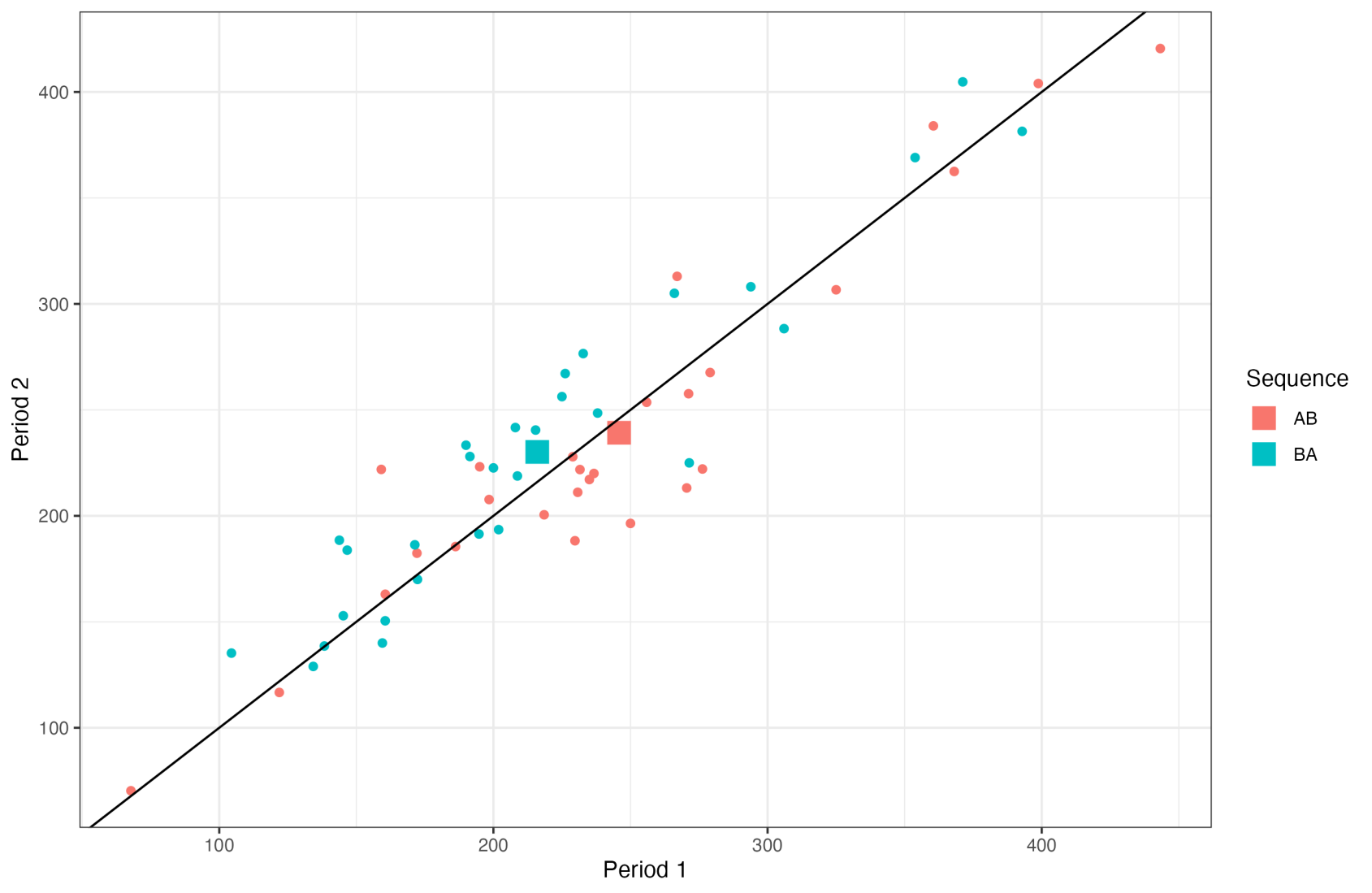
Subject-Profiles Plot



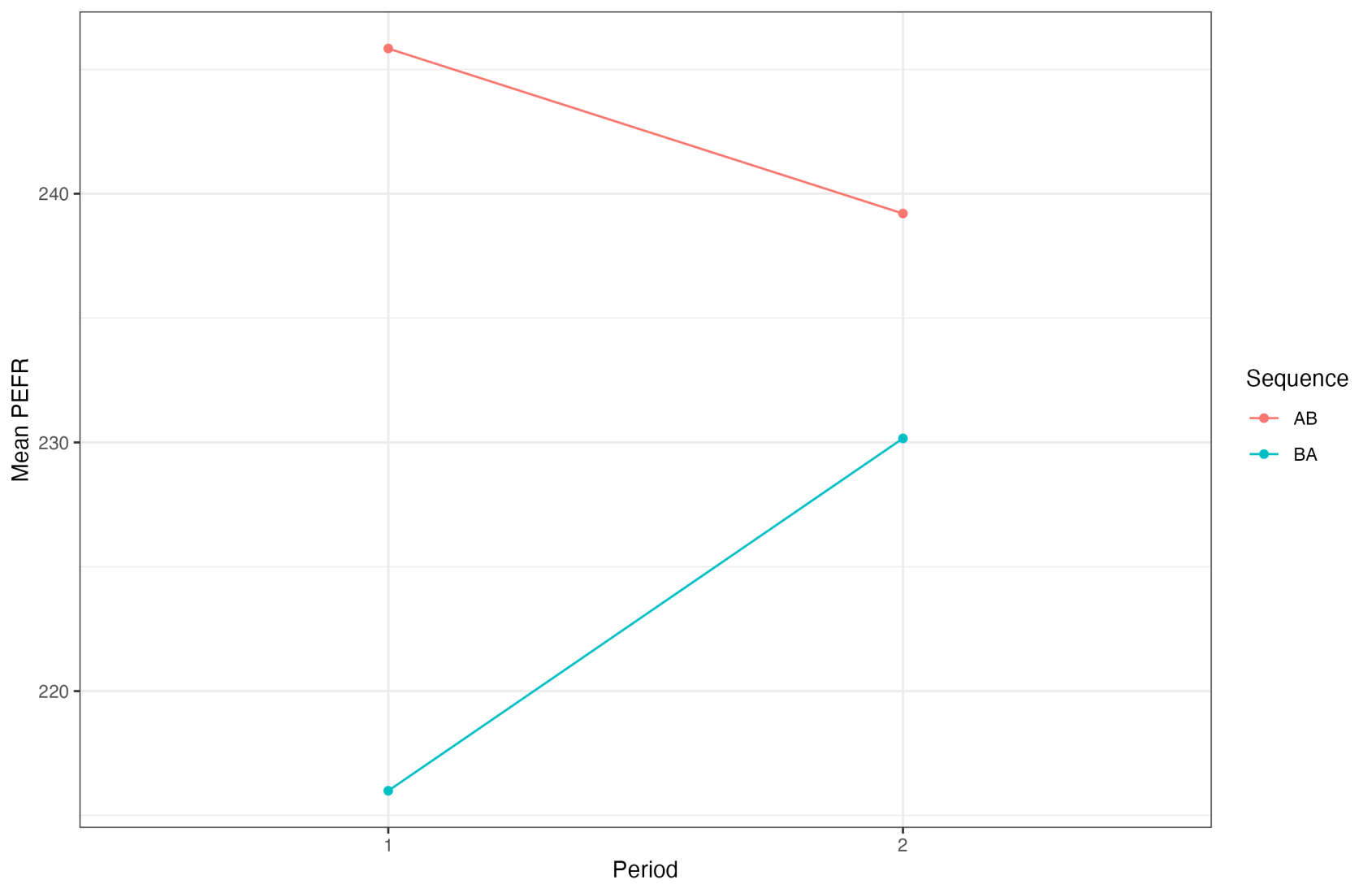
Period 2 vs Period 1



Period 2 vs Period 1 with Centroids



Groups-by-Periods Plot



Analysis of Cross-Over Trial Data

Analysis of Cross-Over Trial Data

t-Tests

Matched-Pairs t-Test

Table 3: Matched-Pairs t-Test on COPD Data

	Group 1	Group 2	n1	n2	t-statistic	df	p
PEFR	A	B	56	56	3.081	55	0.003

Assumptions

Matched-pairs t-test involves two main assumptions:

1. Within-subject differences are independently and randomly distributed around the true treatment effect.
2. Within-subject differences are distributed approximately normally.

Potential assumption violations:

- Period effect
- Sequence effect
- Period-by-treatment interaction
- Carryover
- Patient-by-treatment interaction
- Patient-by-period interaction

Analysis of Cross-Over Trial Data

Mixed Models

Random-Intercepts Model

A random-intercepts mixed model allows us to control for [3] [4]:

- Period effect
- Sequence effect
- Subject-specific effects
- Subject-level variables (e.g. sex, age)

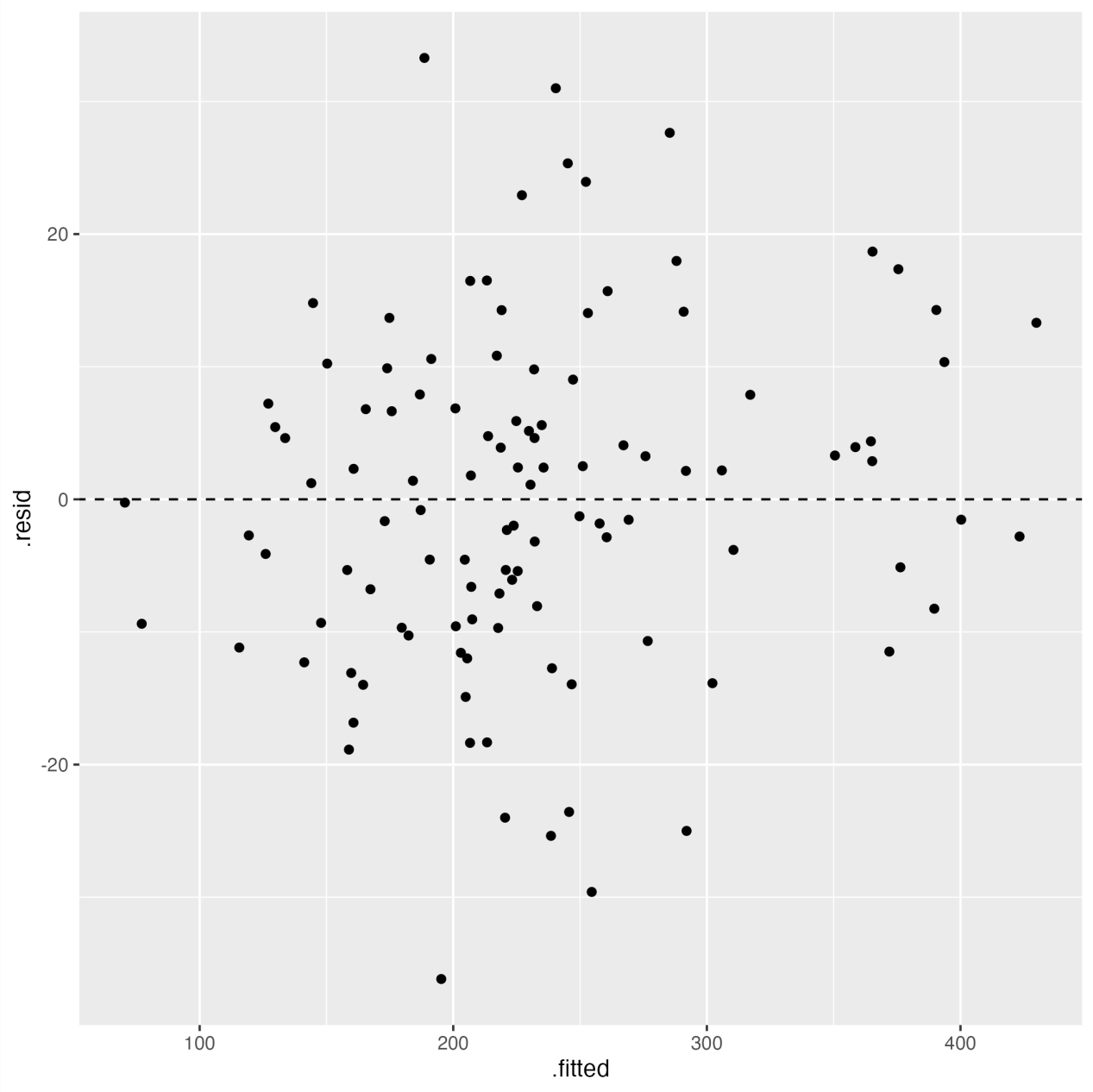
Mixed Model Equation

$$Y = \beta_0 + \beta_1 T + \beta_2 P + \beta_3 Se + \beta_{subject} + \epsilon$$

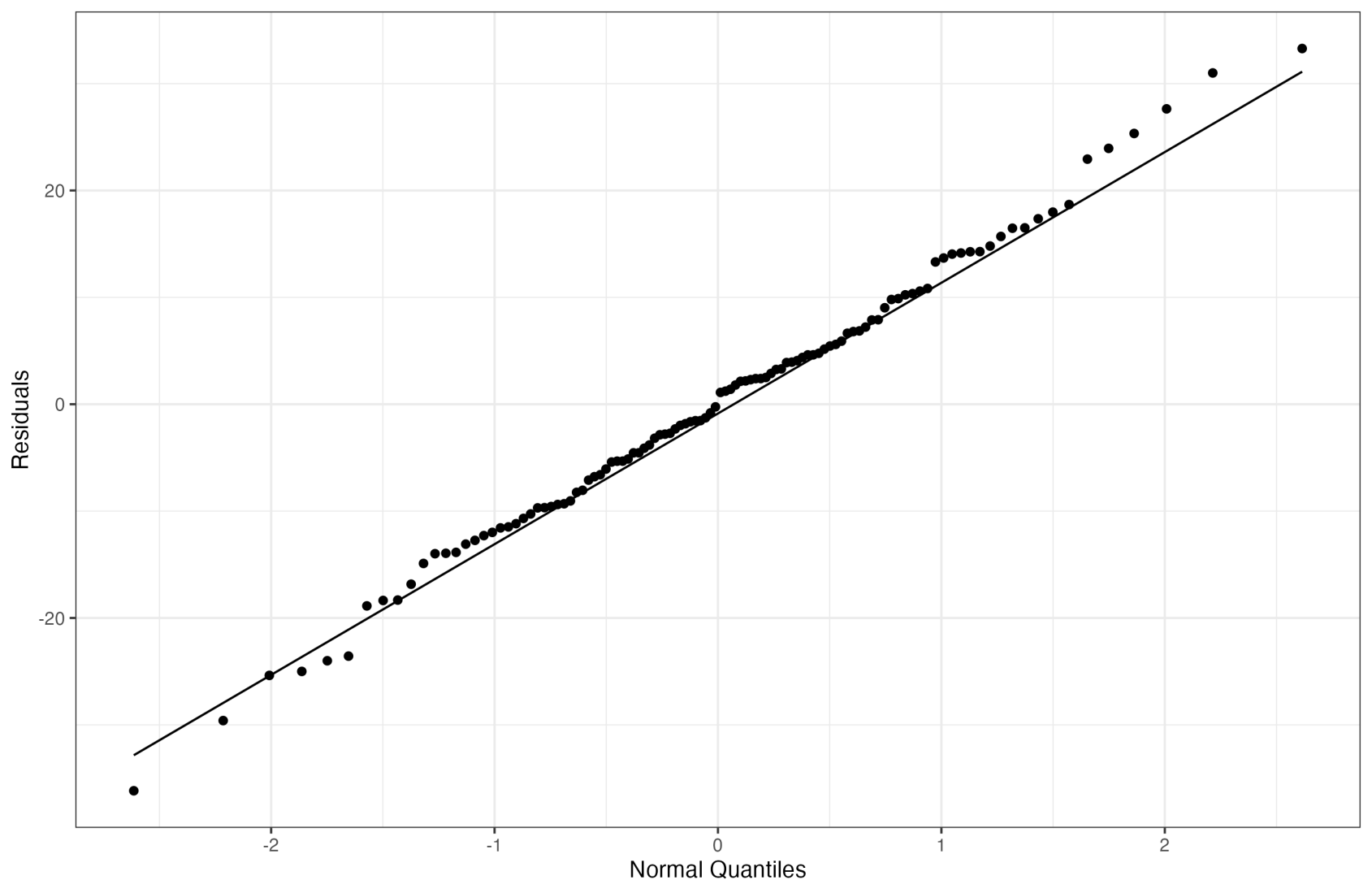
Three key assumptions [5]:

1. Linearity
2. Homoscedasticity
3. Normality of residuals

Verifying Assumptions: Homoscedasticity



Verifying Assumptions: Normality



Mixed Model Estimates

Table 4: Mixed Model Estimates for COPD Data

	Estimate	Std. Error	df	t	p
(Intercept)	245.84	14.96	16.43	56.99	<0.01
TreatB	-10.40	3.42	-3.05	54.00	<0.01
Period2	3.77	3.42	1.10	54.00	0.27
SequenceBA	-19.44	20.50	-0.95	54.00	0.35

Adjusted Means

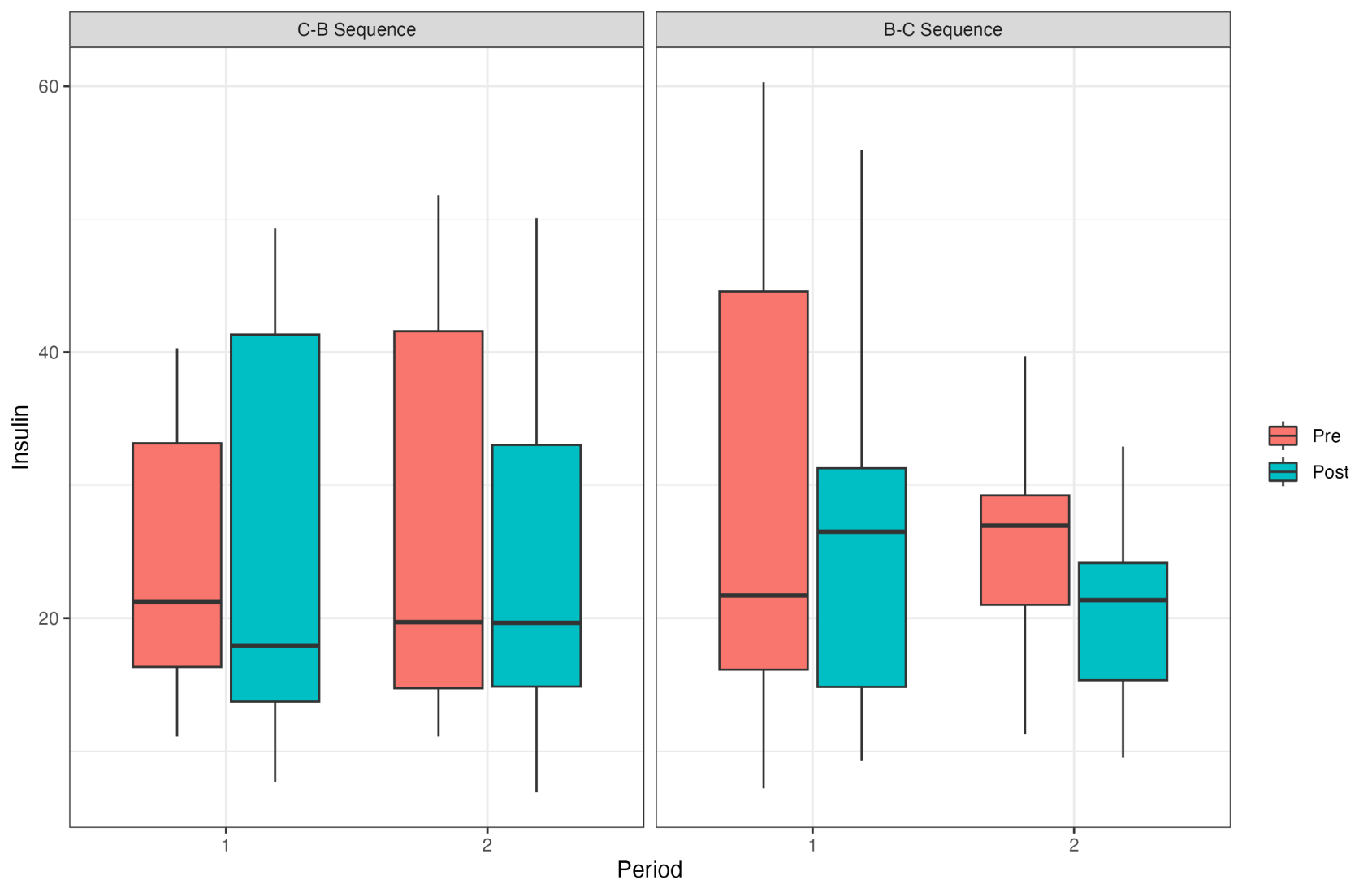
Table 5: LS Means for Mixed Model on COPD Data

Sequence	Difference	Adj. Mean	SE	df	Lower CI	Upper CI
A	.	238.0	10.39	56.99	212.36	263.64
B	.	227.6	10.39	56.99	201.96	253.23
.	A - B	10.4	3.42	54.00	1.96	18.84

Analysis of Cross-Over Trial Data

Controlling for Baseline Measurements

Expanded Boxplot



Expanded Summary Table

Table 6: Summary Table for Protein Data (with Baselines)

		Overall				Period 1				Period 2			
		Pre		Post		Pre		Post		Pre		Post	
Sequence	Subjects	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C-B	10	25.71	13.53	24.55	14.43	24.03	10.70	25.13	16.07	27.39	16.31	23.97	13.45
B-C	10	27.91	14.39	23.12	11.11	29.73	18.94	25.84	13.83	26.10	8.44	20.40	7.27
Total	20	26.81	13.83	23.84	12.74	26.88	15.25	25.48	14.60	26.75	12.66	22.18	10.68

Adjusting for Baseline Values

- ANCOVA incorporates baseline measurements as a covariate in pre-post designs.
- Cross-over designs requires measurements for each subject prior to each treatment period to incorporate baselines.
- Most efficient method is to include *within-subject difference in baselines* as an interaction with the period effect [6].

Mixed Model with Baselines

$$Y = \beta_0 + \beta_1 T + \beta_2 P \cdot X_{diff} + \beta_3 P + \beta_4 X_{diff} + \beta_5 Se + \beta_{subject} + \epsilon$$

Mixed Model Estimates with Baselines

Table 7: Mixed Model on Protein Data Estimates with Baseline Interaction

	Estimate	Std. Error	df	t	p
(Intercept)	25.30	4.23	5.97	20.55	<0.01
TreatmentBEEF	0.71	1.90	0.37	17.00	0.71
Period2	-3.24	1.81	-1.79	17.00	0.09
Pre_diff	0.05	0.26	0.19	20.55	0.85
SequenceB-C	-0.35	5.84	-0.06	17.00	0.95
Period2:Pre_diff	-0.41	0.16	-2.50	17.00	0.02

Bibliography

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