Outline
Introduction
Normality and tests
One sample tests
Two sample tests

Hypotheses tests with continuous variables
Statistical Analysis with R:

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Section 1

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- Introduction
- Type of Tests
- Normality Tests
- One Group Comparison
- Two Groups Comparison
- K Groups Comparison in Independent Samples
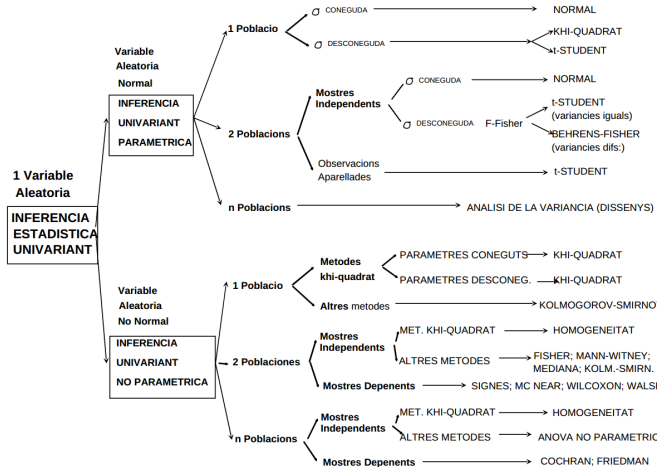- Multiple Comparisons and Multiple Testing

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Section 2

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Introduction

- Once the concept of hypothesis testing is established,
- Researchers face the problem of *which test should be applied at every possible situation*.
- For this, ideally, they should. . .
    - understand the problem and the questions addressed,
    - know available tests for each problem,
    - know (how to check) applicability assumptions of each test,
    - know how robust each test is to assumptions violation.
- Easier to say than to do.
    - Sometimes cheatsheets may be helpful, but be warned against a blind use, that is understand and be critic with the steps.

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Which test is appropriate for which problem

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Example situation (1): Introduction

- Many experimental questions may be answered through hypothesis testing.
- Imagine, for example, a study designed to compare two distinct hypertension control programs.
- 60 individuals with HTA were randomly assigned to either one or the other group (30 per group)
- Blood pressure was measured each month during a year
  - For simplicity we may keep only data at months 1 and 12

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Example situation (2): Data collected

```
hta <- read_excel("datasets/hta.xls")
htaSimple <- hta %>% select(grupo, sexo, tas1, tad1, tas12,
head(as.data.frame(htaSimple))
```

```
##   grupo  sexo tas1 tad1 tas12 tad12
## 1     B VARON  150  100   150    80
## 2     B MUJER  160   90   150    90
## 3     B MUJER  150   90   110    85
## 4     A VARON  120   80   130    90
## 5     A MUJER  150   85   120    80
## 6     B MUJER  140   75   140    90
```

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Example situation (3): Reasonable questions

- The goal of the study is to compare the treatment effect so a reasonable question is:
    - *Is the average decrease in "tad" the same in both groups A and B?*
- Or, if we are testing a new treatment "B", hat is intended to be batter than "A"
    - *Is the average decrease in "tad" greater in group B?*
- *Although they are not planned in this study* other relevant questions may lead to questions that need a test to be answered, such as:
    - *Is the average tad above 150?*
    - *Has the average tad (in group A) decreased in 12 months?*
    - *Is the average tad different in men and women at basal time?*

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Types of tests (1): Confirmatory vs Independence

Distinct classifications can be found in textbooks

- Confirmatory
  - Is average HTA above 150?
  - Is the the tas1 variable normally distributed
- Independence
  - Is sex related to HTA (or is mean(HTA) the same in men or women)
  - Is average HTA decrease the same for both groups?
- *This classification is useful but artificial, not to say that the term "independence" is slightly abused*

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Types of tests (2): Parametric vs Non-parametric

- Parametric tests
  - assume some underlying distribution for the data
  - pose the test in terms of the distribution's parameters
    - E.g. the t-test assumes normality and relies on the normal and t-distribution's parameters
- Non-parametric tests
  - Do not assume an underlying distribution, but they are not assumption-free!
  - Check: Distribution free is not assumption free
- Permutation tests
  - If sample size is not tiny *permutation tests* are a good alternative.

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Hands on: Always start looking at the data

```
library(ggplot2)
library(patchwork)

p1 <- ggplot(hta, aes(y = tas1)) +
  geom_boxplot()

p2 <- ggplot(hta, aes(x = tas1)) +
  geom_histogram()

(p1 / p2)
```

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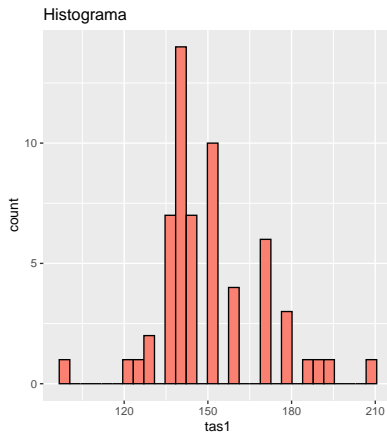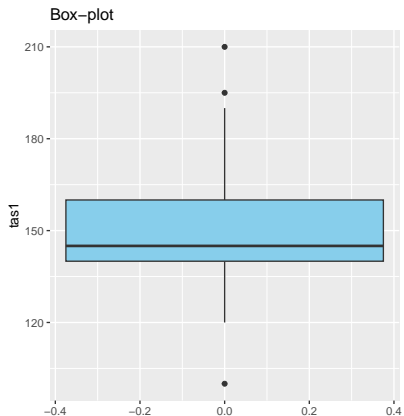# Hands on: Always start looking at the data

```
p_load(ggplot2, patchwork)

p1 <- ggplot(hta, aes(y = tas1)) +
  geom_boxplot(fill = "skyblue") +
  labs(title = "Box-plot", y = "tas1")

p2 <- ggplot(hta, aes(x = tas1)) +
  geom_histogram(bins = 30, fill = "salmon", color = "black") +
  labs(title = "Histograma", x = "tas1")


(p1 | p2)
```

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Data visualization

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Section 3

Normality and tests

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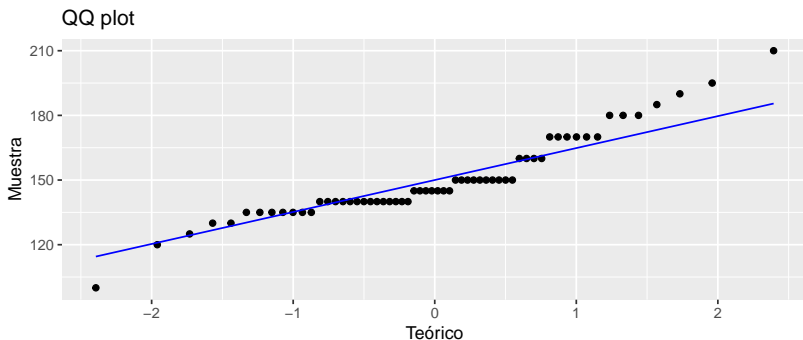One sample tests
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Normality and tests

- The choice of test seems to pivot around the question of ¿is my data normally distributed?
- Leaving apart a tence to repeat what we have been taught, why is this so?
    - If the data is normally distributed there exist some *optimal tests* for some one or two sample problems.
    - Data show often a bell-shaped form that can be assimilated to have a gaussian distribution (it is *normal* to observe this).
    - Iven if data is not bell-shaped, if sample size is big enough th sample mean tends to be bell -shaped as sample size increase.
- In summary, normality is not only "practical" but common.
- As a consequence checking normality has become one of the first steps of any data analysis.
    - It doesn't hurt, but it is clearly over-rated.

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Normality Test

- Normality tests can be used to decide if the data can be considered to follow a normal distribution.
- This is more a theoretical than practical issue because . . .
    - If the sample size is too small, the test is not powerful enough.
    - If the sample size is too big, the test will almost always reject the normality htypothesis
- Normality can be tested
    - Graphically:
        - Check if it is a symmetric distribution
        - Probability graphs (QQ-plots)
    - Using Hypothesis test (Normality)
        - Kolmogorov tests
        - Shapiro-Wilks test

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Normality test: Graphically

```
ggplot(hta, aes(sample = tas1)) +
 stat_qq() +
 stat_qq_line(color = "blue") +
 labs(title = "QQ plot", x = "Teórico", y = "Muestra")
```


QQ plot

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Normality test: Shapiro-Wilks

- In normality test, the null hypothesis is normality
- That is, rejecting the null suggests departure of normality.
    - H_0: The data follow a normal distribution
    - H_1: The data do not follow a normal distribution

```
shapiro.test(hta$tad1)  # Shapiro Wilk test
```

```
##
##  Shapiro-Wilk normality test
##
## data:  hta$tad1
## W = 0.96622, p-value = 0.09512
```

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One sample tests

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One sample tests

- One sample tests refer to a single characteristic of the population such as:
    - *Is it true that the average tad in HTA population is above 150*?
- It is often said that they are less interesting because they are rarely used in most practical situations where the main goal is comparison
- *However*, noticing that a paired tests is equivalent to a one sample tests for the difference makes us realize their relevance.

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One sample parametric vs non-parametric tests

- If we assume normality an appropriate test for a hypothesis about the mean is the *t-test*
    - $H_0 : \mu = \mu_0, \qquad H_1 : \mu \neq \mu_0, \ (\mu > \mu_0, \ \mu < \mu_0)$.
- If we don't assume normality we can rely on
    - Wilcoxon rank test, if data re symmetrical
    - Sign test in other cases

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Hands on one sample tests (1): t-test

- Is tad in HTA patients above 90?
- Notice that even if we are interested in "above 90" the null is "equality"
  - $H_0 : \mu = 90, \qquad H_1 : \mu > 90.$

```
t.test(hta$tad1,mu=90, alternative="greater") # One sample T.test
```

```
##
##  One Sample t-test
##
## data:  hta$tad1
## t = -1.2137, df = 59, p-value = 0.8852
## alternative hypothesis: true mean is greater than 90
## 95 percent confidence interval:
##  86.23664      Inf
## sample estimates:
## mean of x
##  88.41667
```

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Hands on one sample tests (2): Wilcoxon test

- In wilcoxon test the null hypothesis is about the median.

```
wilcox.test(hta$tad1, mu = 90, alternative = "greater")  #
```

```
##
##   Wilcoxon signed rank test with continuity correction
##
## data:  hta$tad1
## V = 429, p-value = 0.8934
## alternative hypothesis: true location is greater than 90
```

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Two sample tests

```
head(hta)
```

```
## # A tibble: 6 x 27
##   numero sexo  grupo  tas1  tad1  tas2  tad2  tas3  tad3  tas4  tad4  tas5
##    <dbl> <chr> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <
## 1      1 VARON B      150   100   150    90   170    90   175    85   140
## 2      2 MUJER B      160    90   170    90   160    80   150    90   150
## 3      3 MUJER B      150    90   110    90   115    90   120    80   125
## 4      4 VARON A      120    80   140    90   140    90   130    90   130
## 5      5 MUJER A      150    85   145    85   160    90   140    80   120
## 6      6 MUJER B      140    75   160    70   135    75   140    70   140
## # i 14 more variables: tas6 <dbl>, tad6 <dbl>, tas7 <dbl>, tad7 <dbl>,
## #   tas8 <dbl>, tad8 <dbl>, tas9 <dbl>, tad9 <dbl>, tas10 <dbl>, tad10 <dbl>
## #   tas11 <dbl>, tad11 <dbl>, tas12 <dbl>, tad12 <dbl>
```

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Homogeneity variance Test

```
library(car)
hta %>%
  group_by(sexo) %>%
  summarise(var = sd(tas1))
```

```
## # A tibble: 2 x 2
##   sexo   var
##   <chr> <dbl>
## 1 MUJER  17.6
## 2 VARON  22.1
```

```
leveneTest(hta$tad1~factor(hta$sexo),center="median")
```

```
## Levene's Test for Homogeneity of Variance (center = "median")
##       Df F value Pr(>F)
## group  1  1.3506 0.2499
##       58
```

- p value is over 0.05
- We can assume homogeneity of variances

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T test when variances are equal

```
t.test(hta$tas1~factor(hta$sexo), var.equal = TRUE )
```

```
##
##   Two Sample t-test
##
## data:  hta$tas1 by factor(hta$sexo)
## t = -0.2471, df = 58, p-value = 0.8057
## alternative hypothesis: true difference in means between group MUJER and gro
## 95 percent confidence interval:
##  -11.603461    9.053519
## sample estimates:
## mean in group MUJER mean in group VARON
##            149.5946            150.8696
```

- Type I Error is over than 0.05
- We cannot reject mean equality

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T test when variances are unequal

```
t.test(hta$tas1~factor(hta$sexo),var.equal=FALSE )
```

```
##
##  Welch Two Sample t-test
##
## data:  hta$tas1 by factor(hta$sexo)
## t = -0.23436, df = 39.098, p-value = 0.8159
## alternative hypothesis: true difference in means between group MUJER and gro
## 95 percent confidence interval:
##  -12.277927    9.727986
## sample estimates:
## mean in group MUJER mean in group VARON
##            149.5946            150.8696
```

- Same conclusions as before
- Test is also known as Welch test

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U Mann-Whitney or Sum Rank non parametric test

```
wilcox.test(hta$tad1~factor(hta$sexo)
    ,alternative='two.sided',exact=TRUE, correct=FALSE)
```

```
##
##  Wilcoxon rank sum test
##
## data:  hta$tad1 by factor(hta$sexo)
## W = 434, p-value = 0.8955
## alternative hypothesis: true location shift is not equal to 0
```

```
hta%>%
  group_by(sexo) %>%
  summarise(median = median(tad1))
```

```
## # A tibble: 2 x 2
##   sexo  median
##   <chr> <dbl>
## 1 MUJER    90
## 2 VARON    90
```

- Null Hypothesis cannot be rejected

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Paired T-test

```
t.test(hta$tas1,hta$tas12,paired=TRUE)
```

```
##
##  Paired t-test
##
## data:  hta$tas1 and hta$tas12
## t = 6.0672, df = 51, p-value = 1.609e-07
## alternative hypothesis: true mean difference is not equal to 0
## 95 percent confidence interval:
##    8.518285 16.943253
## sample estimates:
## mean difference
##        12.73077
```

```
summary(hta$tas1)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   100.0   140.0   145.0   150.1   160.0   210.0
```

```
summary(hta$tas12)
```

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Paired Sign-Rank Wilcoxon Test

```
wilcox.test(hta$tad1,hta$tad12, exact=TRUE, paired=TRUE)

##
##  Wilcoxon signed rank test with continuity correction
##
## data:  hta$tad1 and hta$tad12
## V = 478.5, p-value = 0.05333
## alternative hypothesis: true location shift is not equal to 0
```

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Read diabetes data

```r
require(pacman)
p_load(readxl, dplyr, magrittr)
diabetes <- read_excel("datasets/diabetes.xls")
sapply(diabetes, class)
```

```
##    numpacie        mort     tempsviu        edat         bmi     edatdiag
##   "numeric" "character"   "numeric"   "numeric"   "numeric"   "numeric"
##       tabac         sbp         dbp         ecg         chd
## "character"   "numeric"   "numeric" "character" "character"
```

```r
diabetes_factor <- diabetes %>%
  mutate_if(sapply(diabetes, is.character), as.factor) %>%
  select (-numpacie)

diabetes%>%
  group_by(ecg) %>%
  summarise( n=n(),
    mean = mean(edat),
            sd=sd(edat))
```

```
## # A tibble: 3 × 4
```

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ANOVA

```
anova<-aov(edat~ecg,data=diabetes_factor)
summary(anova)
```

```
##               Df Sum Sq Mean Sq F value  Pr(>F)
## ecg            2   2166  1083.0   8.619 0.00029 ***
## Residuals    146  18347   125.7
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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Multicomparison

```
library(multcomp)
tuk <- glht(anova, linfct = mcp(ecg = "Tukey"))

  print(summary(tuk)) # pairwise tests
```

```
##
##    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: aov(formula = edat ~ ecg, data = diabetes_factor)
##
## Linear Hypotheses:
##                      Estimate Std. Error t value Pr(>|t|)
## Frontera - Anormal == 0  -11.094      4.010  -2.767 0.016497 *
## Normal - Anormal == 0    -14.405      3.543  -4.065 0.000216 ***
## Normal - Frontera == 0    -3.310      2.405  -1.376 0.345699
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

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Multicomparison plot

```
plot(confint(tuk))
```

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Kruskal-Wallis Test

```
diabetes_factor%>%
  group_by(ecg) %>%
  summarise(median = median(edat))
```

```
## # A tibble: 3 x 2
##   ecg       median
##   <fct>      <dbl>
## 1 Anormal       64
## 2 Frontera      53
## 3 Normal        49
```

```
kruskal.test(edat~ecg,data=diabetes_factor)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  edat by ecg
## Kruskal-Wallis chi-squared = 17.483, df = 2, p-value = 0.0001598
```

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Dunn Test for multiple comparison

```
library(dunn.test)
dunn.test(diabetes_factor$edat, diabetes_factor$ecg, method="bonferroni")
```

```
##   Kruskal-Wallis rank sum test
##
## data: x and group
## Kruskal-Wallis chi-squared = 17.4826, df = 2, p-value = 0
##
##
##                          Comparison of x by group
##                                (Bonferroni)
## Col Mean-|
## Row Mean |   Anormal   Frontera
## ---------+----------------------
## Frontera |   2.721182
##          |     0.0098*
##          |
##   Normal |   4.075469   1.467464
##          |     0.0001*     0.2134
##
## alpha = 0.05
```