Repeated Measurements

Concept of repeated measures

- We measure the same outcome measure several times on the same person (or thing)
- This is often done over time
- Usually the measurements are continuous data
- Normally, the measurements are on people

- Often, there is missing data
- The number of measurements per subject may differ
- The timing of measurement per subject may differ

- Measurements within a subject are likely to be **correlated**, and this needs to be taken into account in the analysis

Why do we take repeated measurements on individuals?

- Some research questions requiring repeated measures data:
  - How much of the drug gets into the blood and what happens to these levels over time?
  - Do the quality of life scores differ before and after an intervention?
  - Do haemoglobin levels change over time within a person?
  - Is there a difference in the quality of life scores measured over time between smokers and non smokers?
  - Compare 2 diets, and we measure body weight every month on each person

Why do we take repeated measurements on individuals?

- Monitor change over time
- Inform about potential causal relationship between an exposure and an outcome
- Study designs in which patients are followed up over time
- Efficient use of a limited number of subjects: more measurements from fewer individuals – but you should only take repeated measures if there is a scientific reason for this (don't do it just to get more data!)
- There is less variability within subject than between subjects, which allows statistical inference to be made with fewer subjects

Simple ways to analyse repeated measures data

•A simple but sometimes not very efficient way is to use only one value per person/thing

•Standard and simple methods already covered can then be used

• If repeated measurements data was collected, analysing data at only one time point runs the risk of losing useful information

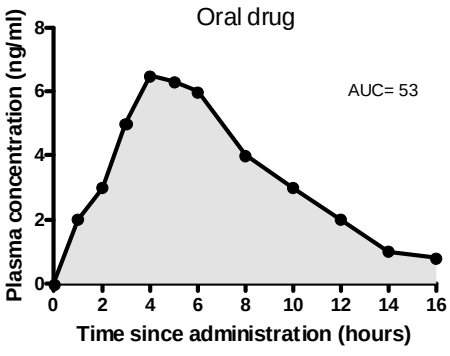
•Selection of **one measurement value per person/thing**:

- •Measurement at a specific visit, or relevant time point
- •Minimum or maximum of all the measurements for one person/object
- •First or Last measurement

•Consider **converting several measurements for a person/object into a single value**:

- •Mean or median
- •Difference between two time points
- •Area Under the Curve (AUC)

Example using AUC



- This is a curve for one person, with a plasma value for every 1 or 2 hours after taking a drug
- The Area Under the Curve is 53
- We have turned 12 values into 1
- Some software can easily give AUC for each person/thing
- Can analyse this using t-test or simple linear regression

Example - Repeated measures data

- Laboratory experiment in 16 samples (8 per group) to evaluate the effect of a new compound.
- 8 samples randomized to the active exposure and 8 to a control
- Samples assessed weekly for 3 weeks (week 0, week 1, week 2)
- Samples measured for a specific cell mutation on a scale (0 to 100)

- Questions:
  - Is there any evidence of a difference in level between the active exposure and control
  - Do differences in between groups depend on the timing (i.e. do you get larger differences earlier than later?)

Example - Repeated measures data

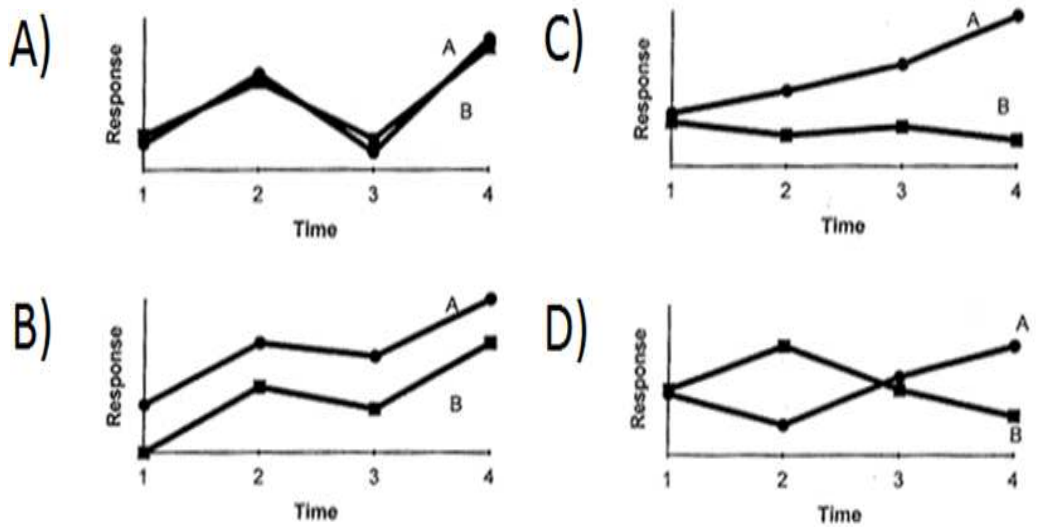
Wide format

ID	Exposure	Measurement of cell mutation (0-100 scale)		
		Week 0	Week 1	Week 2
1	Active	43	34	45
2	Active	23	33	32
3	Control	15	19	21
4	Control	18	18	23
5	Control	27	24	22
6	Control	28	18	26
…				

Long format

ID	Exposure	Week	Measurement of cell mutation (0-100 scale)
1	Active	0	43
1	Active	1	34
1	Active	2	45
2	Active	0	23
2	Active	1	33
2	Active	2	32
3	Control	0	15
3	Control	1	19
3	Control	2	21
…			

Example - Repeated measures data



Accounting for repeated measures outcomes

- Common statistical methods such as two independent sample t-test and linear regression assume independence between observations.

- Some people **incorrectly** treat repeated measurements observations as separate observations
  - having 10 measurements on 10 people is not the same as having 1 measurement on 100 people (just because there are 100 data values in each situation)
  - You still have only 10 people, but the statistical analysis is usually more complex

- Also, do not perform lots of **independent pairwise comparisons** between time points (it increases the error rate)

Repeated measures analysis

- There are specific statistical methods that account for repeated measures
- These methods are often more complex than the standard statistical tests but:
  - They are the **correct way** to analyse repeated measures data
  - They account for the fact that the data has a **dependent structure** (eg measurements are organised in terms of subjects)
  - They will analyse **all** of the actual data you have observed
  - They are more **sensitive** to detecting differences between groups than if we only use one single point
  - They allows us to answer question about the entire profile over time

Two measurements per subject

ID	Measurement of cell mutation (0-100 scale)		Difference After-Before
	Before treatment	After treatment	
1	43	34	-9
2	23	33	10
3	15	19	4
4	18	18	0
5	27	24	-3
6	28	18	-10
…			

If we want to account for paired data and assess whether there is a difference between measurements After and Before treatment consider using:

- Paired t-test
- Wilcoxon signed-ranks test

Two measurements per subject

Paired t-test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	After treatment	24.333	6	7.4476	3.0405
	Before treatment	25.667	6	9.8725	4.0304

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	After treatment - Before treatment	-1.3333	7.6855	3.1376	-9.3988	6.7321	-.425	5	.689

Wilcoxon signed-ranks test

Ranks

		N	Mean Rank	Sum of Ranks
Before treatment - After treatment	Negative Ranks	2[a]	3.25	6.50
	Positive Ranks	3[b]	2.83	8.50
	Ties	1[c]		
	Total	6		

a. Before treatment < After treatment

b. Before treatment > After treatment

c. Before treatment = After treatment

Test Statistics[a]

	Before treatment - After treatment
Z	-.374[b]
Asymp. Sig. (2-tailed)	.786

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

Two measurements per subject

ID	Treatment	Measurement of cell mutation (0-100 scale)	
		Week 0	Week 1
1	Active	43	34
2	Active	23	33
3	Control	15	19
4	Control	18	18
5	Control	27	24
6	Control	28	18
7	Active	45	34
8	Active	35	33

If you want to assess whether there is a difference in the measurements between Active and Control in week 1 adjusting for week 0 consider using:

- Linear regression with treatment as a predictor adjusting for week 0

Linear regression

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1	(Constant)	5.329	2.486		2.144	.085	-1.061	11.720
	Week 0	.089	.103	.129	.872	.423	-.174	.353
	treatment	12.454	2.109	.876	5.906	.002	7.033	17.874

a. Dependent Variable: Week 1

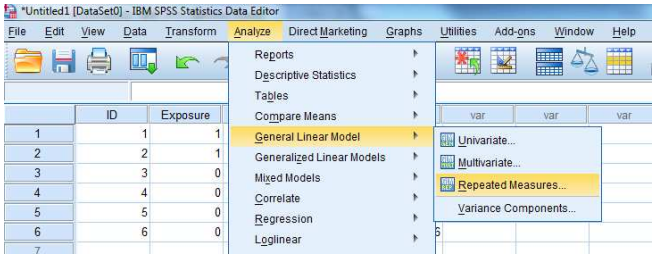
More than 2 measurements per subject

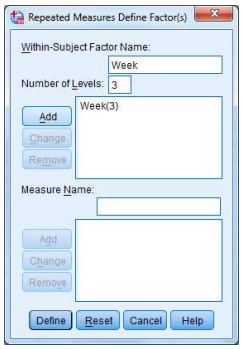
ID	Exposure	Week	Measurement of cell mutation (0-100 scale)
1	Active	0	43
1	Active	1	34
1	Active	2	45
2	Active	0	23
2	Active	1	33
2	Active	2	32
3	Control	0	15
3	Control	1	19
3	Control	2	21
…			

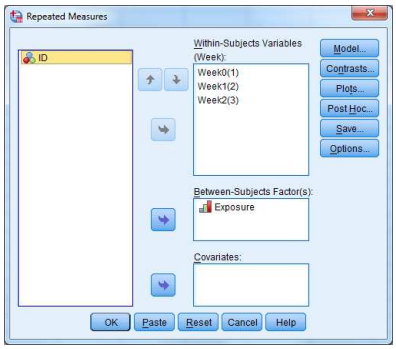
Analysis of repeated measures when the outcome was measured two or more times per subject:

- Repeated Measures ANOVA
- Mixed effects modelling

Repeated Measures ANOVA using SPSS



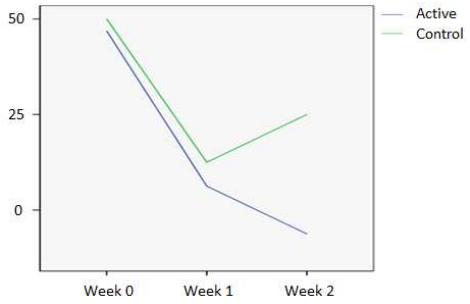




Repeated Measures ANOVA

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	104.167	2	52.083	55.38	.000
Week * Exposure	12.167	2	6.083	6.47	.005



Tests whether there are differences in the outcome between Active and Control simultaneously each week (i.e. whether mean profiles are similar over time)

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Exposure	14.083	1	14.083	14.97	.001

Repeated Measures ANOVA

ID	Exposure	Measurement of cell mutation (0-100 scale)		
		Week 0	Week 1	Week 2
1	Active	43	34	45
2	Active	23	Missing	32
3	Control	15	19	21
4	Control	18	18	23
5	Control	27	24	Missing
6	Control	28	18	26
…				

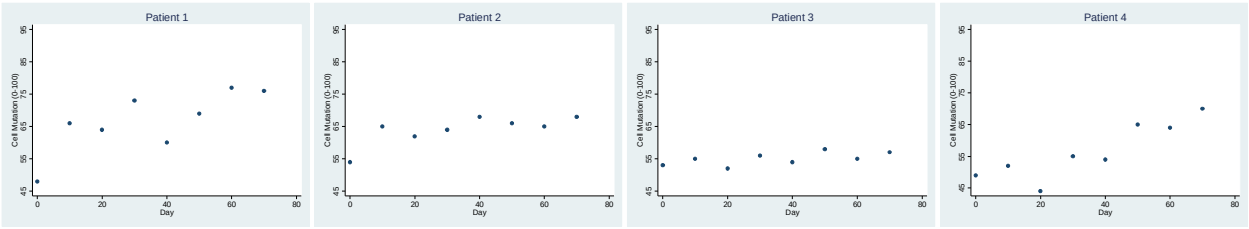
- But when there is missing data, 'repeated measures ANOVA' ignores the entire row (i.e. ID's 2 and 5 would not be included in the analysis at all)
- Need to use an alternative method

Mixed Effects modelling for repeated measures

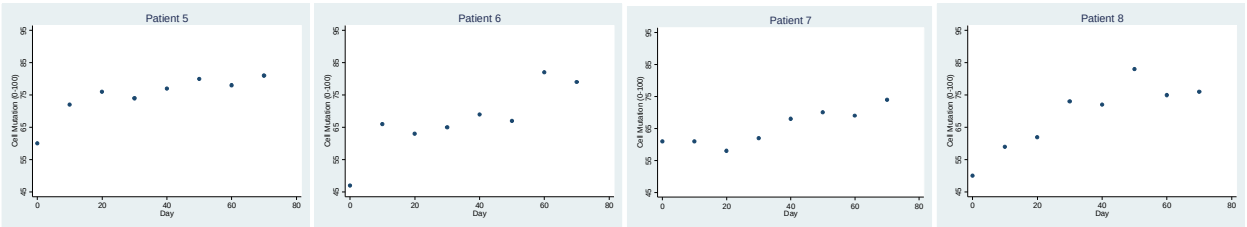
- It is essentially another (more complicated) **type of regression analysis**
- It is used mostly for **continuous outcome measures**
- It can account for **multiple predictors**
- **Predictors** can be any mixture of categorical or continuous
- It accounts for the fact that the outcome measurements are **grouped** within subjects
- It can be used for very complex data
- It is an efficient approach to analyse data when there is **missing data**

Mixed Effects modelling - Example

Active Group

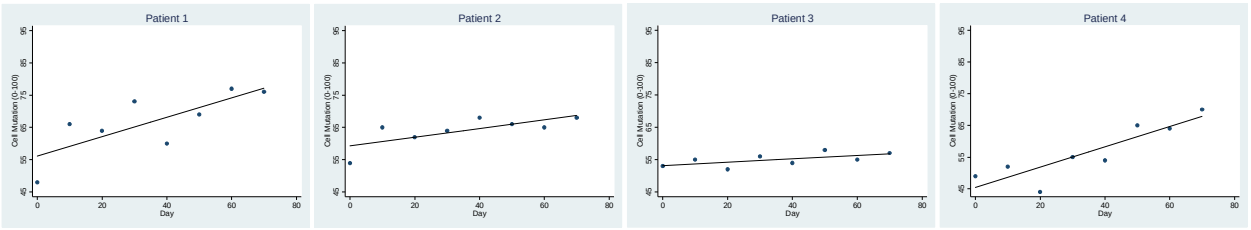


Control Group

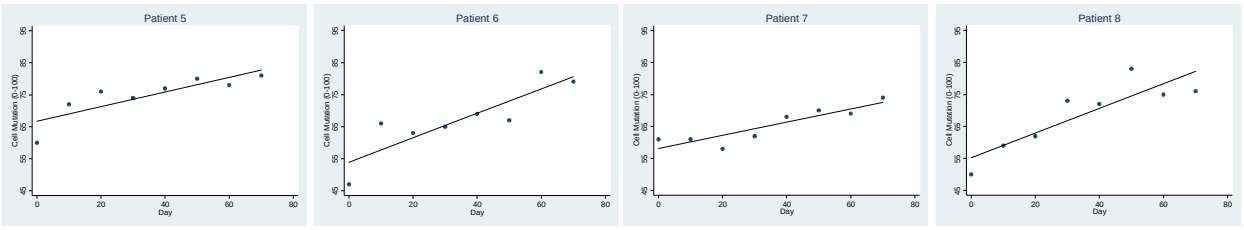


Mixed Effects modelling - Example

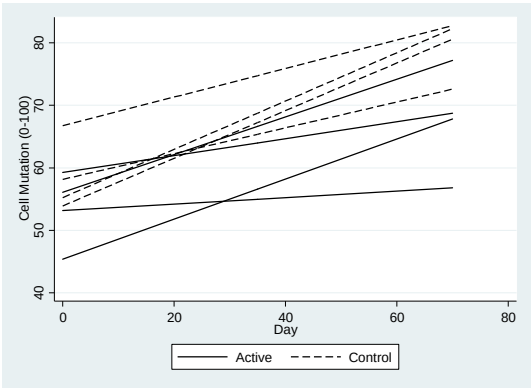
Active Group



Control Group



Mixed Effects modelling - Example



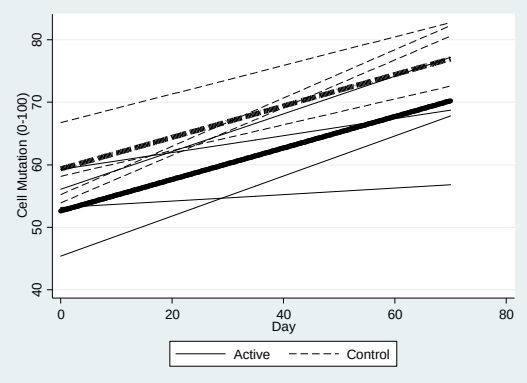
3 questions to ask:

Does the measure change over time?

Is there a difference in the measure between Active treatment and Control?

Does the change over time differ between the 2 groups?

Mixed Effects modelling - Example



The average lines in the Active group and in the Control group:
- Average intercept in each group
- Average slope of all slopes

Random intercept and slope modelling (mixed model):
- Fixed part
- Random part

Random intercept and slope modelling (mixed model)

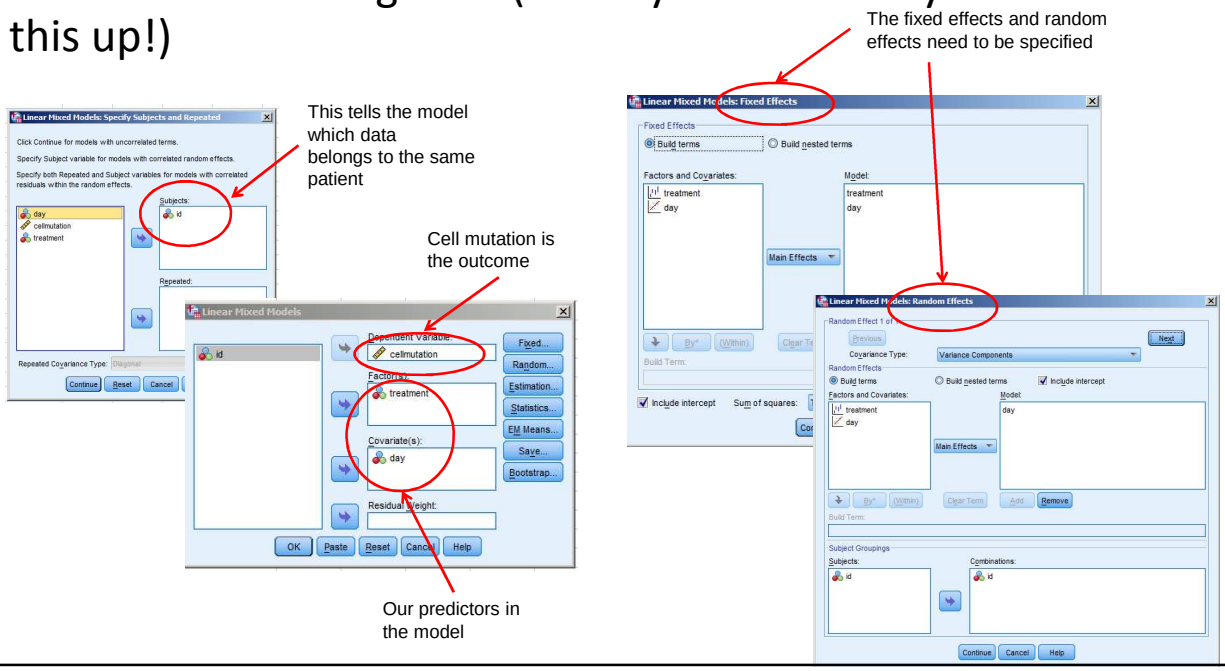
Fixed component:

-The model will provide us with quantities that are fixed across groups (eg, difference in the treatment means; slope expressing changes in the outcome with time)

Random component:

- The model will consider certain quantities random
- Each subject has its own intercept and slope and these quantities can be seen as randomly scattered around a fixed intercept or fixed slope
- The model will estimate the variability of these quantities that are considered random
- These variability is used to adjust in an efficient way for the fact that the data is organised in terms of repeated measures.

Mixed models using SPSS (be very careful how you set this up!)



Mixed model results

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	59.359659	2.258106	9.196	26.287	.000	54.268015	64.451302
treat: Active	-6.719317	3.074642	7.701	-2.185	.062	-13.857675	.419040
treat: Control	0[b]	0	.	.	.	.	.
day	.251339	.035614	10.103	7.057	.000	.172096	.330583

a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

Fixed effects

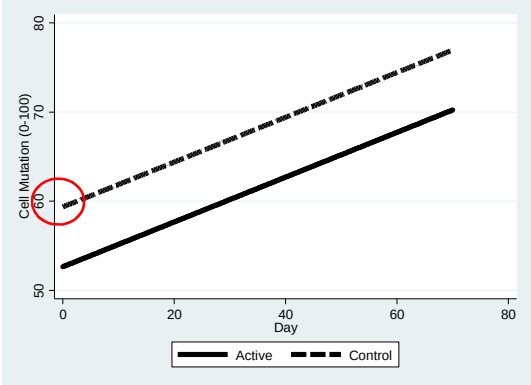
Estimates of Covariance Parameters[a]

Parameter		Estimate	Std. Error
Residual		20.861932	4.310717
Intercept [subject = id]	Variance	13.193014	9.556593
day [subject = id]	Variance	.005180	.004607

a. Dependent Variable: cellmutation.

Random effects

Mixed model results



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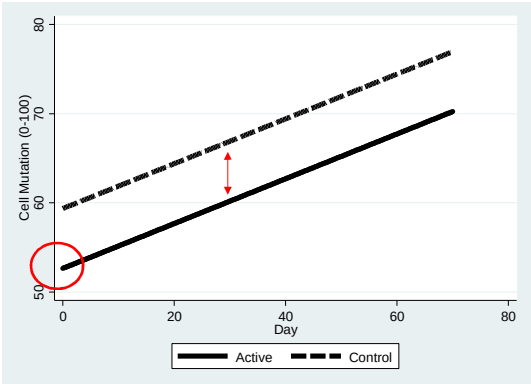
a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

Estimated value of cell mutation on day 0 in the baseline group (Control group)

Mixed model results



59.36 - 6.72 = 52.64

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	59.359659	2.258106	9.196	26.287	.000	54.268015	64.451302
treat: Active	-6.719317	3.074642	7.701	-2.185	.062	-13.857675	.419040
treat: Control	0[b]	0	.	.	.	.	.
day	.251339	.035614	10.103	7.057	.000	.172096	.330583

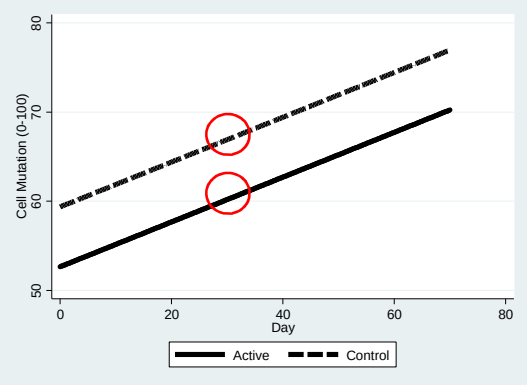
a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

Borderline evidence of a difference in the Active treatment and Control <u>when adjusting for time and taking into account repeated measures</u>

It is estimated that the mean mutation value in the Active treatment to be 6.7 units less (95%CI:-13.86 to 0.42) than in the Control group, when adjusting for time and taking into account repeated measures.

Mixed model results



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treat: Active	-6.719317	3.074642	7.701	-2.185	.062	-13.857675	.419040
treat: Control	0[b]	0	.	.	.	.	.
day	.251339	.035614	10.103	7.057	.000	.172096	.330583

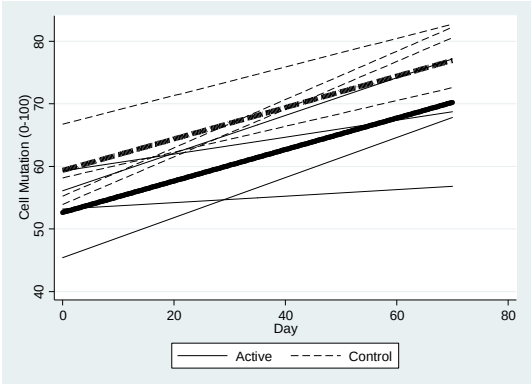
a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

There is evidence that the cell mutation values increase over time adjusting for treatment

It is estimated that for each day increase, the cell mutation increase by 0.25 units (95%CI 0.17 to 0.33), adjusting for treatment and taking into account repeated measures.

Mixed modelling – interaction treatment-time?

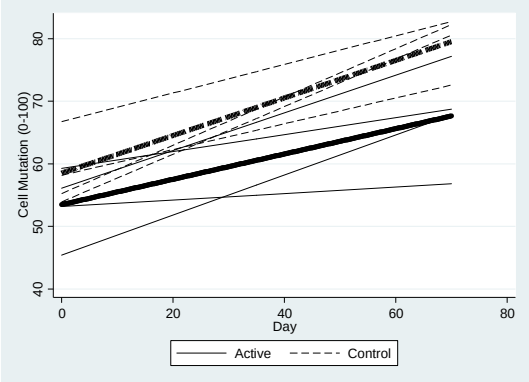


Does the change over time differ between the 2 groups?

Are the slopes for time different between groups?

Is the slope in one group steeper than the other?

Mixed modelling – interaction treatment-time?



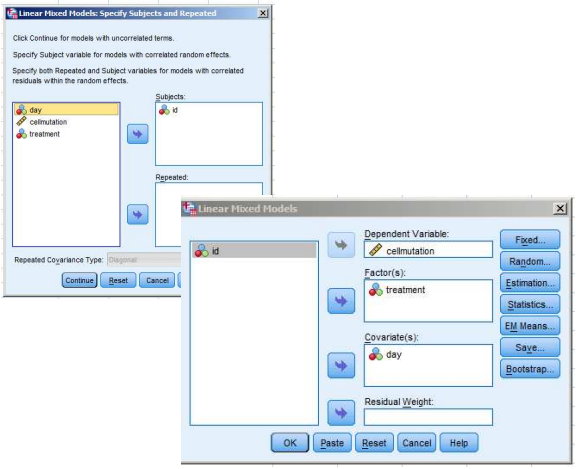
Does the change over time differ between the 2 groups?

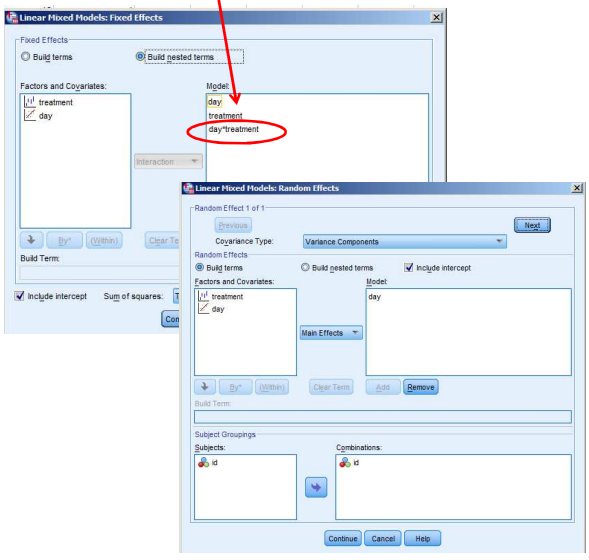
Are the slopes for time different between groups?

Is the slope in one group steeper than the other?

Mixed models using SPSS – interaction treatment-time

Adding treatment-time interaction into the mixed model





Mixed modelling – interaction treatment-time?

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	58.520833	2.335362	11.364	25.059	.000	53.400727	63.640940
treat: Active	-5.041667	3.302700	11.364	-1.527	.154	-12.282591	2.199258
treat: Control	0[b]	0	.	.	.	.	.
day	.300298	.045692	11.364	6.572	.000	.200122	.400473
treat: Active * day	-.097917	.064618	11.364	-1.515	.157	-.239586	.043753
treat: Control * day	0[b]	0	.	.	.	.	.

a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

Estimates of Covariance Parameters[a]

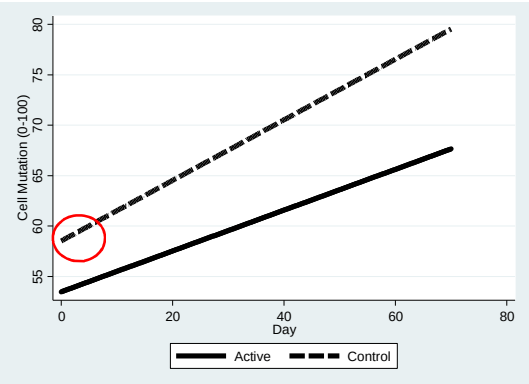
Parameter		Estimate	Std. Error
Residual		20.760219	4.224752
treatment [subject = id]	Variance	13.165566	9.168124
day [subject = id]	Variance	.003408	.003560

a. Dependent Variable: cellmutation.

Is there evidence of an interaction?

No evidence of interaction between treatment and time, adjusting for repeated measures

Mixed modelling – interaction treatment-time?



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treat: Active * day	-.097917	.064618	11.364	-1.515	.157	-.239586	.043753
treat: Control * day	0[b]	0	.	.	.	.	.

a. Dependent Variable: cellmutation.

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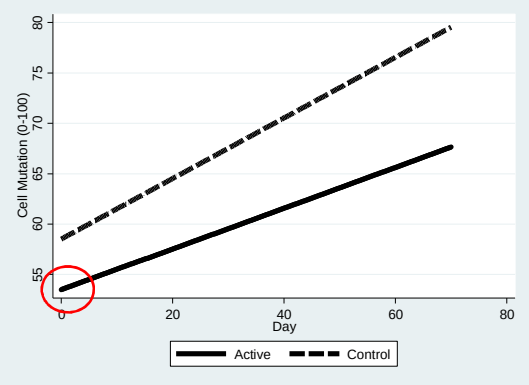
Estimated mean cell mutation in the control group at day 0, adjusting for repeated measures

Mixed modelling – interaction treatment-time?



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treat: Control	0[b]	0	.	.	.	.	.
day	.300298	.045692	11.364	6.572	.000	.200122	.400473
treat: Active * day	-.097917	.064618	11.364	-1.515	.157	-.239586	.043753
treat: Control * day	0[b]	0	.	.	.	.	.

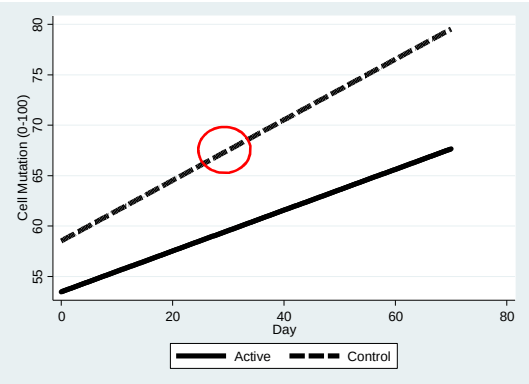
a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

Estimated difference in means between Active and Control groups at day 0, adjusting for repeated measures

58.52 - 5.04 = 53.48

Mixed modelling – interaction treatment-time?



Estimates of Fixed Effects[a]

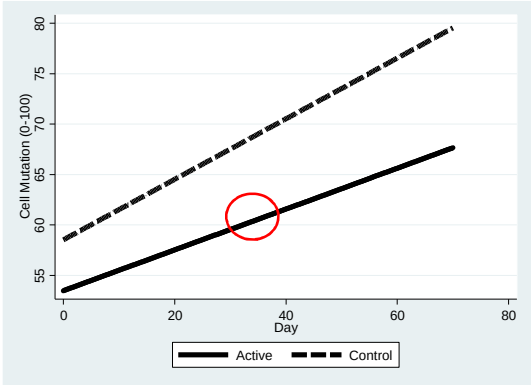
Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	58.520833	2.335362	11.364	25.059	.000	53.400727	63.640940
treat: Active	-5.041667	3.302700	11.364	-1.527	.154	-12.282591	2.199258
treat: Control	0[b]	0	.	.	.	.	.
day	.300298	.045692	11.364	6.572	.000	.200122	.400473
treat: Active * day	-.097917	.064618	11.364	-1.515	.157	-.239586	.043753
treat: Control * day	0[b]	0	.	.	.	.	.

a. Dependent Variable: cellmutation.

b. This parameter is set to zero because it is redundant.

For each unit increase in time, the cell mutation increased on average by 0.30 in the Control group, adjusting for repeated measures

Mixed modelling – interaction treatment-time?



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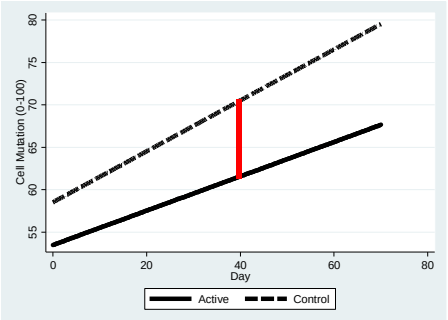
0.30 – 0.098 = 0.202

For each unit increase in time, the cell mutation increased on average by 0.202 in the Active group, adjusting for repeated measures

Mixed modelling – interaction treatment-time?



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Control: 58.52+(0.30*40)= 70.52

Active: (58.52-5.04)+(0.30*40)+(-0.098*40)= 61.56

Active versus Control at day 40 : 61.56 - 70.52 = - 8.96

-8.96 (95%CI: -15.36 to -2.56); p=0.006

Repeated measures analysis - Conclusion

- Repeated measures analysis is more complex than using traditional statistical methods
- Start with simple statistical methods, if possible, and develop into more complex statistical methods
- Mixed models are very flexible and efficient approaches to analyse repeated measures but they can be very complex