筑波大学
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Single-Factor Experiments: Analysis of Variance (ANOVA)

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Before we Begin

- Go to the github repo:
  - https://github.com/Mollinetti/Statistics-R
- Download the script for this class! (in the ‘scripts’ folder, class_4.r!)
- Run the first lines to load/install the required libraries

- Introduction
- What is ANOVA
- Completely Randomized single-factor model
- Single-factor Unbalanced Design
- Single-factor Random Effects model
- Single-factor Blocking design
- Single-factor Repeated measures ANOVA
- Single-factor Model Validation
- Power test for Single-factor ANOVA

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Introduction

- Many single-factor experiments require more than two levels of the factor to be considered
- Randomization of the experiments is now taken into account
- In the medical field, effects of medicines/treatments are verified for multiple samples of populations
- We will now call each level a treatment

Introduction

- Montgomery explains the steps for a experiment:

1. **Conjecture**: the original hypothesis that motivates the experiment.
2. **Experiment**: the test performed to investigate the conjecture.
3. **Analysis**: the statistical analysis of the data from the experiment.
4. **Conclusion**: what has been learned about the original conjecture from the experiment. Often the experiment will lead to a revised conjecture, and a new experiment, and so forth.

Introduction

- Factor levels can be chosen in two ways:
  - Fixed-effects model
  - Random effects/ Components of variance model

Introduction

- Factor levels can be chosen in two ways:
  - Fixed-effects model
    - Specifically choose the a levels
    - Conclusions cannot be extended to treatments that were not considered
  - Random effects/ Components of variance model

Introduction

- Factor levels can be chosen in two ways:
  - Fixed-effects model
  - Random effects/ Components of variance model
    - Random sample from a larger population of treatments
    - Extend the conclusion to all treatments
    - Knowledge about the treatments investigated is not important

Introduction

- Before we start ANOVA, let's remember:
  - Dependent Variable
    - Variable being tested and measured in a scientific experiment
  - Independent Variable
    - Variable that is changed or controlled in a scientific experiment

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What is ANOVA

- ANalysis Of VAriance
- ANOVA partitions the variability into two parts
- Test the hypothesis based on a comparison of two independent estimates of the population variance/mean/covariance

What is ANOVA

- In ANOVA we want to test the significance of the effect of one or more dependent variables against three or more groups of independent variables
- One-way-ANOVA, repeated ANOVA, mixed effects ANOVA, ANCOVA
- two-way-ANOVA, MANOVA, MANCOVA, Single cell design

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Completely Randomized single-factor Model

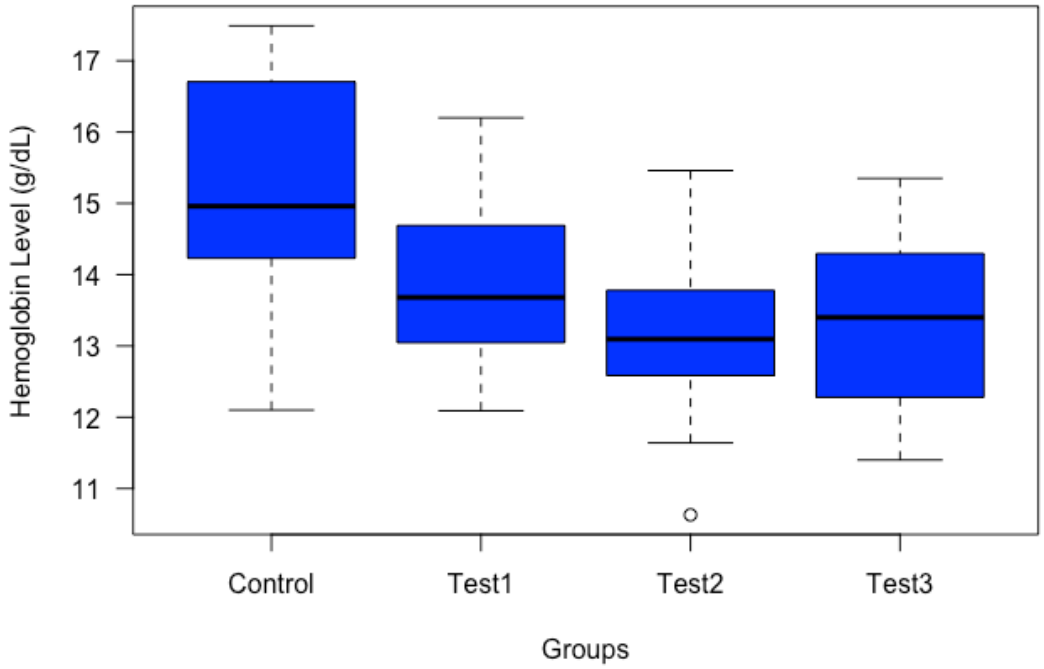
- Consider the following experiment: “We need to verify the effect of some medicine to anemic male patients. We have 4 groups: control, placebo and two variations of the medicine. Nominal hemoglobin levels for male are between 13.5 to 17.5g/dl. Sample size is 20. Age is disregarded

Completely Randomized single-factor Model

- Blood samples are taken in a completely randomized fashion
- Need to reduce any nuisance variable in the experiment
- Human error x Machine error
- Graphical interpretation and statistic interpretation

Completely Randomized single-factor Model

- Let's observe the boxplot of our data



Completely Randomized single-factor Model

- Suppose we have a different levels of a single factor we wish to compare
- Observations follow the linear model:

$$Y_{ij} = \mu_i + \epsilon_{ij} \begin{cases} i = 1,2,\dots,a \\ j = 1,2,\dots,n \end{cases}$$

Where $\mu_i = \mu + \tau_i$

Completely Randomized single-factor Model

- Suppose we have a different levels of a single factor we wish to compare
- Observations follow the linear model:

Mean of the treatment

Random observation → $Y_{ij} = \mu_i + \epsilon_{ij}$ $\begin{cases} i = 1,2,\dots,a \\ j = 1,2,\dots,n \end{cases}$ ← Error

Where $\mu_i = \mu + \tau_i$ ← Effect factor

Mean of the population (overall mean)

Completely Randomized single-factor Model

- Each treatment can be thought of as a normal population with mean μ_i and variance σ^2
- Moreover, each treatment can be understood as a normally distributed variable with mean μ plus a perturbation τ_i

Completely Randomized single-factor Model

- For the fixed effect models, effects τ_i are considered as deviations from overall mean μ
- So the sum of τ_i is expected to be 0
- This is equivalent to testing the following hypotheses

$$H_0: \tau_1 = \tau_2 = \ ... = \tau_a = 0$$

$$H_1: \tau_i \neq 0 \text{ for at least one } i$$

Completely Randomized single-factor Model

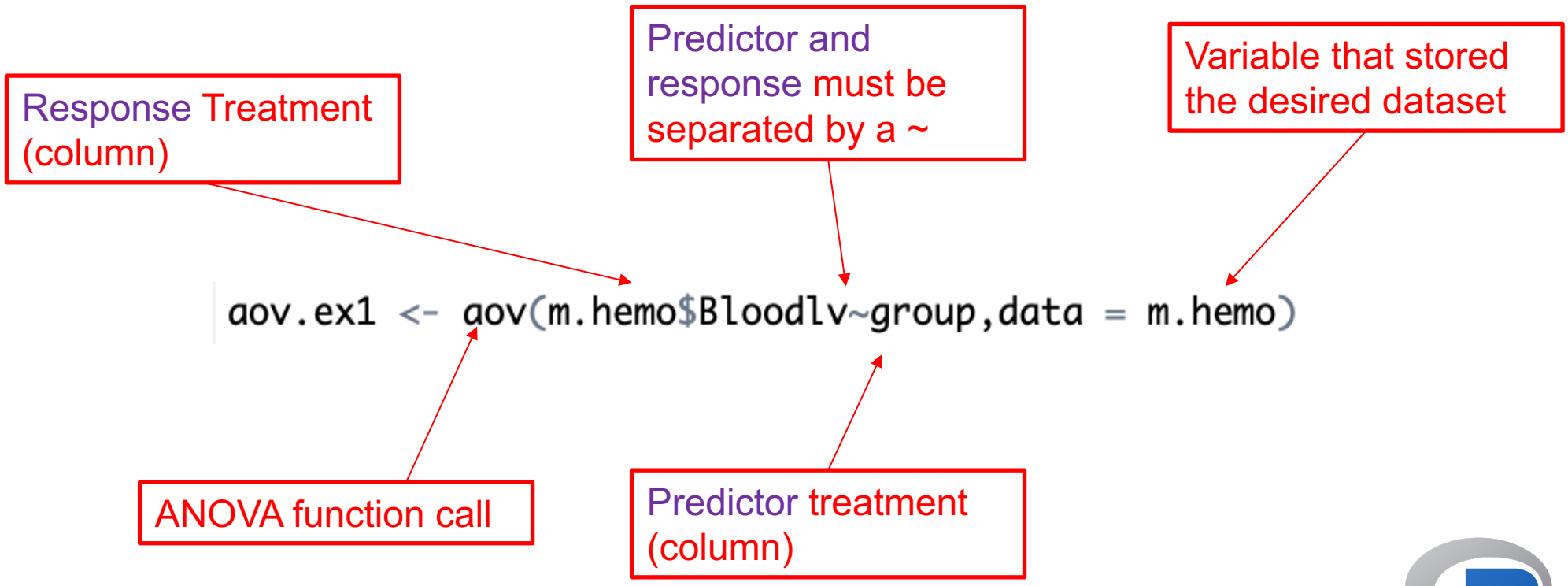
- We have the following point estimators:
  - Total sum of squares: $SS_T = \sum_{i=1}^{a}\sum_{j=1}^{n} y_{ij}^2 - \frac{\bar{y}^2}{N}$
  - Sum square of treatments: $SS_{Tr} = \sum_{i=1}^{a} \frac{y_i^2}{n} - \frac{\bar{y}^2}{N}$
  - Mean square of treatments: $MS_{Tr} = \frac{SS_{Tr}}{(a-1)}$
  - Error sum of squares: $SS_E = SS_T - SS_{Tr}$
  - Error mean square: $MS_E = \frac{SS_E}{a(n-1)}$
  - F-statistic: $F_0 = \frac{MS_{Tr}}{MS_E}$

Completely Randomized single-factor Model

- Importance of the F-statistic
- F-statistic: $F_0 = \frac{MS_{Tr}}{MS_E}$ = variance of the treatment means / mean of the treatment variances
  - Supports the p-value in answering "Is the variance between the means of two population significantly different?"
  - F statistic and Critical F-value
  - If the calculated F statistic is greater than the F critical value, you can reject your null hypothesis

Completely Randomized single-factor Model

- Anatomy of the ANOVA function in R:



Completely Randomized single-factor Model

- Now let's run the ANOVA for our hemoglobin experiment

```
> summary(aov.ex1)
             Df Sum Sq Mean Sq F value   Pr(>F)
m.hemo$Group  3   50.8  16.935   10.97 4.57e-06 ***
Residuals    76  117.3   1.543
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Since P is considerably smaller than 0.1 we can safely reject H_0

Completely Randomized single-factor Model

- Since the Mean Squared error MS_E is an estimator of variance σ, we can build confidence intervals
- Let's verify the confidence interval for each mean μ_i

Completely Randomized single-factor Model

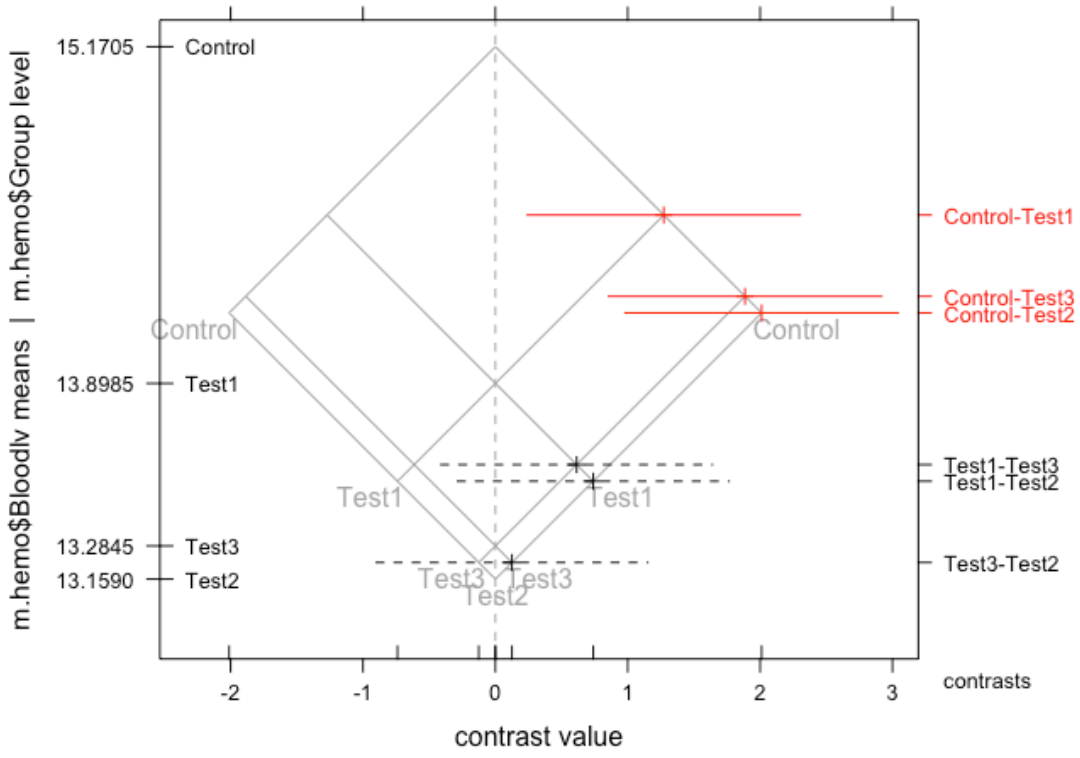
- Since we rejected H_0, we know that there is at least one factor that is different from the others
- How do we know what factor?
  - Fisher's least significant Difference (LSD)
  - Tukey's test
  - mmc
- We'll run a mean-mean multiple comparison (mmc)

Completely Randomized single-factor Model

- Tukey Honest Single Differences (HSD) test
  - Post-hoc analysis of the ANOVA
  - H_0: no significance in the difference of means
  - H_1: significance in the difference of means
- We will first plot the mmc, then plot the Tukey HSD to account for overlapping labels (tiebreaker plot)

Completely Randomized single-factor Model

- Plotting the mmc we get a very straightforward answer:

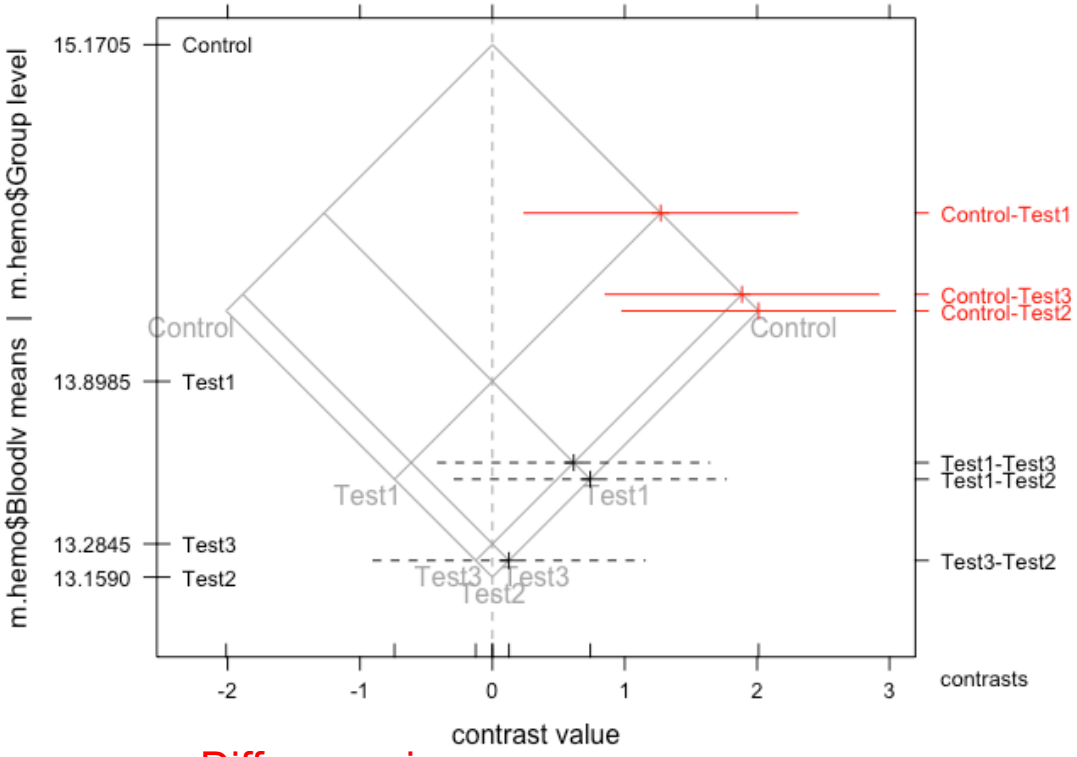


Completely Randomized single-factor Model

- How to read a mca plot:

Crossing of the means of a treatment at the levels of one factor with itself rotated 45 degrees

Mean values of each treatment

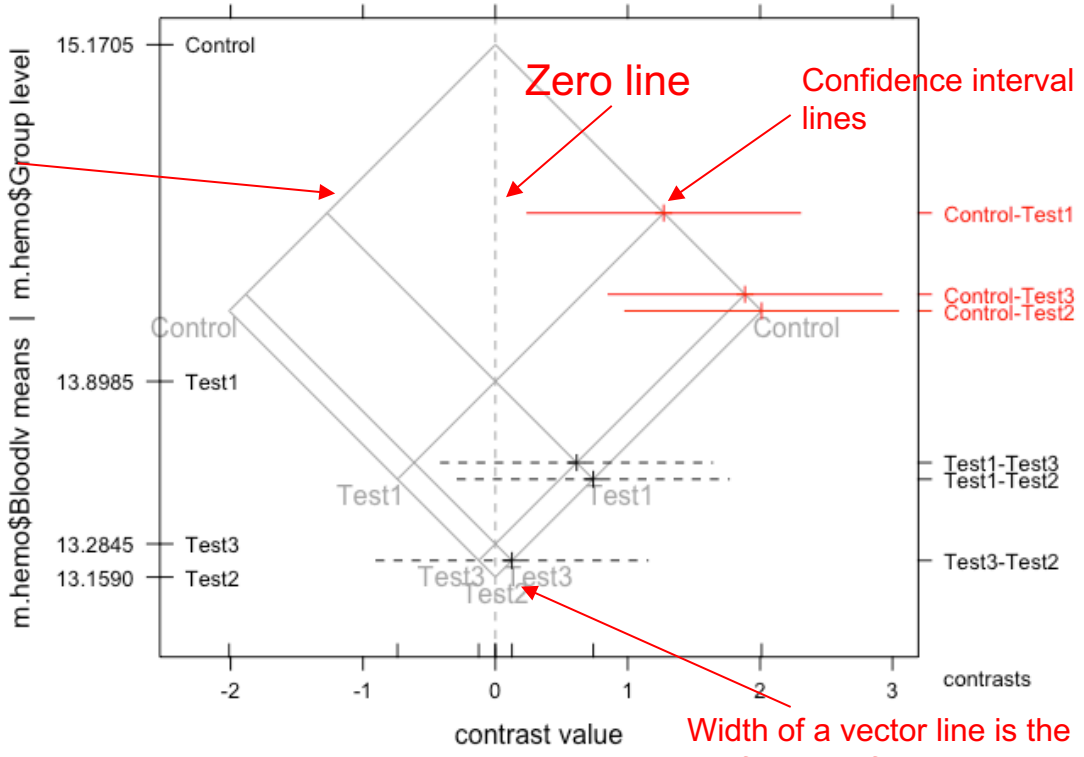


Weighted average of the means comprising each comparison

Difference in mean levels

Completely Randomized single-factor Model

- How to read a mca plot:



Completely Randomized single-factor Model

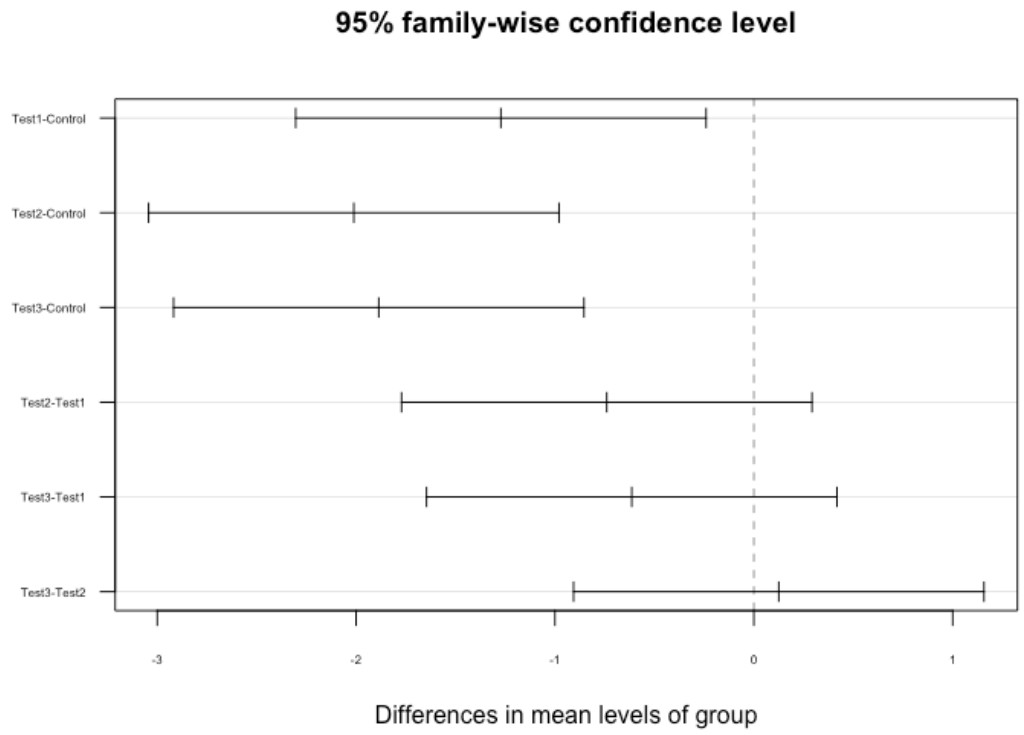
- Contrasts are weighted variables in which their values must sum to 0
- Example: comparing 4 means

μ_1	μ_2	μ_3	μ_4
1	-1	0	0
0	0	1	-1
1	1	-1	-1

We call this an orthogonal contrast

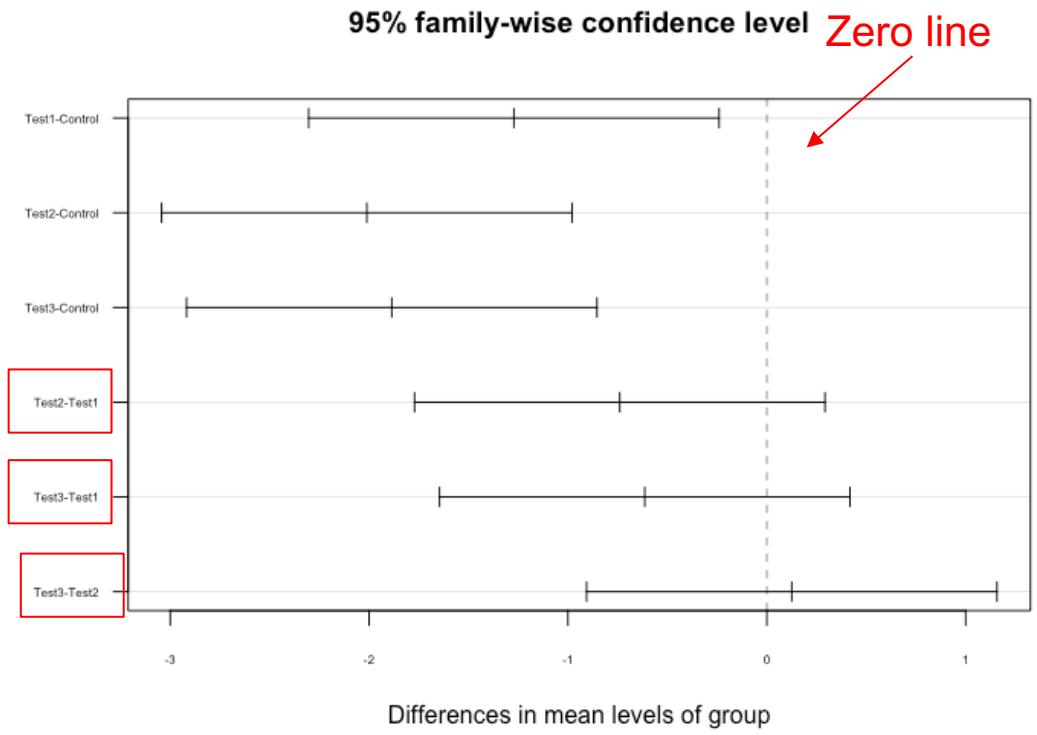
Completely Randomized single-factor Model

- Plotting the Tiebreaker plot:



Completely Randomized single-factor Model

- Plotting the Tiebreaker plot:



Intervals that contain 0 indicate that the difference between the means **IS NOT SIGNIFICANT**

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Unbalanced Design

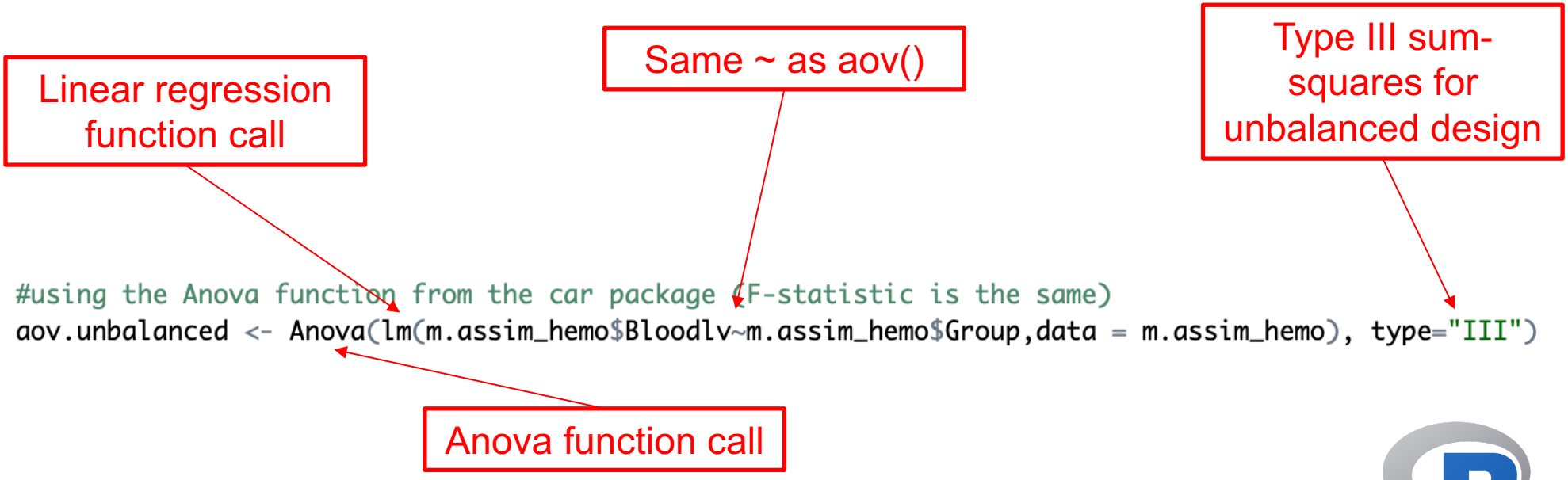
- In some cases, the number of observations taken under each treatment may be different
- We say the the design is unbalanced
- Disadvantages over balanced design:
  - Insensitive to small departures from the assumption of equality
  - More prone to Type-II errors, less power β

Unbalanced Design

- Same steps as the one-way randomized ANOVA
  - Load the assimetric_hemo_exp dataset
  - Don’t forget to drop the empty rows
  - Run the ANOVA
  - Run the mmc
  - Verify the differences

Unbalanced Design

- We will use the Anova() function from the 'car' library, but we must wrap it with a linear regression function call



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Random Effects Model

- Now, treatments are random samples from a population of treatments
- We want to draw conclusions for the entire population of factor levels
- a random factors are chosen

Random Effects Model

- Consider variance of the treatment effects τ_i to be σ_τ^2
- The variance of the response is $\sigma_\tau^2 + \sigma^2$
- So we test Hypotheses about σ_τ^2

$$H_0: \sigma_\tau^2 = 0$$
$$H_1: \sigma_\tau^2 \neq 0$$

Random Effects Model

- We first have to use the restricted maximum likelihood (REML) procedure and then apply ANOVA
- The computational procedure of the ANOVA table is the same as of the fixed-effects*
- Conclusions, however apply for the entire population of treatments
- Load the 'Block_Hemo_exp.csv' dataset

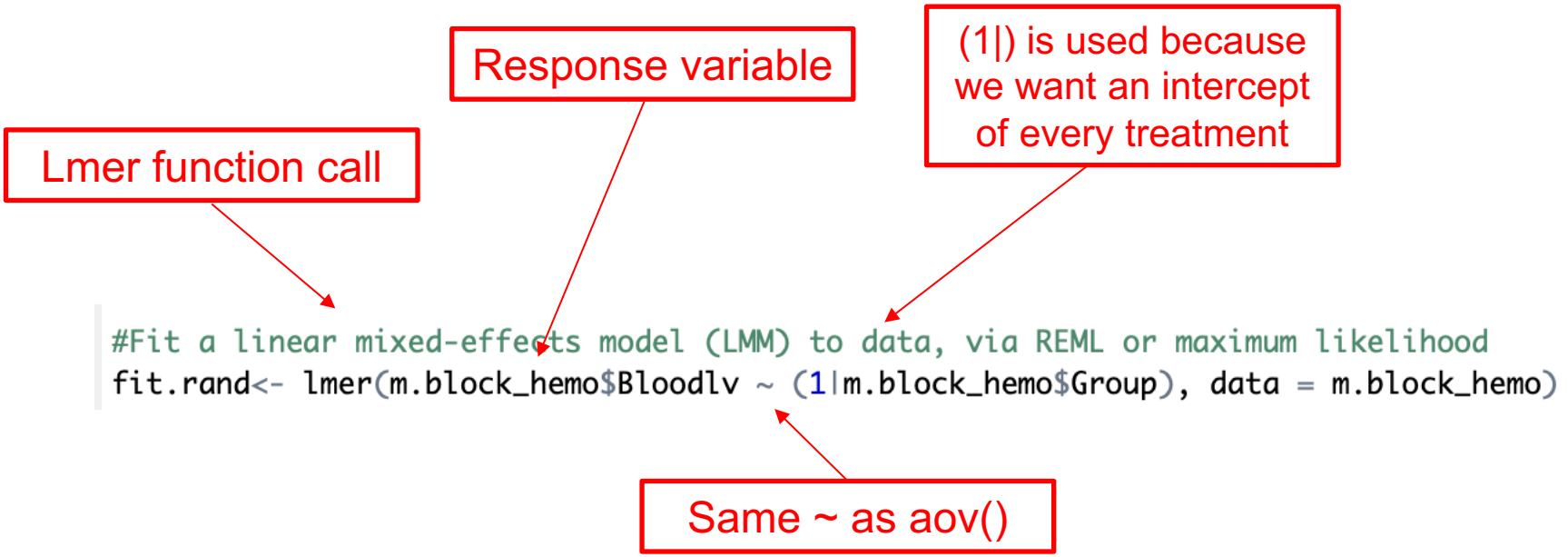
Random Effects Model

- In R, we have to use an entirely different approach from the regular aov function call.
- We call the function **lmer** from the library lme4 (linear mixed random effect)

```
#Fit a linear mixed-effects model (LMM) to data, via REML or maximum likelihood
fit.rand<- lmer(m.block_hemo$Bloodlv ~ (1|m.block_hemo$Group), data = m.block_hemo)
```

Random Effects Model

- In R, we have to use an entirely different approach from the regular aov function call.
- We call the function **lmer** from the library lme4 (linear mixed random effect)



Random Effects Model

- Running the summary we can see the random effect associated to each treatment

```
> summary(fit.rand)
Linear mixed model fit by REML ['lmerMod']
Formula: m.block_hemo$Bloodlv ~ (1 | m.block_hemo$Group)
   Data: m.block_hemo

REML criterion at convergence: 1465.4

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.90058 -0.71495 -0.03995  0.70689  3.04667

Random effects:
 Groups              Name        Variance Std.Dev.
 m.block_hemo$Group (Intercept) 0.2512   0.5012
 Residual                       1.4504   1.2043
Number of obs: 450, groups:  m.block_hemo$Group, 9

Fixed effects:
            Estimate Std. Error t value
(Intercept)  13.6314     0.1764   77.26
```

Random effects (sum them)

Estimate from the expected measurement of a donor from a random treatment (mean)

Random Effects Model

- Now we check the random effect σ_τ^2 associated to each treatment

```
## "estimated" (better: conditional means of) random effects that we computed with the lmer
ranef(fit.rand)
```

- To validate the assumptions of our model we do several plots
  - Residuals x fitted values (Tukey-Ascombe plot)
  - Qq-plot
  - Normalized qq plot

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Blocking Design

- Reduce the variability from a nuisance factor
- Extension of the paired t-test when more than two treatments must be compared
- Selection of b blocks and running a complete replicate of the experiment in each block
- A levels, b blocks

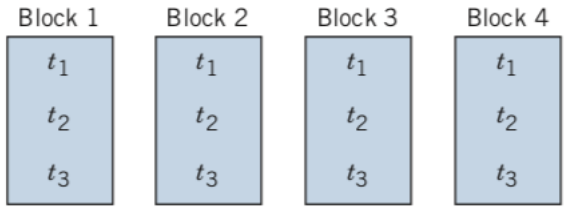


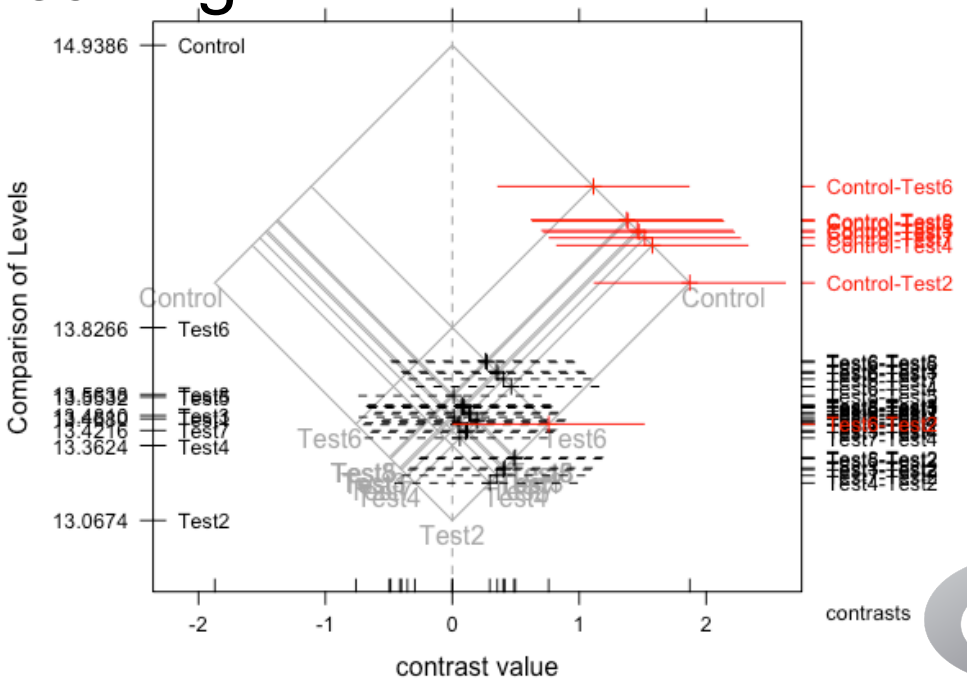
Figure 13-8 A randomized complete block design.

Blocking Design

- When to use block design?
  - When you want to reduce the MS_E
  - When doing a single factor experiment has much more degrees of freedom
- Generally, it is based on trial and error

Blocking Design

- Run the ANOVA with block design at R
- Do the mmc
- Run the HSD for tie-breaking
- Check the plot



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Repeated Measures ANOVA

- The same participant are observed multiple times or under all the levels of a within-subject factor
- One independent variable
  - Within-subject variable
  - Two or more categorical related group
- One dependent variable
  - Interval or ratio level of measurement

- A within-subjects variable is an independent variable that is manipulated by testing each subject at each level of the variable
- Example: suppose we take blood measurements on two situations:
  - After 12 hour fasting
  - Without fasting

Repeated Measures ANOVA

- We test for the following hypotheses:
  - H_0: Means of measures are equal
  - H_1: Means of measures are not equal

$$H_0: \mu_1 = \mu_2 = \ ... = \mu_a = 0$$
$$H_1: \mu_i \neq 0 \text{ for at least one } i$$

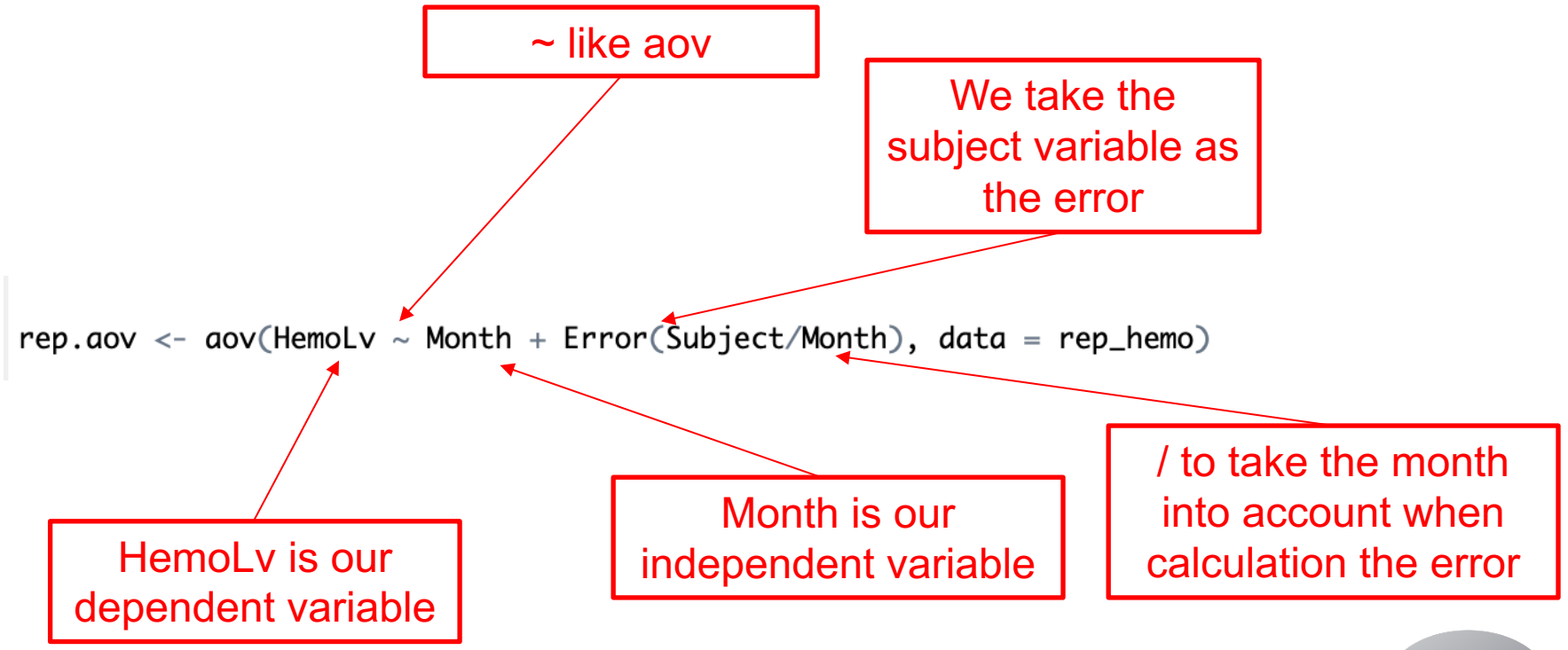
Repeated Measures ANOVA

- Anova model for the repeated measures

```
rep.aov <- aov(HemoLv ~ Month + Error(Subject/Month), data = rep_hemo)
```

Repeated Measures ANOVA

- Anova model for the repeated measures



Repeated Measures ANOVA

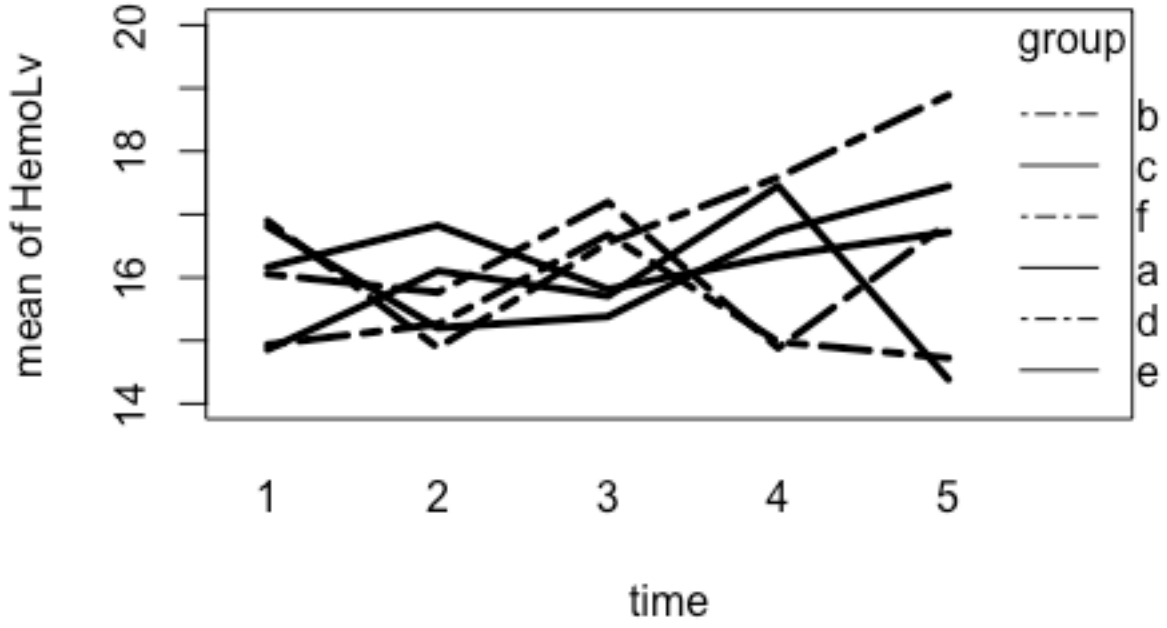
- For the assumption of the repeated measures for one-way ANOVA, we test:
- Normality
  - Shapiro-Wilk test
- Sphericity
  - Mauchly test (for 2 or more factors)

Repeated Measures ANOVA

- Load the 'Rep_hemo_exp.csv' dataset
- Run the anova with a modified model
- Run the shapiro-wilk test
- Plot the means for interpretation

Repeated Measures ANOVA

- Plot of the means



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Model Validation

- Like the t-test, we must test our model for:
  - Normality
  - Independence
  - Heteroscedascity

Model Validation

- Our validation varies according to which ANOVA we are dealing with:
  - One-way, unbalanced, blocking:
    - Normality: Normal Probability plot of residuals
    - Heteroscedascity: Plotting the residuals against time
    - Independence: Linear regression against the residuals
  - Random effects:
    - Normality: normal qq plots Tukey-Ascombe plot
    - Heteroscedascity: residuals against time
    - Independence:qqplot of residuals

Model Validation

- Our validation varies according to which ANOVA we are dealing with:
  - Repeated measures:
    - Normality: Shapiro-Wilk test
    - Independence: Plot of residuals against time
    - Sphericity: Mauchly Test

Model Validation

- R commands for each test:
  - Normality: plot()

```
plot(aov.ex1)
```

  - Heteroscedascity: plot(residuals())

```
plot(aov.ex1$res)
```

  - Independence: lm()

```
lm.aov1<- lm(formula = abs(aov.ex1$res) ~ group)
```

Model Validation

- R commands for each test:
  - Normality: plot()

```
plot(aov.ex1)
```

  - Heteroscedascity: plot(residuals())

```
plot(aov.ex1$res)
```

Residuals column of the anova

  - Independence: lm()

Don't forget the ~

Desired variable

Function call

```
lm.aov1<- lm(formula = abs(aov.ex1$res) ~ group)
```

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Power test for ANOVA

- We will use the power.anova.test in R, for that we need:
  - Number of groups
  - Between group variance
  - Within group variance
  - Confidence level α
  - Sample size or desired power β

Power test for ANOVA

- Using our randomized experiment example:

```
#calculate the within group variance
hemo_wvar <- anova(aov.ex1)["Residuals", "Mean Sq"]
#do the power test
p <- power.anova.test(groups = length(colMeans(hemo)),  between.var = var(hemo_means),
                      within.var = hemo_wvar,  power=0.85, sig.level=0.05, n=NULL)
```

Between group variance

Desired power

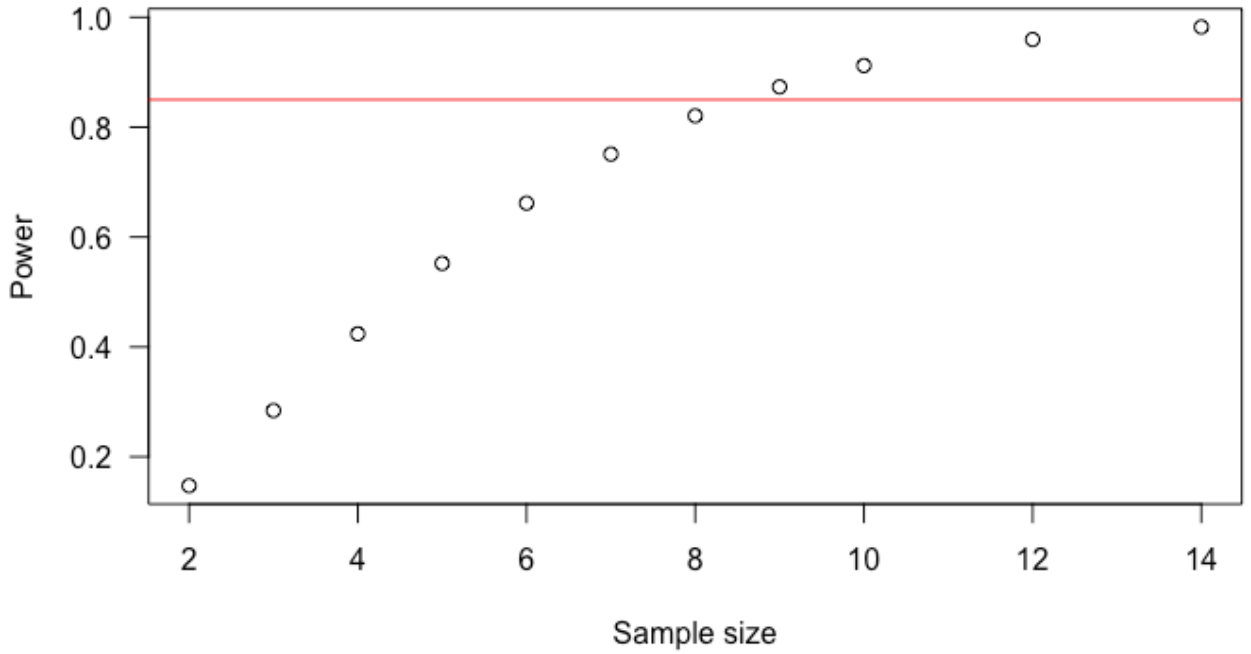
Within group variance

```
n<- ceiling(p$n)
#do the power test with sample size
p2 <- power.anova.test(groups = length(colMeans(hemo)),  between.var = var(hemo_means), within.var = hemo_wvar, sig.level=0.05, n=n)
```

Calculated sample size

Power test for ANOVA

- Plotting different sample sizes to measure the increase in power



Next Episode

- ANOVA is not over yet! We will talk about the general case of ANOVA (MANOVA, two-way)
- However, we must understand how to fit linear models (linear regression)
- Next stop, Linear Regression!
- And a brief explanation about single-factor ANCOVA

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