DSDHT FALL 2017

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Module 11 – Clinical data analysis in R

1. View the following video in which Abhik Seal describes analyzing a small Clinical Trials dataset using various statistical tests (e.g. student's t test, Welch test, Bootstrap method, One - Way ANOVA and Two - Way ANOVA) and inference, statistically significant variables, and interactions between the variables. https://youtu.be/aqKXf7Kr00E
2. See the GIT repository for the data and the code: https://github.com/abhik1368/dsdht/tree/master/ClinicalDataAnalysis data and code also in the File Folder for Module 11: dbpdata.csv and cda.R
3. Follow along the video and run the R code. Check for and resolve any errors.
4. Interpret and analyze the results (a) statistically and (b) as a biomedical data scientist, in terms comprehensible to a biomedical audience.

Code/Output | **Analysis**

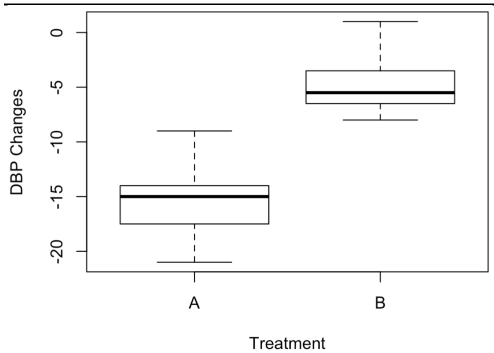
```
dat = read.csv("dbpdata.csv",header=TRUE)
# create the difference
dat$diff = dat$DBP5-dat$DBP1
# print the first a few observation
head(dat)
```

```
  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
```

a) We have data for two treatment groups, a group of people who took a drug “A” (TRT=A), and another group who took a placebo “B”, then we measured their DBP in week 0 (“DBP1”), and weekly for 4 weeks (“DBP2”-“DBP5”). We also tracked age and gender. The column “diff” measures the difference between DBP at last week vs. at the beginning of the study. Can we say the two groups have different “diff” means?

b) Can we find out from the data if drug A is effective in reducing DBP?

```
# call boxplot
boxplot(diff~TRT, dat, xlab="Treatment", ylab="DBP Changes")
```



a) The range for the “diff” data from the two treatment groups does not overlap and the medians appear to be distant enough to indicate the two groups have different means.

b) It appears drug A produced a significant reduction in DBP when compare to the reduction observed for people who took the placebo.

<table>
<tr><th>Code/Output</th><th>Analysis</th></tr>
<tr><td><pre>
# call t-test with equal variance
t.test(diff~TRT, dat, var.equal=T)

        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
</pre></td><td>a) If we assume the treatment groups (samples) were randomly obtained from populations randomly distributed and with equal variance, then the t-test on this data demonstrates that we can reject the null hypothesis that the two samples come from populations with the same mean, at the level of significance of 0.05 (alpha). There is a very small probability, very close to zero, of making a Type I error (p-value is very very small).
b) This is strong evidence that drug A changes DBP more than placebo changes DBP, all above stated assumptions holding. We note that this is a two-tailed t-test, so technically our result does not demonstrate that drug A reduces DBP more than a placebo does.</td></tr>
<tr><td><pre>
# Welch test
t.test(diff~TRT, dat, var.equal=F)

        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
</pre></td><td>c) If we assume the samples were randomly obtained from populations randomly distributed and with UNequal variance, then the Welch t-test on this data demonstrates that we can reject the null hypothesis that the two samples come from populations with the same mean. There is a very small probability, very close to zero, of making a Type I error p-value is very very small).
a) This is further evidence that drug A is more effective in modifying DBP than the placebo is. The assumptions about the populations are less stringent, since they are not required to have the same variance.</td></tr>
<tr><td><pre>
# Ftest
var.test(diff~TRT, dat)

        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
</pre></td><td>a) We want to know if the samples come from populations with the same variance. P-value = 38.19% is not significant at the 95% significance level, so we cannot reject the null hypothesis that the variances are the same.
b) The t-test is better than the Welch t-test to compare the means, which is good, because the p-value for the t-test was almost half smaller than the p-value for the Welch t-test. This is further evidence that drug A is more effective in modifying DBP than the placebo.</td></tr>
</table>

<table>
<tr><th>Code/Output</th><th>Analysis</th></tr>
<tr><td><pre>
wilcox.test(diff~TRT, dat)

      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
</pre></td><td>
a) Can we reject the null hypotheses that the samples came from different populations (different means) regardless of whether the populations are normally distributed and/or have the same variance? The Wilcoxon rank-sum test result demonstrate that yes, we can reject the null hypothesis, at the level of significance of 0.05. There is a very small probability, very close to zero, of making a Type I error (p-value is very very small).
b) This is evern further evidence that drug A is more effective in modifying DBP than the placebo is. The assumptions about the populations are less stringent, since this is a non-parametric test.
</td></tr>
<tr><td><pre>
# data from treatment A
diff.A = dat[dat$TRT=="A",]$diff
# data from treatment B
diff.B = dat[dat$TRT=="B",]$diff
# 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
</pre></td><td>
a) Now we do a one-tail Welch t-test to test the null hypothesis that the difference in DBP after taking the placebo is less than the difference in DBP after taking drug A. Given the data at our disposal, we reject the null hypotheses at the 0.05 significant level. The chance that the placebo cause a difference in DBP less than that of caused by drug A is extremely small (p-value very close to zero).
b) There is strong evidence not only that drug A causes a change to DBP that is higher than the change caused by the placebo, but also that this change is in the direction that we want, i.e., drug A reduces DBP when compared to a placebo. Based on the data at our disposal, the chance of reaching this conclusion in error is close to zero.
</td></tr>
</table>

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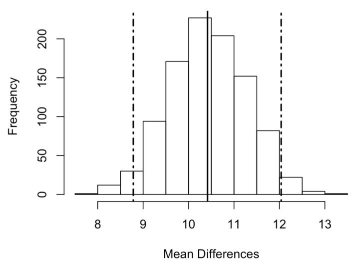
Code/Output

```
# bootstrap to test the significance of the di□ence in DBP
treatment group means
# load the library "bootstrap"
library(bootstrap)
# define a function to calculate the mean difference
#      between treatment groups A to B:
mean.diff = function(bn,dat)
  diff(tapply(dat[bn,]$diff, dat[bn,]$TRT,mean))

# number of bootstrap
nboot     = 1000
# call "bootstrap" function
boot.mean = bootstrap(1:dim(dat)[1], nboot, mean.diff,dat)

# extract the mean differences
x = boot.mean$thetastar
# calcualte the bootstrap quantiles
x.quantile = quantile(x, c(0.025,0.5, 0.975))
# show the quantiles
print(x.quantile)
# make a histogram
hist(boot.mean$thetastar, xlab="Mean Differences", main="")
# add the vertical lines for the quantiles
abline(v=x.quantile,lwd=2, lty=c(4,1,4))
```



Analysis

a) Here we use the bootstrap technique draw 1000 samples from our data and create a distribution of mean differences between DBP decreases observed after 4 weeks taking drug A vs. taking placebo. The mean of the distribution is close to 10.5 indicating that the difference is positive.

b) This is more evidence that drug A is effective in reducing DBP when compared to the placebo.

Code/Output

```
# To see mean changes the months following baseline
aggregate(dat[,3:7], list(TRT=dat$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

Analysis

a) We have compared variations on DBP between month 0 and month 4. What can we tell about month-to-month variations if we look at the monthly DBP mean values?

b) Can we see drug A impact on DBP on a month-to-month basis? We notice that drug A does show a monthly reduction in DBP greater than the placebo, but is it meaningful?

Code/Output

```
#call reshape
Dat = reshape(dat, direction="long",
varying=c("DBP1","DBP2","DBP3","DBP4","DBP5"),
 idvar = c("Subject","TRT","Age","Sex","diff"),sep="")
colnames(Dat) = c("Subject","TRT","Age","Sex","diff","Time","DBP")
Dat$Time = as.factor(Dat$Time)
head(Dat)
```

```
               Subject TRT Age Sex diff Time DBP
1.A.43.F.-9.1        1   A  43   F   -9    1 114
2.A.51.M.-15.1       2   A  51   M  -15    1 116
3.A.48.F.-21.1       3   A  48   F  -21    1 119
4.A.42.F.-14.1       4   A  42   F  -14    1 115
5.A.49.M.-11.1       5   A  49   M  -11    1 116
6.A.47.M.-15.1       6   A  47   M  -15    1 117
```

```
# one-way ANOVA to test the null hypotheses that the means of DBP
at all five times of measurement are equal
# test treatment "A"
datA   = Dat[Dat$TRT=="A",]
test.A = aov(DBP~Time, datA)
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 treatment "B"
datB   = Dat[Dat$TRT=="B",]
test.B = aov(DBP~Time, datB)
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
```

Analysis

a) The month-to-month variations of DBP may not be significant. We will test for the hypothesis that each monthly observation have all the same mean, i.e., we will test for the hypothesis that the month-to-month decrease we observed doesn't represent a real change of any kind as a result of taking drug A or the placebo. We reshape the data for the test and our one-way ANOVA test results show evidence that at the 0.05 significance level we can reject the null hypothesis that the means are equal. That is the case for both drug A and the placebo (surprisingly). The risk of Type I error is close to zero on both cases, but it is 100,000 smaller for drug A.

b) There is very strong evidence not only that drug A is effective in reducing DBP over 4 months, but also that it affects DBP over smaller periods of time, perhaps even monthly.

Code/Output	Analysis

`# To understand the nature of the differences across time, multiple range testing`
`TukeyHSD(test.A)`
Tukey multiple comparisons of means
95% family-wise confidence level
 	
Fit: aov(formula = DBP ~ Time, data = datA)
 	
$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
 	
`TukeyHSD(test.B)`
Tukey multiple comparisons of means
95% family-wise confidence level
 	
Fit: aov(formula = DBP ~ Time, data = datB)
 	
$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
	a) Now that we know that at least one of the means in the 4 months for both drug A and the placebo is different, i.e, at least one month showed variation as a results of drugs A and placebo, we want to look at what month transitions exactly show significant changes in mean values. We use the Tukey test and for drug A the difference between any two months in the range of months from 1-5 shows that we can reject the null hypotheses that there is not mean difference at the 0.05 significance level. For the placebo the same is not true. For months 2-1, 3-2, 4-3, and 5-4, we cannot reject the null hypothesis at the 0.05 significance level.
	b) This is more evidence that drug A is effective. Now we see evidence that it modifies DBP on a monthly basis. Interestingly, while the placebo doesn't modify DBP on a monthly basis, it does modify DBP over a period of two or more months (we can reject the null hypothesis at a 0.05 significance level for the placebo when comparing 2 moths that are more than 1 month apart.)

`# statistical signicance of the interaction betweeb A and B`
`mod2 = aov(DBP~ TRT*Time, Dat)`
`summary(mod2)`
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 the interactions between TRT and Time`
	a) Here we test if the changes observed in DBP values are the result of changes in each independent variable alone (the dependent variables being time and drug) and to what extent these changes can be justified by the interaction between the two dependent variables. The p-values for the ANOVA with interaction test shows that yes, we can, at the 5% significance level. In regards to the plots, the first one shows DBP decreases for both drug types; the second plot shows some interaction between drug and time, since

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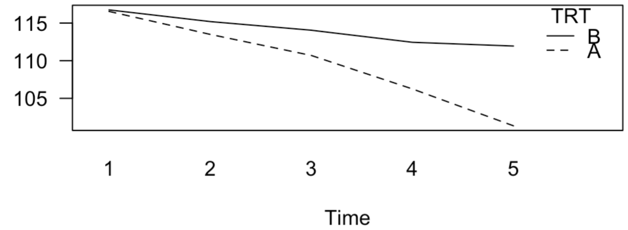
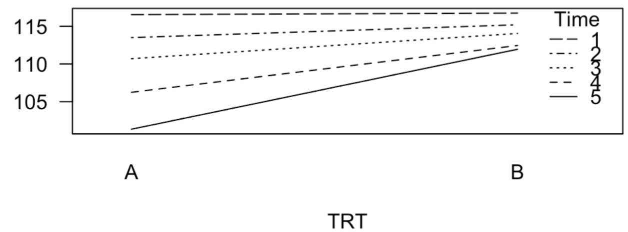
Code/Output

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






```
# Use multiple comparison approach for testing main effects.
TukeyHSD(aov(DBP ~ TRT*Time,Dat))
```

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = DBP ~ TRT * Time, data = Dat)

$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

$`TRT:Time`
          diff        lwr        upr     p adj
B:1-A:1   0.20  -2.0737649   2.4737649 0.9999998
A:2-A:1  -3.05  -5.3237649  -0.7762351 0.0011351
B:2-A:1  -1.35  -3.6237649   0.9237649 0.6684315
```

Analysis

the lines in the plot are not parallel. The Tukey multiple comparisons of means shows that at the 5% significance we can reject the null for most comparisons of means between samples across drug types and time periods.

b) There is strong evidence that the decrease in observed DBP values is associated not with time only, but with intake of drug A vs. the intake of the placebo.

Code/Output	Analysis

<pre>A:3-A:1 -5.85 -8.1237649 -3.5762351 0.0000000
B:3-A:1 -2.50 -4.7737649 -0.2262351 0.0188264
A:4-A:1 -10.30 -12.5737649 -8.0262351 0.0000000
B:4-A:1 -4.10 -6.3737649 -1.8262351 0.0000014
A:5-A:1 -15.20 -17.4737649 -12.9262351 0.0000000
B:5-A:1 -4.60 -6.8737649 -2.3262351 0.0000000
A:2-B:1 -3.25 -5.5237649 -0.9762351 0.0003579
B:2-B:1 -1.55 -3.8237649   0.7237649 0.4723958
A:3-B:1 -6.05 -8.3237649 -3.7762351 0.0000000
B:3-B:1 -2.70 -4.9737649 -0.4262351 0.0072480
A:4-B:1 -10.50 -12.7737649 -8.2262351 0.0000000
B:4-B:1 -4.30 -6.5737649 -2.0262351 0.0000003
A:5-B:1 -15.40 -17.6737649 -13.1262351 0.0000000
B:5-B:1 -4.80 -7.0737649 -2.5262351 0.0000000
B:2-A:2   1.70 -0.5737649   3.9737649 0.3355035
A:3-A:2 -2.80 -5.0737649 -0.5262351 0.0043660
B:3-A:2   0.55 -1.7237649   2.8237649 0.9988534
A:4-A:2 -7.25 -9.5237649 -4.9762351 0.0000000
B:4-A:2 -1.05 -3.3237649   1.2237649 0.8990806
A:5-A:2 -12.15 -14.4237649 -9.8762351 0.0000000
B:5-A:2 -1.55 -3.8237649   0.7237649 0.4723958
A:3-B:2 -4.50 -6.7737649 -2.2262351 0.0000001
B:3-B:2 -1.15 -3.4237649   1.1237649 0.8372192
A:4-B:2 -8.95 -11.2237649 -6.6762351 0.0000000
B:4-B:2 -2.75 -5.0237649 -0.4762351 0.0056388
A:5-B:2 -13.85 -16.1237649 -11.5762351 0.0000000
B:5-B:2 -3.25 -5.5237649 -0.9762351 0.0003579
B:3-A:3   3.35   1.0762351   5.6237649 0.0001963
A:4-A:3 -4.45 -6.7237649 -2.1762351 0.0000001
B:4-A:3   1.75 -0.5237649   4.0237649 0.2948066
A:5-A:3 -9.35 -11.6237649 -7.0762351 0.0000000
B:5-A:3   1.25 -1.0237649   3.5237649 0.7590918
A:4-B:3 -7.80 -10.0737649 -5.5262351 0.0000000
B:4-B:3 -1.60 -3.8737649   0.6737649 0.4247400
A:5-B:3 -12.70 -14.9737649 -10.4262351 0.0000000
B:5-B:3 -2.10 -4.3737649   0.1737649 0.0975920
B:4-A:4   6.20   3.9262351   8.4737649 0.0000000
A:5-A:4 -4.90 -7.1737649 -2.6262351 0.0000000
B:5-A:4   5.70   3.4262351   7.9737649 0.0000000
A:5-B:4 -11.10 -13.3737649 -8.8262351 0.0000000
B:5-B:4 -0.50 -2.7737649   1.7737649 0.9994658
B:5-A:5 10.60 8.3262351 12.8737649 0.0000000</pre>	