L27: 3+ Sample testing with continuous outcomes

April 9, 2020

Announcements

Please fill out the survey!

Skipping around

Reminder, if you are following the textbook, to stick with continuous outcomes, we are going to skip ahead to chapter 24 (ANOVA) and chapter 23(regression) and bring in some outside information about non-parametric testing.

Summary of Continouous outcomes so far - the flavors of T

In R, the t.test function will allow you to conduct any of the t-tests we have covered so far

- One sample T test comparing a sample mean to a hypothesized null (if we know σ and have a large sample we could also consider a Z test) t.test(data, mu=value)
- Two sample T test comparing samples from independent populations t.test(continousvar~categoricalvar) or t.test(data1, data2)
- Two sample T test comparing paired (non-independent) groups of observations t.test(continuousvar~categoricalvar, paired=TRUE) or t.test(data1, data2, paired=TRUE)

Three sample testing

Now that we have looked at how to compare one sample to a value and means between two samples, let's extend this to a case where we have 3 samples or 3 groups that we are interested in comparing.

Today's lecture

- Introducing ANOVA - what is the null hypothesis of this test?
- Visualizing 3 sample data
- Using ANOVA to test for a difference
- Example

ANOVA

ANOVA

ANOVA or analysis of variance can be thought of as an extension of the two-sample t-test to three or more samples.

If we had 3 groups to compare, we could do this by using the two-sample t test multiple times This would result in $\binom{3}{2}$ comparisons:

$\mu_1 \neq \mu_2$ $\mu_2 \neq \mu_3$ $\mu_1 \neq \mu_3$

The problem with that approach is that we would end up with 3 p-values, one for each test performed. That doesn't tell us how likely it is that three sample means are spread apart as far as they are. If we are comparing more than 3 groups, this problem is compounded by creating even more comparisons.

We need a method that allows us to have an overall measure of confidence in all our conclusions about comparisons. This is a common problem of multiple comparisons.

ANOVA

Here we are testing a null hypothesis that all the means are the same, for 3 samples this would be

H_0: $\mu_1 = \mu_2 = \mu_3$

Our alternative hypothesis is that at least one of the means is not equal to the others

Even though your hypothesis involves means, the test compares the variability between groups to the variability within groups

ANOVA

Analysis of variance (ANOVA) is also referred to as the F test.

The ANOVA is based on two kinds of variability: - The variability among sample means or how much the individual group means vary around the overall mean - The variability within groups, how much do individual observation values vary around the group mean

If the variability within the k different populations is small relative to the variability among their respective means, this suggests that the population means are in fact different.

ANOVA

Loosely expressed:

$$F = \left(\frac{variation among sample means}{variation among observations in the same sample} \right)$$

Note: You will NOT need to know the full formula for the F-test, you will need to know how to do one in R

Data

What would the data look like in a data frame?

Data

What would the data look like in a data frame?

- One "grouping" variable (categorical)
- One continuous response variable

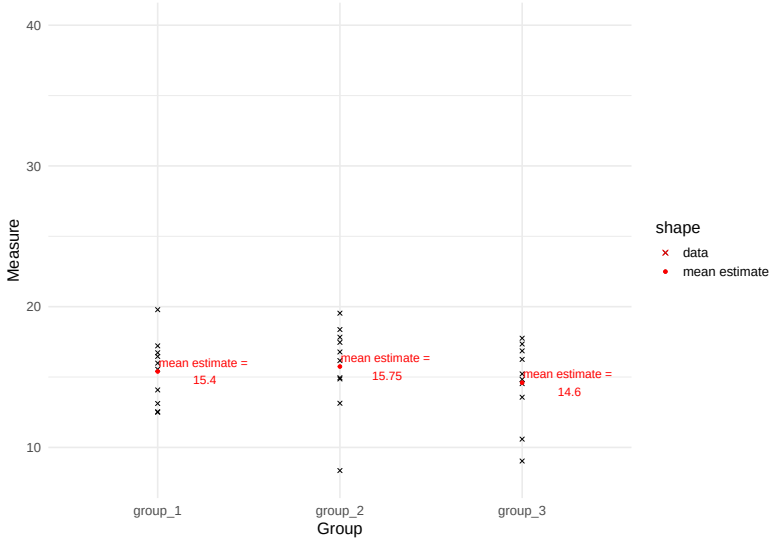
ANOVA asks if there is an association between the grouping variable and the response variable.

Visualizing first

What graphical strategies have we learned to look at variability within and between groups?

A) Is there a difference between these means?

Describe why you do or do not think so.



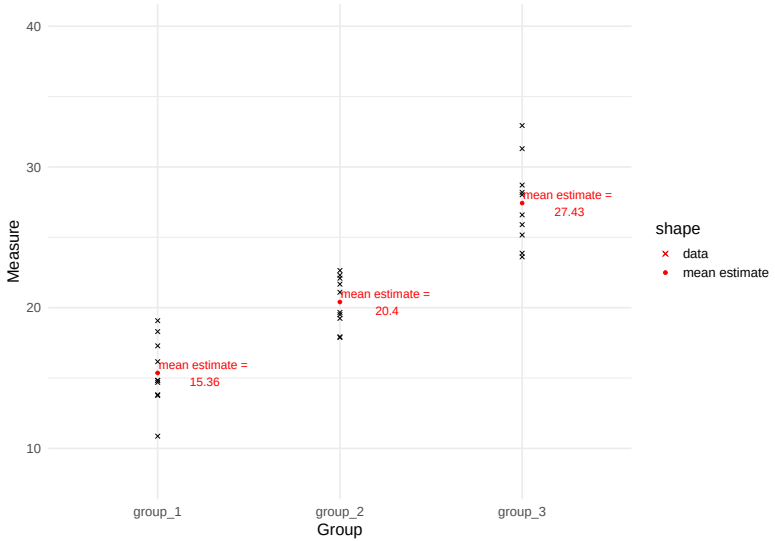
Summary of the plots

Plot (A)

- The means (red dots) were not very different across the groups. This means the variation between the group means was small.
- The distribution of the data (black Xs) was wide enough that the distribution of points for each group overlapped almost completely. This means that the variation within each group was relatively wide

B) Is there a difference between these means?

Describe why you do or do not think so.



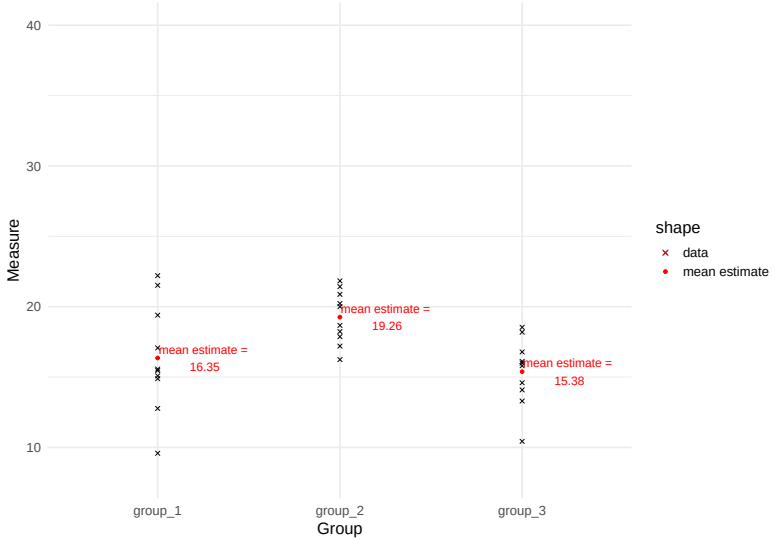
Summary of the plots

Plot (B)

- The means are quite different across the groups. The variation between the group means would be larger than in plot (A)
- The distribution of the data overlaps between groups 1 and 2 and 2 and 3, but not 1 and 3. The variation within each group is as wide as it was in Plot (A) but doesn't mask the mean differences, especially between group 1 and 3

C) Is there a difference between these means?

Describe why you do or do not think so.



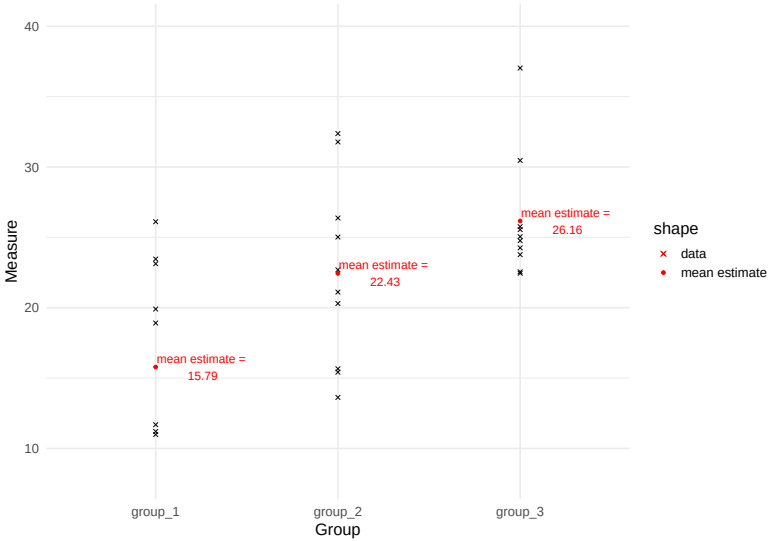
Summary of the plots

Plot (C)

- Here, the means for group 1 and 3 look similar, but the mean for group 2 appears a bit higher than the other two, though there is still overlap between the data from all the groups
- Is there evidence that at least one of the means is different?

D) Is there a difference between these means?

Describe why you do or do not think so.



Summary of the plots

Plot (D)

- Plot (D) looked like Plot (B) but with more variation within groups
- This variation makes the difference between the means harder to detect

Overall summary

- What we informally did on the previous slides was compare the variation between group means to the variation within the groups
- This focus on variation is why this test is called ANOVA: an ANalysis Of VAriance
- When the ratio of between vs. within variation is large enough then we detect a difference between the groups
- When the ratio isn't large enough we don't detect the difference.
- This ratio is our test statistic, denoted by F

Descriptive plots

Descriptive plots

What other ways to present the data visually have we learned that might be useful before we move on to testing?

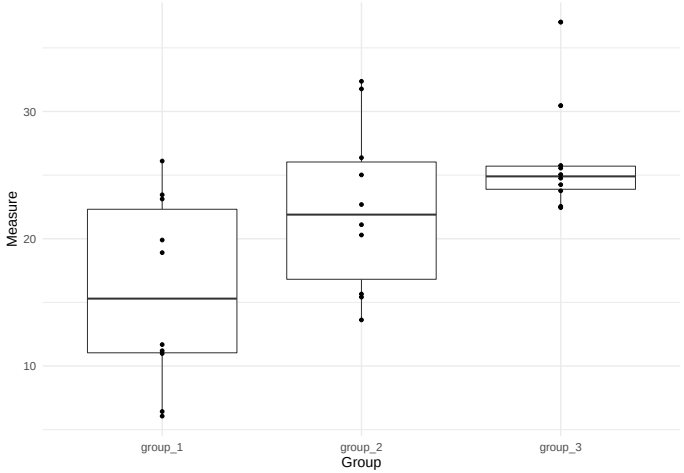
Descriptive plots

How would you want to plot these data before you conduct a test?

- Option 1: Box plot for each level of the grouping variable (with overlaid data points)

```
ggplot(diff_3_narrow, aes(x = Group, y = Measure)) +
geom_boxplot() +
geom_point() +
theme_minimal(base_size = 15)
```

Box plot



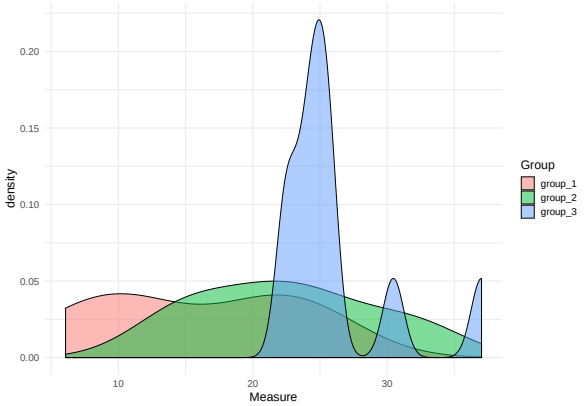
Descriptive plots

How would you want to plot these data before you conduct a test?

- Option 2: Density plot for each level of the grouping variable

```
ggplot(diff_3_narrow, aes(x = Measure)) +
geom_density(aes(fill = Group), alpha = 0.5) +
theme_minimal(base_size = 15)
```

Density plot



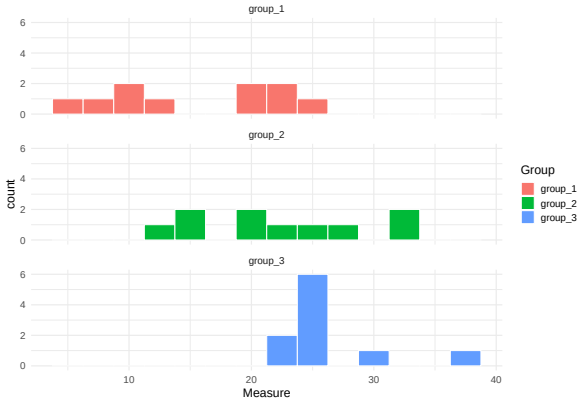
Descriptive plots

How would you want to plot these data before you conduct a test?

- Option 3: Histogram for each level of the grouping variable

```
ggplot(diff_3_narrow, aes(x = Measure)) +
geom_histogram(aes(fill = Group), col = "white", binwidth = 2.5) +
theme_minimal(base_size = 15) +
facet_wrap(~ Group, nrow = 3)
```

Histograms with facet wrap



Testing with ANOVA

The hypotheses

Null hypothesis

$H_0 : \mu_1 = \mu_2 = ...\mu_K$, where K is the number of levels of the grouping variable

- Can you also state the null hypothesis in words?

The hypotheses

Alternative hypothesis

H_a : not all μ_1, μ_2,..., μ_K are equal

- In words: Not all means are the same. Or, at least one of the means differs from the others.

The test statistic (ANOVA F Statistic)

$$F = \frac{\text{variation among group means}}{\text{variation among individuals in the same group}}$$

- Numerator is, fundamentally, the variance of the sample means
- Denominator is, fundamentally, an average of the group variances.
- The F statistic follows an F distribution
- Computation details are at the end of the book chapter (these computation details will not be tested)

The F distribution

- Skewed right
- Take only positive values
- The F distribution depends on the number of means being compared and the sample size for each of the groups
- Let k be the number of groups being compared and $N_{Total} = n_1 + n_2 + ... + n_k$ (the total sample size across all the groups)
- Then the F statistic follows an F distribution with $k - 1$ degrees of freedom in the numerator and $N_{Total} - k$ degrees of freedom in the denominator
- The p-value of the ANOVA F statistic is always the area to the right of the test statistic

ANOVA in R: use `aov()`, then `tidy()` it up!

- `aov()` stands for analysis of variance

The general syntax for the ANOVA is:

aov(outcomevariable ~ groupvaraible, data=dataset)

We will save the output of this as an object and then use tidy(object) to get the output we want.

reference: https://broom.tidyverse.org/reference/anova_tidiers.html

ANOVA in R: use `aov()`, then `tidy()` it up!

We will focus on two parts of the output from this package:

- `statistic` is the F test statistic, the ratio of the variation between means vs. the variation within groups.
- `p.value` is the p-value for the test.

p of an f statistic in R

You can check that you can calculate the p-value from the F distribution. Remember, that you need to specify a degrees of freedom for the numerator and for the denominator:

pf(value, df1=numerator degrees of freedom, df2= denominator degrees of freedom, lower.tail=F)

This general pattern of syntax should look familiar by now....

Conditions for using ANOVA

Conditions for ANOVA

Condition 1: k independent SRSs, one from each of k populations.

- The most important assumption, because this method, like the others in Part III of the course, depends on the premise of having taken a random sample.

Conditions for ANOVA

Condition 2: Each of the k populations has a Normal distribution with an unknown mean μ_i.

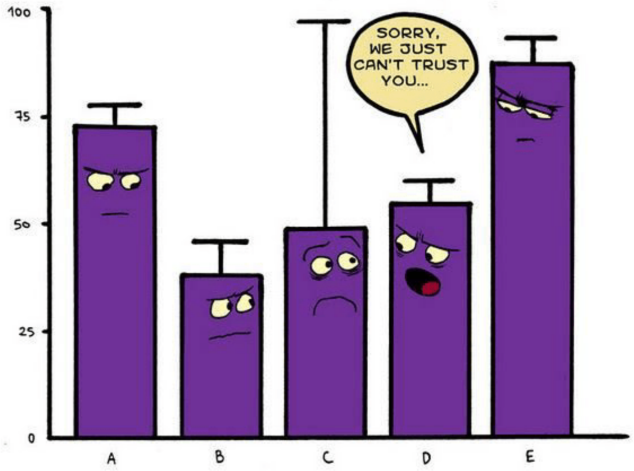
- This assumption is less necessary
- The ANOVA test is robust to non-Normality.
- Remember that the ANOVA is based on comparing the differences of sample means
- What did the CLT tell us about variability of sample means when the samples are not normally distributed?

Conditions for ANOVA

Condition 3: All the populations have the same standard deviation σ, whose value is unknown.

- Hardest condition to satisfy and check
- If this condition is not satisfied ANOVA is often okay if the sample sizes are large enough and if they are similar across the groups
- Can use `group_by()` and `summarize()` to calculate the sample SDs to see if they're similar and indicative that the population parameters are too
- General rule: we want the largest sample standard deviation to be less than twice as largest as the smallest one. I.e., $s_{max}/s_{min} < 2$

Conditions for ANOVA



Example: Cannabis to treat brain cancer

Example: Cannabis to treat brain cancer (in mice)

High-grade glioma is an aggressive type of brain cancer with a low long-term survival rate. Cannabinoids, which are chemical compounds found in cannabis, are thought to inhibit glioma cell growth. Researchers transplanted glioma cells in otherwise-healthy mice, and then randomly assigned these mice to 4 cancer treatments: irradiation alone, cannabinoids, alone, irradiation combined with cannabinoids, or no cancer treatment. The treatments were administered for 21 days, after which the glioma tumor volume (in cubic millimeters) was assessed in each mouse using brain imaging.

The data

```
head(cancer_data)
```

```
##       treatment tumor_volume
## 1  Irradiation           30
## 2  Irradiation           46
## 3  Irradiation           46
## 4  Irradiation           95
## 5 Cannabinoids           12
## 6 Cannabinoids           14
```

Organize the data

- Think about how you want the data to look.
- I want to plot the raw data points and display the mean for each treatment group
- I also want to specify the order that the treatment groups show up in the graph

```
# specify the order of the treatment groups for plotting
library(forcats)
cancer_data <- cancer_data %>%
  mutate(trt_order = fct_relevel(treatment, c("Neither", "Irradiation",
                                              "Cannabinoids", "Both")))
```

Look at summary statistics

```
# calculate the means and SD for each group
summary_stats <- cancer_data %>%
  group_by(trt_order) %>%
  summarise(mean_vol = mean(tumor_volume),
            sd_vol = sd(tumor_volume),
            samp_size = n())

summary_stats
```

```
## # A tibble: 4 x 4
##   trt_order    mean_vol sd_vol samp_size
##   <fct>           <dbl>  <dbl>     <int>
## 1 Neither          48.3  24.8          7
## 2 Irradiation      54.2  28.2          4
## 3 Cannabinoids     26    16.6          5
## 4 Both              6     2.76         6
```

Graph the data

ggplot(cancer_data, aes(x = trt_order, y = tumor_volume)) +

geom_jitter(pch = 4, width = 0.1) +

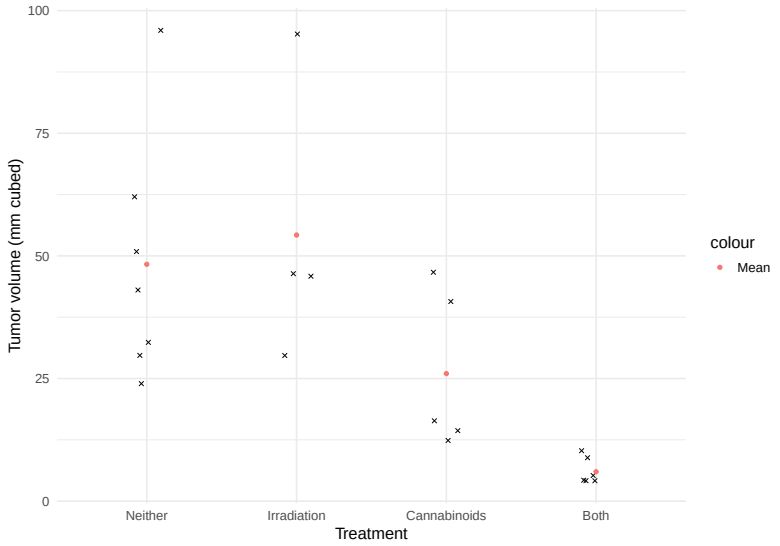
geom_point(data = summary_stats, aes(y = mean_vol, col = "Mean"), pch = 19) +

labs(y = "Tumor volume (mm cubed)", x = "Treatment") +

theme_minimal(base_size = 15)

note: geom_jitter() with width = 0.1 randomly "jitters" the location of the points along the x axis so that we can see each of them since some have the exact same values.

Graph the data



ANOVA in R: use aov(), then tidy() it up!

```
library(broom)
```

```
## Warning: package 'broom' was built under R version 3.5.3
```

```
cancer_anova <- aov(formula = tumor_volume ~ treatment, data = cancer_data)
tidy(cancer_anova)
```

```
## # A tibble: 2 x 6
##   term         df sumsq meansq statistic  p.value
##   <chr>     <dbl> <dbl>  <dbl>     <dbl>    <dbl>
## 1 treatment     3 8060.  2687.      6.70  0.00313
## 2 Residuals    18 7218.   401.     NA    NA
```

This F says that the variation between the means is nearly 7 times as large as the variation within the groups.

This p-value is equal to 0.3%. There is a 0.3% chance of observing the F statistic we observed (or more extreme) under the null hypothesis that all the means are

P value of the F test

Remember

- k is the number of groups being compared and $N_{Total} = n_1 + n_2 + ... + n_k$ is the total sample size across all the groups.
- The F statistic follows an F distribution with $k - 1$ degrees of freedom in the numerator and $N_{Total} - k$ degrees of freedom in the denominator
- The p-value of the ANOVA F statistic is always the area to the right of the test statistic

```
pf(6.699489, df1 = 3, df2 = 22 - 4, lower.tail = F)
```

```
## [1] 0.003131703
```

What now?

- The p-value equaled 0.003, indicating a difference. But what groups are actually different?

After a significant ANOVA...

next steps. . .

- You could look at all pairwise differences (i.e., comparing each combination of treatments), but we have to be careful because we will find differences "just by chance" if we compare enough groups.

Remember this guy?

FOOD FOR THOUGHT

Cornell Food Researcher's Downfall Raises Larger Questions For Science

September 26, 2018 · 3:07 PM ET

BRETT DAHLBERG

FROM WXXI

A brief reminder about p-hacking

Remember, one of the issues with multiple comparisons is that when you repeatedly question the same dataset, you can end up finding "significant" results by chance alone.

We talked about this before as p-hacking or p-fishing or data dredging

This along with other issues that are sometimes unconscious can lead to bias in what is found and what is published.

Ioannidis, John P.A. (August 30, 2005). "Why Most Published Research Findings Are False". PLoS Medicine. https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0020124

Adjustment for multiple comparisons

We could do a series, comparing each combination of groups in pairs or $\binom{k}{2}$ comparisons.

To compensate for making multiple comparisons and set the overall probability of making a type I error at 0.05, we can adjust our α to $\alpha*$ for each comparison by dividing by the number of comparisons we are making.

$$\alpha* = \left(\frac{0.05}{\binom{k}{2}} \right)$$

We then use $\alpha*$ as the significance level for each individual comparison. So for a comparison of 3 groups we would use an α of 0.0167 as the significance level for each comparison.

This modification is known as the Bonferroni correction. Bonferroni is fairly basic and can become unwieldy - what happens if you have a lot of groups?

Tukey's honest significant differences (Tukey's HSD)

John Wilder Tukey

Tukey's honestly significant differences (Tukey's HSD)

- Tukey's test maintains a 5% experimentwise or "family" error rate.
- Even if you make many pairwise comparisons, the overall error rate is 5% (at most)
- Overcomes the issue of multiple testing. Recall: If you conducted 100 tests with a 5% error rate (i.e., $\alpha = 0.05$) AND the H_0 was always true, how many p-values would you expect to be < 0.05?
- The Tukey's error rate is 5% overall, no matter how many tests you do. Thus it overcomes the problem of multiple testing

TukeyHSD() to calculate the differences in R

You can think of the TukeyHSD() as a wrap-around for the anova, you can either nest the statements like this:

TukeyHSD(aov(outcome ~ group))

or save the ANOVA as an object and use that in the statement:

modelresult<-aov(outcome ~ group)

TukeyHSD(modelresult, overall_alpha)

TukeyHSD() to calculate the differences in R

Here is the R code and output for the cancer example:

```
diffs <- TukeyHSD(cancer_anova, conf.level = 0.05) %>% tidy()
diffs
```

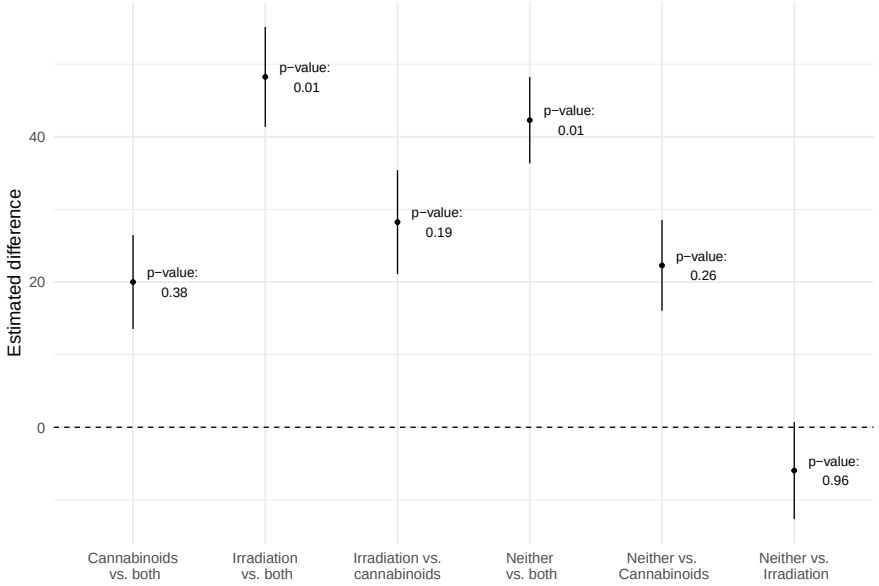
```
## # A tibble: 6 x 6
##   term      comparison              estimate conf.low conf.high adj.p.valu
##   <chr>     <chr>                      <dbl>    <dbl>     <dbl>       <dbl
## 1 treatment Cannabinoids-Both          20.      13.5      26.5       0.378
## 2 treatment Irradiation-Both           48.3     41.4      55.1       0.0075
## 3 treatment Neither-Both               42.3     36.4      48.2       0.0066
## 4 treatment Irradiation-Cannabinoi~    28.2     21.1      35.4       0.190
## 5 treatment Neither-Cannabinoids       22.3     16.0      28.5       0.263
## 6 treatment Neither-Irradiation        -5.96   -12.6       0.720     0.964
```

`TukeyHSD()` to calculate the differences in R

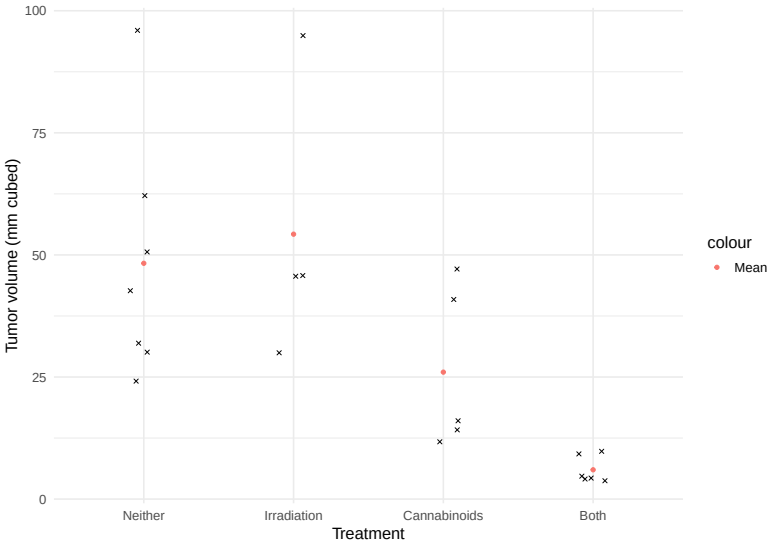
Each row in the table corresponds to a pairwise test. So the first row is looking at the difference between Cannabinoids vs. Both treatments. The estimated difference in means is 20 and the 95% CI is 13.54 to 26.45. The adjusted p-value is 0.38.

- "Adjusted" means that it is adjusted for conducting multiple tests. The unadjusted p-value would be smaller. You can tell the unadjusted p-value would be < 0.05 because the 95% CI doesn't include 0.
- Thus, when you have an adjusted test you can't use the CI to infer the value of the p-value!

Visualize the pairwise differences



Review raw data for comparison



Parting humor XKCD.com