Analysis of numerical data
S4

Katarzyna Jóźwiak
k.jozwiak@nki.nl

November 20, 2018

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The Netherlands Cancer Institute
Antoni van Leeuwenhoek Hospital

Hypothesis tests: numerical and ordinal data

- 1 group: one-sample *t*-test, sign test.
- 2 groups:
  - Dependent (paired/related): paired *t*-test, Wilcoxon signed rank test, sign test
  - Independent (unrelated): unpaired *t*-test, Wilcoxon rank sum test.
- More than 2 groups:
  - Dependent (paired/related): repeated measures ANOVA, Friedman's ANOVA
  - Independent (unrelated): one-way ANOVA, Kruskal-Wallis test.

Single group

One-sample t-test (parametric):

H_0: population mean = parameter value
H_1: population mean $\neq$ parameter value
(or H_1: population mean > parameter value,
or H_1: population mean < parameter value)

- Example: sample from a single group of individuals (high school students) with their standardized test scores in writing.

H_0: population mean writing score = 5
H_1: population mean writing score $\neq$5

Reject H_0 if:

$$\frac{\text{sample mean-parameter value}}{\text{SEM}} < -c \text{ or } \frac{\text{sample mean-parameter value}}{\text{SEM}} > c$$

with $c > 0$.

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Single group

One-sample t-test (parametric):

- Variable (e.g., writing test score)
  - is numerical,
  - is normally distributed with a given (usually unknown) variance.
- If population variance unknown: test statistic follows a Student-t distribution:
  - 5% significance level: $SEM = s/\sqrt{n}$ and $c = t_{n-1,1-\alpha/2} = t_{n-1,0.975}$ (s =standard deviation of sample observations, n =sample size)
- If population variance known (σ^2) or sample size very large: test statistic follows a normal distribution (z-test):
  - 5% significance level: $SEM = \sigma/\sqrt{n}$ and $c = z_{1-\alpha/2} = z_{0.975} = 1.96$

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Two related groups

Paired data (matched pairs):

- One group of individuals and variable measured on each individual in two circumstances.
  E.g. measurement while taking active treatment and while taking placebo; blood pressure measured before and after a particular treatment.
- Two samples of different individuals but linked to each other.
  E.g. matched patients in case-control study

Example: sample of patients in which hours of sleep under a sleeping drug is measured one night, and hours of sleep under a placebo is measured a different night for each.

Two related groups

Paired t-test (parametric):

H_0: population mean group 1 - population mean group 2 = parameter value

- For the hours of sleep example:

  H_0: pop. mean hours sleep (drug) - pop. mean hours sleep (placebo) = 0
  H_1: pop. mean hours sleep (drug) - pop. mean hours sleep (placebo) $\neq$ 0

- Since data are paired, we reduce the two samples to a single sample of differences:
  - Difference in hours sleep = hours sleep under drug - hours sleep under placebo
- Variable (difference in hours sleep)
  - is numerical,
  - is normally distributed with a given (usually unknown) variance.
- Hypotheses become:

  H_0: population mean difference hours sleep = 0
  H_1: population mean difference hours sleep $\neq$ 0

- We can use a one-sample t-test.
- Ratio paired t-test: If ratio (treatment/control) seems better to quantify effect of treatment.

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Two related groups

Paired t-test (parametric):

- Example: Concentration of antibody (μg/ml) to type II group B Streptococcus in 20 volunteers before and after immunisation
  - H_0 : population mean concentration before immunisation = population mean concentration after immunisation

Paired Samples Test

		Paired Differences			
					95% Confidence ...
		Mean	Std. Deviation	Std. Error Mean	Lower
Pair 1	Antibody after inmunisation - Antibody before inmunisation	1.18000	2.85281	.63791	-.15516

Paired Samples Test

		Paired ...			
		95% Confidence ...			
		Upper	t	df	Sig. (2-tailed)
Pair 1	Antibody after inmunisation - Antibody before inmunisation	2.51516	1.850	19	.080

Two related groups

Wilcoxon signed rank test (nonparametric):

H_0: population median of differences between paired observations = parameter value

- For the hours of sleep example:

  H_0: population median difference hours sleep = 0
  H_1: population median difference hours sleep $\neq$ 0

  - Intuition: if the population median of differences is zero, then approximately half of the values of differences between the two samples should be below zero.
- No normality required, but it does assume (rough) symmetry.
- Robust to outliers
- Applicable to numerical and ordinal data

Two related groups

Wilcoxon signed rank test (nonparametric):

- How does it work?
  - Compute differences between paired observations and ignore differences equal to the parameter value (sample size is reduced to n_r)
  - Assign ranks to the absolute values of the differences (for ties, calculate the average rank)
  - Reassign the + and - signs to the ranks
  - Sum up positive ranks and negative ranks separately
  - Test statistic W is the smaller value of the sum of positive ranks and sum of negative ranks
- Reject H_0 if:
  - Small sample size: $W \geq c$ with $c > 0$
  - Large sample size: $Z < -c$ or $Z > c$ where $Z = \frac{W - \mu_W}{\sigma_W}$, $\mu_W = \frac{n_r(n_r+1)}{4}$, $\sigma_W = \sqrt{\frac{n_r(n_r+1)(2n_r+1)}{24}}$;
    W is approximately normally distributed.

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Two related groups

Wilcoxon signed rank test (nonparametric):

- H_0: population median of differences between paired observations equals 0

group 1	group 2	diff	abs(diff)	rank	sign
6	7	-1	1	1	neg
8	3	5	5	6	pos
5	5	0	0	-	-
5	2	3	3	3.5	pos
7	9	-2	2	2	neg
9	6	3	3	3.5	pos
9	5	4	4	5	pos

sum of positive ranks = 15; sum of negative ranks = 3

Two related groups

Wilcoxon signed rank test (nonparametric):

- Critical values

	level of significance for a one-tailed test					
	.10	.05	.025	.01	.005	.001
	level of significance for a two-tailed test					
N	.20	.10	.05	.02	.01	.002
4	0					
5	2	0				
6	4	2	0			
7	6	3	2	0		
8	8	5	3	1	0	
9	11	8	5	3	1	
10	14	10	8	5	3	0

$W = 3$, $n_r = 6$, $c = 0$ for two-sided test and $\alpha = 0.05 \rightarrow W > c$ thus we reject the null hypothesis

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Two related groups

Wilcoxon signed rank test (nonparametric):

- Example: Weight measured before and after radiotherapy in 18 patients
  - H_0 : population median weight before radiotherapy = population median weight after radiotherapy

Test Statistics[a]

	postRT_weight - preRT_weight
Z	-2.132[b]
Asymp. Sig. (2-tailed)	.033
Exact Sig. (2-tailed)	.032
Exact Sig. (1-tailed)	.016
Point Probability	.002

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

Two related groups

The sign test (nonparametric):

H_0: population median of differences between paired observations = parameter value

- For the hours of sleep example:

  H_0: population median difference hours sleep = 0
  H_1: population median difference hours sleep $\neq$ 0
- No normality required, no symmetry required
- Robust to outliers
- Applicable to numerical and ordinal data
- Less powerful than Wilcoxon signed rank test when the population is symmetric since it ignores magnitudes completely

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Two related groups

The sign test (nonparametric):

- How does it work?
  - Compute the differences between paired observations and omit differences equal to the parameter value (sample size is reduced to n_r)
  - Count the number of positive and negative differences
  - The test statistic W is the number of positive differences or the number of negative differences, whichever is smaller
- Reject H_0 if: $p-$value$<\alpha$ and $p-$value=P(W or less differences) for one-sided test or $p-$value=2P(W or less differences) for two-sided test;
  W has a binomial distribution with n_r trials and $p = 1/2$.

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Two related groups

The sign test (nonparametric):

- Example: Weight measured before and after radiotherapy in 18 patients
  - H_0 : population median weight before radiotherapy = population median weight after radiotherapy

Test Statistics[a]

	postRT_weight - preRT_weight
Exact Sig. (2-tailed)	.143[b]
Exact Sig. (1-tailed)	.072
Point Probability	.047

a. Sign Test

b. Binomial distribution used.

Two unrelated groups

- Samples from two independent (unrelated) groups of individuals.
  - Example 1: birth length of children born to $n_1 = 40$ heavy smokers (group 1) and to $n_2 = 42$ non-smokers (group 2).
  - Example 2: BMI of two groups of children, each child being randomly allocated to receive either a dietary supplement (group 1) or placebo (group 2).

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Two unrelated groups

Unpaired (two-sample) *t*-test (parametric):

H_0: two populations have the same means

- For the birth length example:

  H_0: population mean length group 1 = population mean length group 2
  H_1: population mean length group 1 $\neq$ population mean length group 2

Reject H_0 if:

$$\frac{\text{sample mean group 1 - sample mean group 2}}{SEM} < -c$$

or

$$\frac{\text{sample mean group 1 - sample mean group 2}}{SEM} > c$$

with $c > 0$.

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Two unrelated groups

Unpaired (two-sample) *t*-test (parametric):

- Variable (e.g., weight)
  - is numerical in each group,
  - is normally distributed in each group with (usually unknown) variances.
- If population variances are equal but unknown: test statistic follows a Student-*t* distribution:
  - 5% significance level: $SEM = s\sqrt{1/n_1 + 1/n_2}$ and $c = t_{n_1+n_2-2,1-\alpha/2} = t_{n_1+n_2-2,0.975}$ ($s = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}$) pooled standard deviation of the two groups; s_i is the standard deviations sample observations group i; n_i is the sample size of group i, with i = 1, 2)

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Two unrelated groups

Unpaired (two-sample) *t*-test (parametric):

- If population variances known (σ_1^2, σ_2^2) or sample size very large: test statistic follows a normal distribution (*z*-test):
  - 5% significance level: $SEM = \sqrt{\sigma_1^2/n_1 + \sigma_2^2/n_2}$ and $c = z_{1-\alpha/2} = z_{0.975} = 1.96$
- If population variances unequal and unknown: test statistic follows a Student-*t* distribution:
  - 5% significance level: $SEM = \sqrt{s_1^2/n_1 + s_2^2/n_2}$ and $c = t_{df,1-\alpha/2}$ with $df = \frac{(s_1^2/n_1+s_2^2/n_2)^2}{\frac{(s_1^2/n_1)^2}{n_1-1}+\frac{(s_2^2/n_2)^2}{n_2-1}}$
- Levene's test with H_0 : variances across groups are the same

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Two unrelated groups

Unpaired (two-sample) *t*-test (parametric):

- Example: BMI in patients with heart disease and healthy people
  - H_0 : population mean BMI patients = population mean BMI healthy people

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
bmi	Equal variances assumed	.096	.757	2.498	1042	.013	.930	.372	.199	1.661
	Equal variances not assumed			2.554	196.741	.011	.930	.364	.212	1.649

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Two unrelated groups

Unpaired (two-sample) *t*-test (parametric):

- Example: BMI in patients with heart disease and healthy people

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
bmi	Equal variances assumed	.096	.757	2.498	1042	.013	.930	.372	.199	1.661
	Equal variances not assumed			2.554	196.741	.011	.930	.364	.212	1.649

Equal variances are assumed since $F = 0.096$, $p = 0.757 \rightarrow$ we interpret results given in the first row, $t(1042) = 2.498$, $p = 0.013$

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Two unrelated groups

Wilcoxon rank sum (two-sample) test (nonparametric):

H_0: two populations have the same medians

- For the birth length example:

  H_0: population median length group 1 = population median length group 2

  H_1: population median length group 1 $\neq$ population median length group 2
- No normality required but the population distribution of the two groups assumed to have the same shape
- Applicable to numerical and ordinal data

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Two unrelated groups

- Wilcoxon rank sum (two-sample) test (nonparametric):
  - How does it work?
    - Rank the data ignoring grouping
    - Sum up ranks for each group separately
    - The test statistic W is the smaller of the sum of ranks for group 1 and sum of ranks for group 2
  - Reject H_0 if:
    - Small sample size: $W \geq c$ with $c > 0$
    - Large sample size: $Z < -c$ or $Z > c$ where $Z = \frac{W - \mu_W}{\sigma_W}$, $\mu_W = \frac{n_1(n_1+n_2+1)}{2}$, $\sigma_W = \sqrt{\frac{n_1 n_2 (n_1+n_2+1)}{12}}$, n_1 is the sample size of the group that has smaller sum of ranks;
      W is approximately normally distributed.

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Two unrelated groups

Mann-Whitney U (two-sample) test (nonparametric):

H_0: two populations have the same medians

- For the birth length example:

  H_0: population median length group 1 = population median length group 2

  H_1: population median length group 1 $\neq$ population median length group 2
- No normality required but the population distribution of the two groups assumed to have the same shape
- Applicable to numerical and ordinal data

Two unrelated groups

- Mann-Whitney U (two-sample) test (nonparametric):
  - How does it work?
    - Rank the data ignoring grouping
    - Sum up ranks for each group separately (R_1 and R_2)
    - The test statistic U is the smaller value of U_1 and U_2:

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - R_2$$

      where n_1, n_2 are the sample sizes of the groups.
  - Reject H_0 if:
    - Small sample size: $U \geq c$ with $c > 0$
    - Large sample size: $Z < -c$ or $Z > c$ where $Z = \frac{U-\mu_U}{\sigma_U}$, $\mu_U = \frac{n_1 n_2}{2}$, $\sigma_U = \sqrt{\frac{n_1 n_2(n_1+n_2+1)}{12}}$;
      U is approximately normally distributed.

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Two unrelated groups

- Wilcoxon rank sum test and Mann-Whitney U test give identical results
- Example: Age measured in patients with colon cancer who underwent open surgery or laparoscopy
  - H_0 : population median age open surgery = population median age laparoscopy

Test Statistics[a]

	age
Mann-Whitney U	35.000
Wilcoxon W	90.000
Z	-1.134
Asymp. Sig. (2-tailed)	.257
Exact Sig. [2*(1-tailed Sig.)]	.280[b]
Exact Sig. (2-tailed)	.271
Exact Sig. (1-tailed)	.135
Point Probability	.008

a. Grouping Variable: surgery

b. Not corrected for ties.

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More than two related groups

- Related groups:
  - One group of individuals and variable measured on each individual in more than two circumstances.
    E.g. measurement while taking low dose of a drug, high dose of a drug or placebo.
  - More than two samples of different individuals but linked to each other.
- Making pairwise comparisons between groups is not efficient because the type I error rate becomes high
  - Better to carry out a single global test to determine whether the means/medians differ in ANY groups.

More than two related groups

Friedman's ANOVA (nonparametric):

H_0: all populations have the same medians
H_1: at least one population has a different median

- Example: we measure the outcome variable x of n individuals at k different conditions or k different time points.
- No normality required
- Applicable to numerical and ordinal data

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More than two related groups

Friedman's ANOVA (nonparametric)

- How does it work?
  - Rank the data separately for each individual / group of related observations
  - Sum up ranks for each group separately (R_j for group j)
  - The test statistic F is

$$F = \left(\frac{12}{nk(k+1)} \sum_j R_j^2 \right) - 3n(k+1)$$

- Reject H_0 if:
  - Small sample size: $F \geq c$ with $c > 0$
  - Large sample size: $F < -c$ or $F > c$ with $c > 0$ knowing that F has a chi-square distribution with df=$k - 1$

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More than two unrelated groups

- Examples:
  - Samples from 3 independent groups of patients, each with a type of sickle cell disease. For each patient, the steady-state haemoglobin levels are measured.
  - RNA samples from 12 mice of 3 different strains (4 mice/strain). Identify genes that differ in expression levels among these strains.
- Making pairwise comparisons between groups (e.g. with t-test) is not efficient because the type I error rate becomes high
  - Better to carry out a single global test to determine whether the means/medians differ in ANY groups.

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More than two unrelated groups

One-way analysis of variance (ANOVA) (parametric):

H_0: all populations have the same means
H_1: at least one population has a different mean

(Homogeneity or heterogeneity across populations/groups)

- Example: the populations means haemoglobin level for each type of sickle cell disease are the same, or at least one is different.
- Variable (e.g., haemoglobin level, gene expression)
  - is numerical in each group
  - is normally distributed in each group and variances are the same across groups
  - moderate departures from normality may be ignored but unequal variances cannot $\rightarrow$ check homogeneity of variances (Levene's test with H_0 : variances across groups are the same)
- Groups are defined by the levels of a single factor (e.g. different sickle cell disease; gender).

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More than two unrelated groups

One-way analysis of variance (ANOVA) (parametric):

- We measure the outcome variable x (e.g. haemoglobin level) and compare its mean in the k groups defined by the levels of a single factor (e.g. type of sickle cell disease). The outcome is measured n times in total.
- The variance of all observations ignoring subdivision into groups (total sample variance) is $s^2 = \sum_j \sum_i (x_{ij} - \bar{x})^2/(n-1)$
- One-way ANOVA partitions the sum of squares $SS = \sum_j \sum_i (x_{ij} - \bar{x})^2$ into:
  - Between-groups SS (k - 1 d.f.): $SS_M = \sum_j n_j(\bar{x}_j - \bar{x})^2$
  - Within-groups SS or residual SS (n - k d.f.): $SS_R = \sum_j \sum_i (x_{ij} - \bar{x}_j)^2$
  - x_{ij} is the i observation in j group, $\bar{x}_j$ is the mean of group j, $\bar{x}$ is the grand mean
- The amount of variation per degree of freedom is the mean square (MS).

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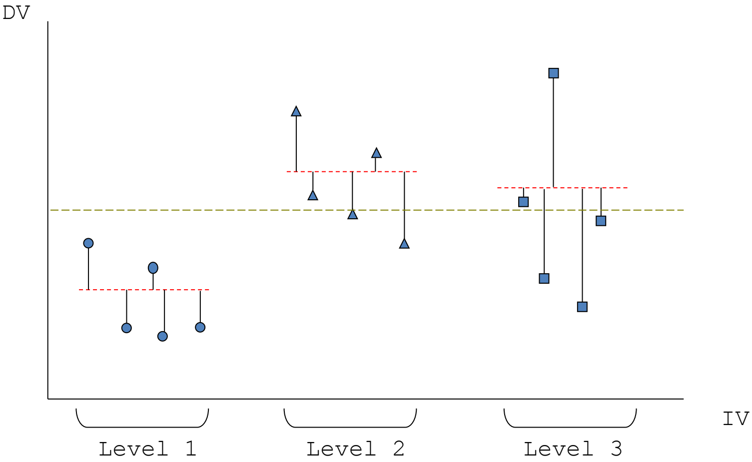
More than two unrelated groups

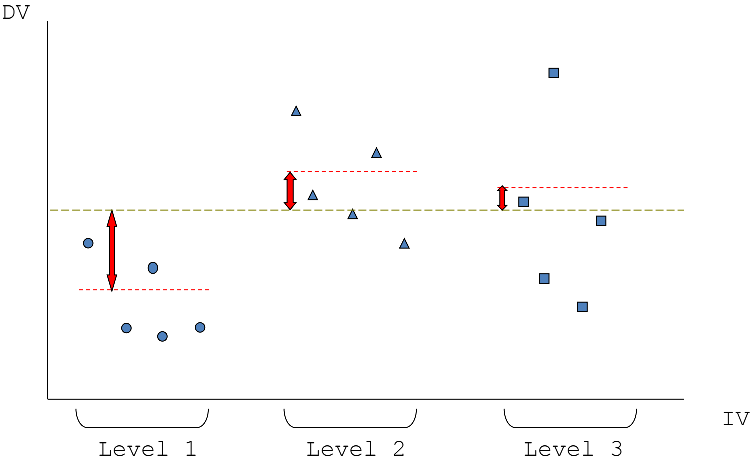
One-way analysis of variance (ANOVA) (parametric):



SS_R uses the differences between the observed data and the group means

More than two unrelated groups

One-way analysis of variance (ANOVA) (parametric):



SS_M uses the differences between the grand means and the group means

More than two unrelated groups

One-way analysis of variance (ANOVA) (parametric):

- Reject H_0 at the 5% significance level if:

$$F = \frac{\text{Between-groups SS}/(k-1)}{\text{Within-groups SS}/(n-1)} = \frac{\text{Between-groups MS}}{\text{Within-groups MS}} > F_{k-1,n-k,0.95}$$

  - The F test statistic is found by dividing the between group variance by the within group variance.
  - When there are only 2 groups, results of the one-way ANOVA exactly equal to results of t-test.

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More than two unrelated groups

One-way analysis of variance (ANOVA) (parametric):

- Example: FEV1 in current smokers, ex-smokers and non-smokers
  - H_0 : population mean FEV1 current smokers = population mean FEV1 ex-smokers = population mean FEV1 non-smokers

Test of Homogeneity of Variances

fev1

Levene Statistic	df1	df2	Sig.
1.058	2	189	.349

ANOVA

fev1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4344.302	2	2172.151	5.454	.005
Within Groups	75273.942	189	398.275		
Total	79618.245	191			

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More than two unrelated groups

Kruskal-Wallis test (nonparametric):

H_0: all populations have the same medians
H_1: at least one population has a different median

- The population distribution of all groups assumed to have the same shape
- We measure the outcome variable x (e.g. haemoglobin level) and compare its median in the k groups defined by the levels of a single factor (e.g. type of sickle cell disease). The outcome is measured n times in total.
- No normality required
- Applicable to numerical or ordinal data

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More than two unrelated groups

Kruskal-Wallis test (nonparametric):

- How does it work?
  - Rank the data ignoring grouping
  - Sum up ranks for each group separately (R_j)
  - The test statistic H is

$$H = \left(\frac{12}{n(n+1)} \sum_j \frac{R_j^2}{n_j} \right) - 3(n+1)$$

- Reject H_0 if:
  - Small sample size: $H \geq c$ with $c > 0$
  - Larger sample size: $H < -c$ or $H > c$ with $c > 0$ knowing that H has a chi-squared distribution with df=$k-1$

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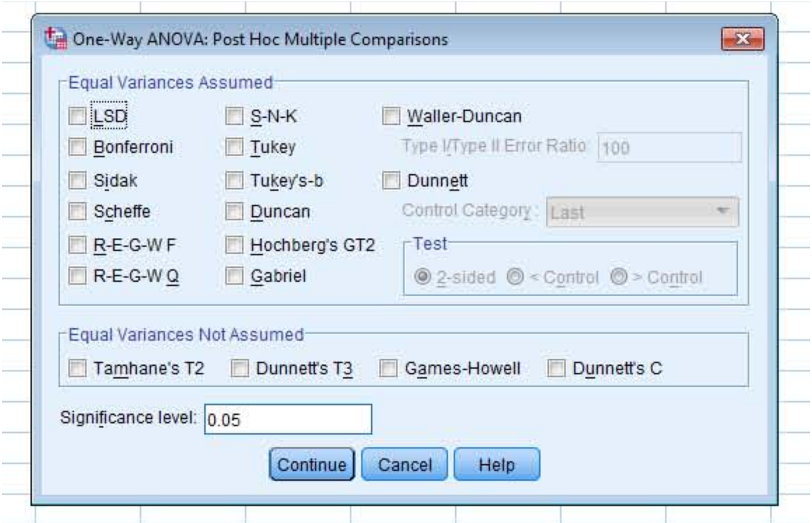
Multiple comparisons

- Multiple comparisons: compare all different pairwise combinations of the groups
  - With k groups we have $\frac{k(k-1)}{2}$ pairs of groups to compare
- There are methods that control for the increased familywise error rate, i.e., make all pairwise comparisons while maintaining the experimentwise error rate at the pre-established α level

Multiple comparisons

- SPSS Post Hoc Multiple Comparisons option



Multiple comparisons

- Fisher's least significant difference (LSD) test: does not correct for multiple comparisons, is equivalent to performing multiple *t*-tests on the data
- Bonferroni method: for each pairwise comparison α/m is used as a significance level and overall Type I error rate is α; m is the number of all possible comparisons
  - in SPSS: each p-value for Bonferroni test is a p-value for LSD test multiplied by the number of comparisons
  - powerful method for a small number of comparisons

Multiple comparisons

- Tukey's HSD(Honestly Significance Difference) test: for each pairwise comparison the test statistic $Q = \frac{\bar{x}_i - \bar{x}_k}{\sqrt{MS_w/n}}$ is used where $i \neq k$, $\bar{x}_i$ and $\bar{x}_k$ are the group means we compare, n is the sample size of each group and MS_w is the within-groups variance value from, e.g, the ANOVA method we obtained at the first phase
  - correction for unequal sized groups: MS_w is divided by $n_h = \frac{m}{1/n_1 + 1/n_2 + \ldots + 1/n_m}$
  - powerful method for a large number of comparisons
- Dunnett's test: makes pairwise comparisons of each group to a control or reference group so we have $k - 1$ comparisons

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Multiple comparisons

- Example: FEV1 in current smokers, ex-smokers and non-smokers

Multiple Comparisons

Dependent Variable: fev1

	(I) smoking	(J) smoking	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Bonferroni	ex-smokers	non-smokers	-2.527	3.412	1.000	-10.77	5.71
		current-smokers	10.550*	3.734	.016	1.53	19.57
	non-smokers	ex-smokers	2.527	3.412	1.000	-5.71	10.77
		current-smokers	13.077*	4.197	.006	2.94	23.22
	current-smokers	ex-smokers	-10.550*	3.734	.016	-19.57	-1.53
		non-smokers	-13.077*	4.197	.006	-23.22	-2.94
Dunnett t (2-sided)[b]	ex-smokers	current-smokers	10.550*	3.734	.010	2.30	18.80
	non-smokers	current-smokers	13.077*	4.197	.004	3.81	22.35

*. The mean difference is significant at the 0.05 level.

b. Dunnett t-tests treat one group as a control, and compare all other groups against it.

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Parametric or nonparametric?

- Parametric approaches:
  - when a variable is normally distributed
    - dependent groups: check normality for variable of differences
    - independent groups: check normality for each variable separately
  - when $n > 30$
- Nonparametric approaches (variable's observations are replaced with their ranks):
  - when a variable is not normally distributed
  - when a variable is ordinal or have many outliers
  - when n is small
  - when median is a better representation of the study

NKI-AVL
The Netherlands Cancer Institute
Antoni van Leeuwenhoek Hospital