

BI476: Biostatistics - Case Studies

Lec04: Clinical Trial Data Analysis

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Outline

- 1 Parametric tests
 - t-test
 - ANOVA
 - ANCOVA
 - Repeated Measure ANOVA
- 2 Nonparametric tests
- 3 Permutation or Resampling Approaches

A Clinical Trial on Diastolic Blood Pressure (DBP)

Here we present a data set of diastolic blood pressure measured in small clinical trials in hypertension from the mid-to-late 1960s and for approximately a decade thereafter. Diastolic blood pressure (DBP) was measured (mmHg) in the supine position at baseline (i.e., “DBP1”) before randomization and monthly thereafter up to 4 months as indicated by DBP2, DBP3, DBP4 and DBP5. Patients’ age and sex were recorded at baseline and represent potential covariates.

The primary objective in the analysis of this dataset is to test whether treatment A (new drug) may be effective in lowering DBP as compared to B (placebo) and to describe changes in DBP across the times at which it was measured.

Importing the dataset

```
options(contrast=c("contr.sum", "contr.poly"))
dbp <- read.table("data/dbp.txt", header=T)
dbp$diff <- dbp$DBP5 - dbp$DBP1
head(dbp)
```

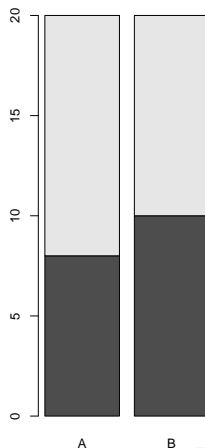
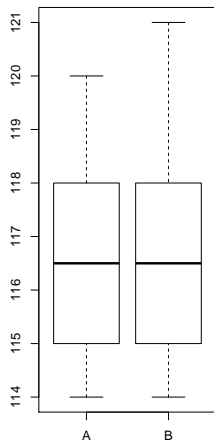
```
##      Subject TRT DBP1 DBP2 DBP3 DBP4 DBP5 Age Sex diff
## 1          1   A  114  115  113  109  105  43   F   -9
## 2          2   A  116  113  112  103  101  51   M  -15
## 3          3   A  119  115  113  104   98  48   F  -21
## 4          4   A  115  113  112  109  101  42   F  -14
## 5          5   A  116  112  107  104  105  49   M  -11
## 6          6   A  117  112  113  104  102  47   M  -15
```

```
table(dbp$TRT)
```

```
##
##   A   B
## 20  20
```

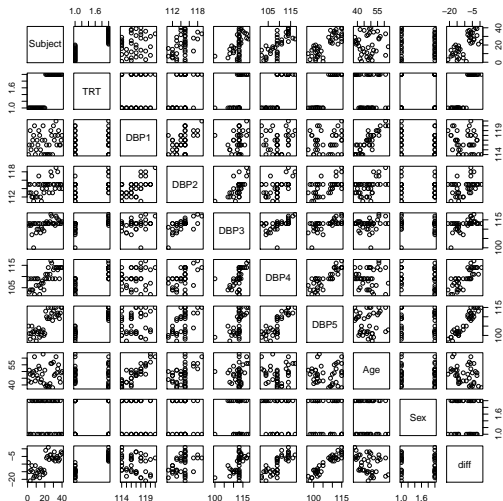
Baseline Information

```
par(mfrow=c(1,2))  
boxplot(DBP1 ~ TRT, data=dbp)  
barplot(table(dbp$Sex, dbp$TRT))
```



Pairwise plot

`pairs (dbp)`



Next Section ...

1 Parametric tests

- t-test
- ANOVA
- ANCOVA
- Repeated Measure ANOVA

2 Nonparametric tests

3 Permutation or Resampling Approaches

Parametric tests

- 2-groups: t-test for continuous outcome in completely randomized parallel design.
- 2-groups: Paired t-test for continuous outcome in crossover design.
- 3+-groups: One-way ANOVA for continuous outcome in completely randomized parallel design.
- 2+-groups: Two-way ANOVA for continuous outcome in factorial design.
- 3+-groups: One-way repeated-measures ANOVA for continuous outcome in randomized block design.
- 2-groups: Chisquare test or Fisher's exact test for binary outcome in completely randomized parallel design.
- 2-groups: McNemar's test for binary outcome in crossover design.
- 3+-groups: Cochran's Q-test for binary outcome in crossover design.

Student's t-test for parallel design

Comparing two treatment group means with equal variances

- **Assumption:** Y_1 and Y_2 are independent and normally distributed with common variance σ^2 .
- **Design:** Randomized parallel design
- **Hypothesis:** $H_0 : \mu_1 = \mu_2$ vs. $H_1 : \mu_1 \neq \mu_2$
- **Process**
 - ▶ Compute the test statistic:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{s\sqrt{1/n_1 + 1/n_2}}$$

, where

$$\bar{y}_i = \sum_{j=1}^{n_i} y_{ij}/n_i, s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}, s_i^2 = \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2 / (n_i - 1)$$

- ▶ Reject H_0 if $t > t_{\alpha/2, n_1+n_2-2}$

```
t.test(..., var.equal=TRUE)
```

t-test with equal variances

```
t.test(diff ~ TRT, data=dbp, var.equal=TRUE)

##
## Two Sample t-test
##
## data: diff by TRT
## t = -12.15, df = 38, p-value = 1.169e-14
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -12.132758 -8.667242
## sample estimates:
## mean in group A mean in group B
## -15.2 -4.8
```

Welch's t-test for parallel design

Comparing two treatment group means with unequal variances

- 1 Compute the t statistic

$$T = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}}$$

- 2 The degree of freedom

$$\nu = \left[\frac{c}{n_1 - 1} + \frac{(1 - c)^2}{n_2 - 1} \right]^{-1}$$

where

$$c = \frac{s_1^2/n_1}{s_1^2/n_1 + s_2^2/n_2}$$

- 3 Reject H_0 if $|T| > t_{\alpha/2, \nu}$

```
t.test(..., var.equal=FALSE)
```

t-test with unequal variances

```
t.test(diff ~ TRT, data=dbp, var.equal=FALSE)

##
##  Welch Two Sample t-test
##
## data:  diff by TRT
## t = -12.15, df = 36.522, p-value = 2.149e-14
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -12.135063  -8.664937
## sample estimates:
## mean in group A mean in group B
##           -15.2           -4.8
```

Wait...Are the two variances equal?

```
var.test(diff ~ TRT, data=dbp)

##
##  F test to compare two variances
##
## data:  diff by TRT
## F = 1.5036, num df = 19, denom df = 19, p-value = 0.3819
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.595142 3.798764
## sample estimates:
## ratio of variances
##           1.503597
```

One-sided t-test

Since “B” is a placebo, the one-sided t-test may be more appropriate to test the treatment effect:

```
# data from treatment A and B
diff.A <- dbp$diff[dbp$TRT=='A']
diff.B <- dbp$diff[dbp$TRT=='B']
# call t.test for one-sided test
t.test(diff.A, diff.B, alternative="less")
```

```
##
## Welch Two Sample t-test
##
## data: diff.A and diff.B
## t = -12.15, df = 36.522, p-value = 1.074e-14
## alternative hypothesis: true difference in means is less than 0
## 95 percent confidence interval:
##      -Inf -8.955466
## sample estimates:
## mean of x mean of y
##      -15.2      -4.8
```

One-way ANOVA

The single factor A has k levels: A_1, A_2, \dots, A_k , and n patients are allocated to each treatment group. We can obtain the samples:

$$y_{i1}, \dots, y_{in}, i = 1, \dots, k$$

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Fundamental statistics

One-way ANOVA

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$$y_{i1}, \dots, y_{in}, i = 1, \dots, k$$

Fundamental statistics

- Grand mean: $\bar{y}_* = \frac{1}{kn} \sum_{i=1}^k \sum_{j=1}^n y_{ij}$
- Marginal mean: $\bar{y}_i = \frac{1}{n} \sum_{j=1}^n y_{ij}$;

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Fundamental statistics

- Grand mean: $\bar{y}_* = \frac{1}{kn} \sum_{i=1}^k \sum_{j=1}^n y_{ij}$
- Marginal mean: $\bar{y}_i = \frac{1}{n} \sum_{j=1}^n y_{ij}$;
- Total sum of squares (SST): $SS_T = \sum_{i=1}^k \sum_{j=1}^n (y_{ij} - \bar{y}_*)^2$;
- Between-group sum of squares (SSB): $SS_B = n \sum_{i=1}^k (\bar{y}_i - \bar{y}_*)^2$;
- Within-group sum of squares (SSE): $SS_E = \sum_{i=1}^k \sum_{j=1}^n (y_{ij} - \bar{y}_i)^2$;

One-way ANOVA Table

Table : One-way ANOVA Table with k groups and n subjects

Variance	SS	DF	MS	F -value
Between-groups	SS_b	$k - 1$	MS_b	$F = MS_b / MS_w$
Within-groups	SS_w	$n - k$	MS_w	
Total	SS_T	$n - 1$	MS_T	

One-way ANOVA for Time Changes

Since the treatment period in the DBP trial was measured at months 1, 2, 3 and 4 post baseline. To see the mean changes over the periods:

```
aggregate(dbp[,3:7], list(TRT=dbp$TRT), mean)
```

```
##      TRT    DBP1  DBP2  DBP3  DBP4  DBP5
## 1     A 116.55 113.5 110.70 106.25 101.35
## 2     B 116.75 115.2 114.05 112.45 111.95
```

Now we can employ the one-way ANOVA to test the change over time. But the first thing is to “reshape” the data:

```
Dbp <- reshape(dbp, direction="long",
               varying=paste("DBP", 1:5, sep=""),
               idvar = c("Subject", "TRT", "Age", "Sex", "diff"), sep="")
colnames(Dbp) <- c("Subject", "TRT", "Age", "Sex", "diff", "Time", "DBP")
rownames(Dbp) <- NULL
Dbp$Time <- as.factor(Dbp$Time)
head(Dbp, 3)
```

```
##      Subject TRT Age Sex diff Time DBP
## 1          1  A  43  F   -9    1 114
## 2          2  A  51  M  -15    1 116
## 3          3  A  48  F  -21    1 119
```

One-way ANOVA for Two Different Treatments

```
# test for treatment A
dbpA <- Dbp[Dbp$TRT=='A',]
test.A <- aov(DBP ~ Time, dbpA)
summary(test.A)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Time          4 2879.7    719.9     127 <2e-16 ***
## Residuals    95   538.5      5.7
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# test for treatment B
dbpB <- Dbp[Dbp$TRT=='B', ]
test.B <- aov(DBP ~ Time, dbpB)
summary(test.B)
```

```
##              Df Sum Sq Mean Sq F value  Pr(>F)
## Time          4   311.6    77.89   17.63 7.5e-11 ***
## Residuals    95   419.8     4.42
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Post-hoc Tests for ANOVA of treatment A

TukeyHSD (test.A)

```
##      Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = DBP ~ Time, data = dbpA)
##
## $Time
##      diff          lwr          upr      p adj
## 2-1   -3.05   -5.143586   -0.9564144 0.0009687
## 3-1   -5.85   -7.943586   -3.7564144 0.0000000
## 4-1  -10.30  -12.393586   -8.2064144 0.0000000
## 5-1  -15.20  -17.293586  -13.1064144 0.0000000
## 3-2   -2.80   -4.893586   -0.7064144 0.0030529
## 4-2   -7.25   -9.343586   -5.1564144 0.0000000
## 5-2  -12.15  -14.243586  -10.0564144 0.0000000
## 4-3   -4.45   -6.543586   -2.3564144 0.0000005
## 5-3   -9.35  -11.443586   -7.2564144 0.0000000
## 5-4   -4.90   -6.993586   -2.8064144 0.0000000
```

Post-hoc Tests for ANOVA of treatment B

TukeyHSD (test.B)

```
##      Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = DBP ~ Time, data = dbpB)
##
## $Time
##      diff      lwr      upr    p adj
## 2-1 -1.55 -3.398584  0.2985843 0.1440046
## 3-1 -2.70 -4.548584 -0.8514157 0.0009333
## 4-1 -4.30 -6.148584 -2.4514157 0.0000000
## 5-1 -4.80 -6.648584 -2.9514157 0.0000000
## 3-2 -1.15 -2.998584  0.6985843 0.4207789
## 4-2 -2.75 -4.598584 -0.9014157 0.0007122
## 5-2 -3.25 -5.098584 -1.4014157 0.0000400
## 4-3 -1.60 -3.448584  0.2485843 0.1223788
## 5-3 -2.10 -3.948584 -0.2514157 0.0176793
## 5-4 -0.50 -2.348584  1.3485843 0.9433857
```

Two-way ANOVA With Interaction

The DBP trial has two factors: treatment and Time. Under this situation, one-way ANOVA (within treatment groups across Time) cannot capture the interaction between these two factors.

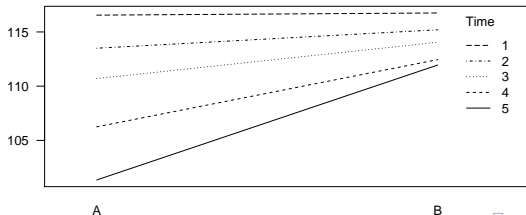
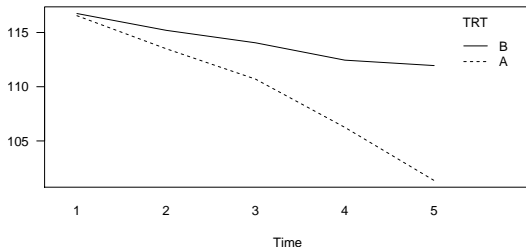
Therefore, a two-way or multi-way ANOVA is needed to analyze the interaction before making statistical inferences about the main effects.

```
mod <- aov(DBP ~ TRT*Time, Dbp)
summary(mod)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## TRT           1  972.4    972.4   192.81 <2e-16 ***
## Time          4 2514.1    628.5   124.62 <2e-16 ***
## TRT:Time       4  677.1    169.3    33.56 <2e-16 ***
## Residuals    190  958.2         5.0
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


Plot Interaction Between Time and Treatment

```
par(mfrow=c(2,1), mar=c(5,3,1,1))  
with(Dbp, interaction.plot(Time,TRT,DBP,las=1,legend=T))  
with(Dbp, interaction.plot(TRT,Time,DBP,las=1,legend=T))
```



Post-Hoc Analysis

```
TukeyHSD(aov(DBP ~ TRT*Time, Dbp))
```

```
##      Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = DBP ~ TRT * Time, data = Dbp)
##
## $TRT
##      diff      lwr      upr p adj
## B-A 4.41 3.783529 5.036471      0
##
## $Time
##      diff      lwr      upr      p adj
## 2-1 -2.300 -3.683042 -0.9169576 0.0000816
## 3-1 -4.275 -5.658042 -2.8919576 0.0000000
## 4-1 -7.300 -8.683042 -5.9169576 0.0000000
## 5-1 -10.000 -11.383042 -8.6169576 0.0000000
## 3-2 -1.975 -3.358042 -0.5919576 0.0011017
## 4-2 -5.000 -6.383042 -3.6169576 0.0000000
## 5-2 -7.700 -9.083042 -6.3169576 0.0000000
## 4-3 -3.025 -4.408042 -1.6419576 0.0000001
## 5-3 -5.725 -7.108042 -4.3419576 0.0000000
## 5-4 -2.700 -4.083042 -1.3169576 0.0000022
##
```

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- One-way/two-way/Multi-way ANOVA with uncontrollable but measurable independent variable X ;
- The outcome Y is continuous;
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- Here we does not control for X in the trials, but conduct a post-hoc analysis.

Example

- Initial blood pressure (X) for the BP reductions (Y) in comparing different blood pressure medications (G).
- Parallel-group clinical trials with pre-treatment baselines.

One-way ANCOVA

$$(x_{i1}, y_{i1}), \dots, (x_{is}, y_{is}), i = 1, \dots, r$$

One-way ANCOVA

$$(x_{i1}, y_{i1}), \dots, (x_{is}, y_{is}), i = 1, \dots, r$$

Fundamental statistics

One-way ANCOVA

$$(x_{i1}, y_{i1}), \dots, (x_{is}, y_{is}), i = 1, \dots, r$$

Fundamental statistics

- $\bar{x}_{i*} = \frac{1}{s} \sum_{j=1}^s x_{ij}$; $\bar{x}_{**} = \frac{1}{rs} \sum_{i=1}^r \sum_{j=1}^s x_{ij}$
- $\bar{y}_{i*} = \frac{1}{s} \sum_{j=1}^s y_{ij}$; $\bar{y}_{**} = \frac{1}{rs} \sum_{i=1}^r \sum_{j=1}^s y_{ij}$

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- $\bar{y}_{i*} = \frac{1}{s} \sum_{j=1}^s y_{ij}; \bar{y}_{**} = \frac{1}{rs} \sum_{i=1}^r \sum_{j=1}^s y_{ij}$
- $SST(x) = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{**})^2;$
- $SSA(x) = s \sum_{i=1}^r (x_{i*} - x_{**})^2;$
- $SSE(x) = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{i*})^2;$

One-way ANCOVA

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- $SSE(x) = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{i*})^2;$
- $SST(y) = \sum_{i=1}^r \sum_{j=1}^s (y_{ij} - y_{**})^2;$
- $SSA(y) = s \sum_{i=1}^r (y_{i*} - y_{**})^2;$
- $SSE(y) = \sum_{i=1}^r \sum_{j=1}^s (y_{ij} - y_{i*})^2;$

One-way ANCOVA

$$(x_{i1}, y_{i1}), \dots, (x_{is}, y_{is}), i = 1, \dots, r$$

Fundamental statistics

- $\bar{x}_{i*} = \frac{1}{s} \sum_{j=1}^s x_{ij}; \bar{x}_{**} = \frac{1}{rs} \sum_{i=1}^r \sum_{j=1}^s x_{ij}$
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- $SST(x) = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{**})^2;$
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- $SST(y) = \sum_{i=1}^r \sum_{j=1}^s (y_{ij} - y_{**})^2;$
- $SSA(y) = s \sum_{i=1}^r (y_{i*} - y_{**})^2;$
- $SSE(y) = \sum_{i=1}^r \sum_{j=1}^s (y_{ij} - y_{i*})^2;$
- $SPT = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{**})(y_{ij} - y_{**});$
- $SPA = s \sum_{i=1}^r (x_{i*} - x_{**})(y_{i*} - y_{**});$
- $SPE = \sum_{i=1}^r \sum_{j=1}^s (x_{ij} - x_{i*})(y_{ij} - y_{i*}).$

One-way ANCOVA: Procedure

- 1 Compute the above statistics and group them into a table:

Variance	$SS(x)$	$SS(y)$	SP	DF
inter-group	$SSA(x)$	$SSA(y)$	SPA	$r - 1$
intra-group	$SSE(x)$	$SSE(y)$	SPE	$r(s - 1)$
Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$

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inter-group	$SSA(x)$	$SSA(y)$	SPA	$r - 1$
intra-group	$SSE(x)$	$SSE(y)$	SPE	$r(s - 1)$
Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$

- 2 Compute the intra-group regression coefficient $\beta = \frac{SPE}{SSE(x)}$;

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intra-group	$SSE(x)$	$SSE(y)$	SPE	$r(s - 1)$
Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$

- 2 Compute the intra-group regression coefficient $\beta = \frac{SPE}{SSE(x)}$;
- 3 If significant, adjust for the linear regression:

$$\bar{y}_{i*}(x = \bar{x}_{**}) = \bar{y}_{i*} - \beta(\bar{x}_{i*} - \bar{x}_{**})$$

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inter-group	$SSA(x)$	$SSA(y)$	SPA	$r - 1$
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Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$

- 2 Compute the intra-group regression coefficient $\beta = \frac{SPE}{SSE(x)}$;
- 3 If significant, adjust for the linear regression:

$$\bar{y}_{i*}(x = \bar{x}_{**}) = \bar{y}_{i*} - \beta(\bar{x}_{i*} - \bar{x}_{**})$$

- 4 $Q_E = SSE(y) - \frac{(SPE)^2}{SSE(x)}$;

One-way ANCOVA: Procedure

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Variance	$SS(x)$	$SS(y)$	SP	DF
inter-group	$SSA(x)$	$SSA(y)$	SPA	$r - 1$
intra-group	$SSE(x)$	$SSE(y)$	SPE	$r(s - 1)$
Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$

- 2 Compute the intra-group regression coefficient $\beta = \frac{SPE}{SSE(x)}$;
- 3 If significant, adjust for the linear regression:

$$\bar{y}_{i*}(x = \bar{x}_{**}) = \bar{y}_{i*} - \beta(\bar{x}_{i*} - \bar{x}_{**})$$

- 4 $Q_E = SSE(y) - \frac{(SPE)^2}{SSE(x)}$;
- 5 $Q_T = SST(y) - \frac{(SPT)^2}{SST(x)}$;

One-way ANCOVA: Procedure

- 1 Compute the above statistics and group them into a table:

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- 6 $Q_A = Q_T - Q_E$;

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- 7 $MQ_A = \frac{Q_A}{r-1}$; $MQ_E = \frac{Q_E}{r(s-1)}$

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- 7 $MQ_A = \frac{Q_A}{r-1}$; $MQ_E = \frac{Q_E}{r(s-1)}$
- 8 $F = \frac{MQ_A}{MQ_E} \sim F_{r-1, r(s-1)-1}$

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Total	$SST(x)$	$SST(y)$	SPT	$rs - 1$



Table : Adjusted ANCOVA Table

Variance	SS	DF	MSS	F	Significance
inter-group	Q_A	$r - 1$	MQ_A	F	
intra-group	Q_E	$r(s - 1) - 1$	MQ_E		
Total	Q_T	$rs - 2$			

One-way ANCOVA: Procedure

Variance	$SS(x)$	$SS(y)$	SP	DF
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Conclusion

Two-way ANCOVA: No Interaction

- Two factors $A : A_1, \dots, A_r$ and $B : B_1, \dots, B_s$;

Two-way ANCOVA: No Interaction

- Two factors $A : A_1, \dots, A_r$ and $B : B_1, \dots, B_s$;
- Continuous covariate X ;

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- Two factors $A : A_1, \dots, A_r$ and $B : B_1, \dots, B_s$;
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Table : Adjusted ANCOVA Table Without Interaction

Variance	SS	DF	MSS	F	Significance
inter-A	Q_A	$r - 1$	MQ_A	F_A	
inter-B	Q_B	$s - 1$	MQ_B	F_B	
intra-group	Q_E	$(r - 1)(s - 1) - 1$	MQ_E		
Total	Q_T	$rs - 2$			

Two-way ANCOVA: With Interaction

- Two factors $A : A_1, \dots, A_r$ and $B : B_1, \dots, B_s$;

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Table : Adjusted ANCOVA Table With Interaction

Variance	SS	DF	MSS	F	Significance
inter-A	Q_A	$r - 1$	MQ_A	F_A	
inter-B	Q_B	$s - 1$	MQ_B	F_B	
inter-AB	Q_{AB}	$(r - 1)(s - 1)$	MQ_{AB}	F_{AB}	
intra-group	Q_E	$rs(m - 1) - 1$	MQ_E		
Total	Q_T	$rsm - 2$			

Analysis of DBP Change from Baseline with ANCOVA

- We now analyze the change from baseline in DBP at the end of trial which is defined as “diff”.
- We start from the full model containing all “covariates”.
- Perform backward stepwise model selection to simplify the model:

```
# start with the full model
m0 <- lm(diff ~ TRT*Age*Sex, dbp)
# stepwise model selection
m1 = step(m0)

## Start:  AIC=79.52
## diff ~ TRT * Age * Sex
##
##              Df Sum of Sq    RSS    AIC
## - TRT:Age:Sex  1      2.7059 198.47  78.07
## <none>                195.76  79.52
##
## Step:  AIC=78.07
## diff ~ TRT + Age + Sex + TRT:Age + TRT:Sex + Age:Sex
##
##              Df Sum of Sq    RSS    AIC
## - TRT:Sex      1      1.3256 199.79  76.336
## - TRT:Age      1      9.5638 208.03  77.952
## <none>                198.47  78.070
```

ANCOVA Analysis of the Changes from Baseline

```
# fit the reduced model
m2 <- lm(diff ~ TRT + Age, dbp)
# output the anova result
anova(m2)

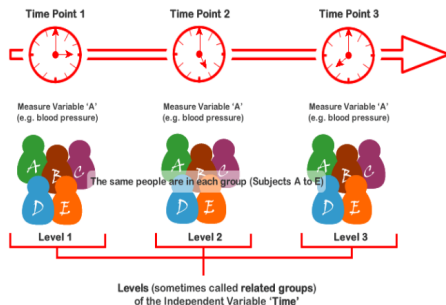
## Analysis of Variance Table
##
## Response: diff
##          Df Sum Sq Mean Sq  F value    Pr(>F)
## TRT        1 1081.60  1081.60  176.0395 1.228e-15 ***
## Age         1   51.07   51.07    8.3119 0.006525 **
## Residuals  37   227.33    6.14
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

What is repeated-measure ANOVA?

- The equivalent of the one-way ANOVA for correlated/non-independent groups
 - ▶ Extension of paired t -test;
 - ▶ Within-subjects ANOVA;
 - ▶ ANOVA for correlated samples;

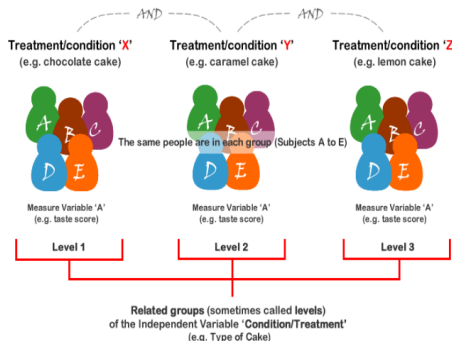
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When to use repeated-measure ANOVA?

- Changes in mean scores over three or more time points;
- Differences in mean scores under three or more different conditions.

Logics of Repeated Measures ANOVA

- ANOVA partitions total variability (SS_T) into between-groups variability (SS_b) and within-groups variability (SS_w)

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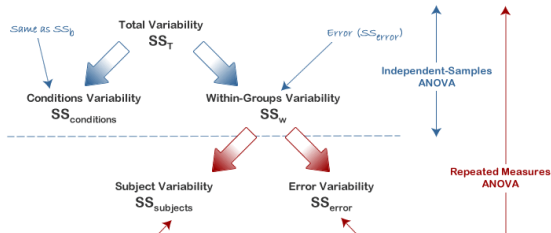
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- Independent ANOVA:** $F = \frac{MS_b}{MS_w} = \frac{MS_b}{MS_{error}}$
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- $SS_{error} = SS_w - SS_{subject} = SS_T - SS_{condition} - SS_{subject}$

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- $SS_{error} = SS_w - SS_{subject} = SS_T - SS_{condition} - SS_{subject}$
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- **Repeated measures ANOVA:** $F = \frac{MS_b}{MS_w} = \frac{MS_{condition}}{MS_{error}}$
- $SS_{error} = SS_w - SS_{subject} = SS_T - SS_{condition} - SS_{subject}$
- $MS_{error} = SS_{error} / (k - 1)(n - 1)$, where k is the number of groups (time points or treatments), and n is the number of subjects.
- $SS_{subject} = k \times \sum_{i=1}^n (\bar{x}_i - \bar{x})^2$, where \bar{x}_i is the mean for subject i .

Repeated-measures ANOVA for DBP Trial

```
Dbp2 <- reshape(dbp, direction="long",  
  varying=paste("DBP", 2:5, sep=""),  
  idvar = c("Subject", "TRT", "Age", "Sex", "DBP1"), sep="")  
rownames(Dbp2) <- NULL  
head(Dbp2)
```

##	Subject	TRT	DBP1	Age	Sex	diff	time	DBP
## 1	1	A	114	43	F	-9	2	115
## 2	2	A	116	51	M	-15	2	113
## 3	3	A	119	48	F	-21	2	115
## 4	4	A	115	42	F	-14	2	113
## 5	5	A	116	49	M	-11	2	112
## 6	6	A	117	47	M	-15	2	112

Repeated-measures ANOVA for DBP Trial

```
m2 <- aov(DBP ~ DBP1 + TRT*time + Error(Subject/time), data=Dbp2)
summary(m2)
```

##

Error: Subject

	Df	Sum Sq	Mean Sq
DBP1	1	978.1	978.1

##

Error: Subject:time

	Df	Sum Sq	Mean Sq
time	1	535.7	535.7

##

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
DBP1	1	8.0	8.0	1.567	0.213
TRT	1	252.0	252.0	49.397	6.47e-11 ***
time	1	1169.5	1169.5	229.242	< 2e-16 ***
TRT:time	1	96.9	96.9	19.000	2.39e-05 ***
Residuals	153	780.5	5.1		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Repeated-measures ANOVA: Pros

- Lower cost
 - ▶ Fewer samples needed
- Increased power
 - ▶ Eliminating the cross-subject variation off the systematic error.

Repeated-measures ANOVA: Cons



Sphericity Assumption of Repeated-measures ANOVA

- The variances of the differences between all possible pairs of groups (i.e., levels of the independent variable) are equal.
- Similar to homogeneity of variance assumption.
- Concerned with different scores.

Testing for sphericity

Mauchly's test, similar to Levene's test.

```
mauchly.test(anova.result)
```

The common solution of deviation is to adjust for degrees of freedom.

- Greenhouse-Geisser correction.

aov () function

```
aov(formula, data=data.frame)
```

Table : Special Symbols

Symbol	Usage
~	The delimiter between response variable and explanatory variables.
+	The delimiter between explanatory variables.
:	The interaction between explanatory variables.
*	All possible interaction terms.
^	The highest order of interaction.
.	All the independent variables.

ANOVA Design Settings

Table : ANOVA Design

Expression	Meaning
$y \sim A$	One-way ANOVA.
$y \sim x + A$	One-way ANCOVA with one covariate x .
$y \sim A * B$	Two-way ANOVA for factorial design.
$y \sim x1 + x2 + A * B$	Two-way ANOVA with two covariates in a factorial design.
$y \sim B + A$	Randomized block design (B is the block factor).
$y \sim A + \text{Error}(\text{Subject})$	Repeated measures ANOVA design.

Pearson's Chi-square test

Comparing categorical outcomes across different treatment groups

- Setting: m treatments, n categories of outcomes
- Compute the test statistic:

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i} \sim \chi^2(df = (m - 1)(n - 1))$$

- Use Yates' correction for lack of continuity When the expected frequencies are too low:

$$\chi_{yates}^2 = \sum_i \frac{(|O_i - E_i| - 0.5)^2}{E_i} \sim \chi^2(df = (m - 1)(n - 1))$$

- Reject H_0 if $\chi^2 > \chi^2(\alpha, df = (m - 1)(n - 1))$

```
prop.test(..., correct=FALSE)
```

Next Section ...

1 Parametric tests

- t-test
- ANOVA
- ANCOVA
- Repeated Measure ANOVA

2 Nonparametric tests

3 Permutation or Resampling Approaches

Nonparametric tests

- 2-groups: Mann-Whitney rank-sum test for continuous outcome in completely randomized parallel design.
- 2-groups: Wilcoxon signed rank test for continuous outcome in crossover design.
- 3+-groups: Kruskal-Wallis rank-sum test for continuous outcome in completely randomized parallel design.
- 3+-groups: Friedman's rank sum test for continuous outcome in randomized block design.
- 2-groups: McNemar's test for binary outcome in crossover design.
- 3+-groups: Cochran's Q-test for binary outcome in crossover design.

Wilcoxon-Mann-Whitney test

Comparing two treatment groups with non-normal data

- The t -test is usually quite robust against departures from normality.
- However, when the departure is extreme, use Mann-Whitney-Wilcoxon U-test (a.k.a Wilcoxon rank-sum test)
- Proposed by Wilcoxon (1945) for equal sample sizes
- Extended to arbitrary sample sizes by Mann and Whitney (1947)
- Virtually identical to performing ordinary parametric two-sample t -test on the combined ranks.

```
wilcox.test(...)
```

Wilcoxon rank-sum test

Nonparametric version of t-test

When the assumption of normality and equal variances are violated, we may use the nonparametric test - Wilcoxon rank-sum test.

```
wilcox.test(diff ~ TRT, data=dbp)

## Warning in wilcox.test.default(x = c(-9L, -15L, -21L, -14L, -11L,
## -15L, : cannot compute exact p-value with ties

##
## Wilcoxon rank sum test with continuity correction
##
## data: diff by TRT
## W = 0, p-value = 6.286e-08
## alternative hypothesis: true location shift is not equal to 0
```

Kruskal-Wallis Test

3+-groups of continuous outcome in randomized parallel design

- Kruskal-Wallis H test; One-way ANOVA on ranks
- Extension of Mann-Whitney U test

Method

- 1 Rank all data from all groups together
- 2 Compute the statistic:

$$H = (N - 1) \frac{\sum_{i=1}^g n_i (\bar{r}_{i*} - \bar{r})^2}{\sum_{i=1}^g \sum_{j=1}^{n_i} (r_{ij} - \bar{r})^2} \sim \chi^2(df = g - 1)$$

where

- ▶ n_i : number of observations in group i ;
- ▶ r_{ij} : rank (among all) of observation j from group i ;
- ▶ N : total number of observations across all groups;
- ▶ $\bar{r}_{i*} = \frac{\sum_{j=1}^{n_i} r_{ij}}{n_i}$ is the average rank of all observations in group i ;
- ▶ $\bar{r} = \frac{N+1}{2}$ is the average of all r_{ij}

Friedman's Q test

3+-groups of continuous outcome in randomized block design

- randomized block design (随机区组设计)
- not necessarily symmetric (不需要满足对称假设)
- Two-way ANOVA by ranks?

Method

- Given data $\{x_{ij}\}_{n \times k}$ with n rows (blocks) and k columns (treatments);
- Replace the data with new matrix $\{r_{ij}\}$, where r_{ij} is the rank of x_{ij} within block i ($r_{ij} = 1, \dots, k$).
- Calculate the values:
 - ▶ $\bar{r}_{*j} = \frac{1}{n} \sum_{i=1}^n r_{ij}$
 - ▶ $\bar{r} = \frac{1}{nk} \sum_{i=1}^n \sum_{j=1}^k r_{ij}$
 - ▶ $SS_t = n \sum_{j=1}^k (\bar{r}_{*j} - \bar{r})^2$
 - ▶ $SS_e = \frac{1}{n(k-1)} \sum_{i=1}^n \sum_{j=1}^k (r_{ij} - \bar{r})^2$
- Calculate the test statistic $Q = \frac{SS_t}{SS_e}$. Q does not need to be adjusted for tied values in the data.
- If $n > 15$ or $k > 4$, $Q \sim \chi^2_{df=k-1}$

Cochrane's Q test

2+ correlated groups of dichotomous outcomes

- Similar to Friedman's test, but with binary outcomes.
- Randomized block design.
- H_0 : The treatments are all equally effective.

Method

The test statistic

$$T = k(k-1) \frac{\sum_{j=1}^k (X_{*j} - \frac{N}{k})^2}{\sum_{i=1}^b X_{i*} (k - X_{i*})} \sim \chi^2_{df=k-1}$$

where

- k : number of treatments;
- X_{*j} : total for j^{th} treatment;
- b : number of blocks;
- X_{i*} : row total for the i^{th} block;
- N : grand total

Next Section ...

1 Parametric tests

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Permutation method

- 1 Iteratively permute the randomization (treatment assignment)
- 2 Compute the statistic of interest for each permuted sample
- 3 Generate the empirical distribution of the statistics
- 4 Compare the original statistic with the permuted ones to determine the empirical p -value
- 5 Reject or not reject the null hypothesis

Exercise

Use `sample()` function to realize the permutation approach.

Bootstrapping method

Bootstrapping is a resampling procedure extensively used in statistics when any of the assumptions underlying the validity of the t -test don't hold for the data under analysis.

- 1 Iteratively drawing samples with replacement from the data.
- 2 Calculating the statistic of interest for each sample
- 3 Generating the empirical resampling distribution of the statistic
- 4 Percentile points corresponding to the Type-I error level and the sided-ness of the alternative hypothesis of the resampling distribution are then used in the assessment of statistical significance.

```
bootstrap::bootstrap()
```

Bootstrapping approach

```
library(bootstrap)
mean.diff <- function(bn, dbp)
  -diff(tapply(dbp$diff[bn], dbp$TRT[bn], mean))
# number of bootstrap
nboot <- 1000
boot.mean <- bootstrap(1:dim(dbp)[1], nboot, mean.diff, dbp)
# extract the mean difference
x <- boot.mean$thetastar
quantile(x, c(0.025, 0.975))

##          2.5%          97.5%
## -12.070833   -8.759351
```

Conclusion

What conclusion can you make from the above result?