

Statistics Course with R - Day 7

UEB

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hta dataset

```
hta <- read_excel("datasets/hta.xls")
hta[1:5,1:9]
```

```
## # A tibble: 5 x 9
##   numero sexo grupo  tas1  tad1  tas2  tad2  tas3  tad3
##   <dbl> <chr> <chr> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1     1  VARON  B      150   100   150    90   170    90
## 2     2  MUJER  B      160    90   170    90   160    80
## 3     3  MUJER  B      150    90   110    90   115    90
## 4     4  VARON  A      120    80   140    90   140    90
## 5     5  MUJER  A      150    85   145    85   160    90
```

Checking Normality

Graphically

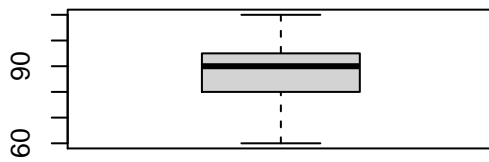
```
oldpar<-par(mfrow=c(1,1)) # Guarda los parámetros para el dibujo
par(mfrow=c(2,2)) # Dibuja cuatro gráficos por grafico
with(hta, boxplot(tad1, main="Box-plot" ) )

with(hta, hist(tad1) )

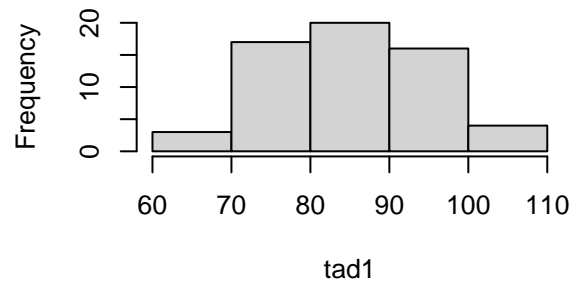
with(hta, qqnorm(tad1, main="Normal QQplot" ) );with(hta, qqline(tas1) )

par(oldpar) # Vuelve a los parámetros de dibujo originales
```

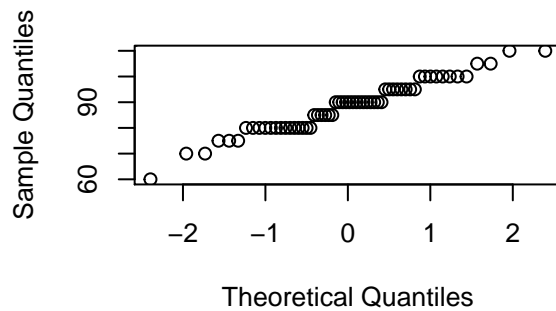
Box-plot



Histogram of tad1



Normal QQplot



Normality Test

```
with(hta,shapiro.test(tad1) ) # Shapiro Wilk test
```

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

One sample Test

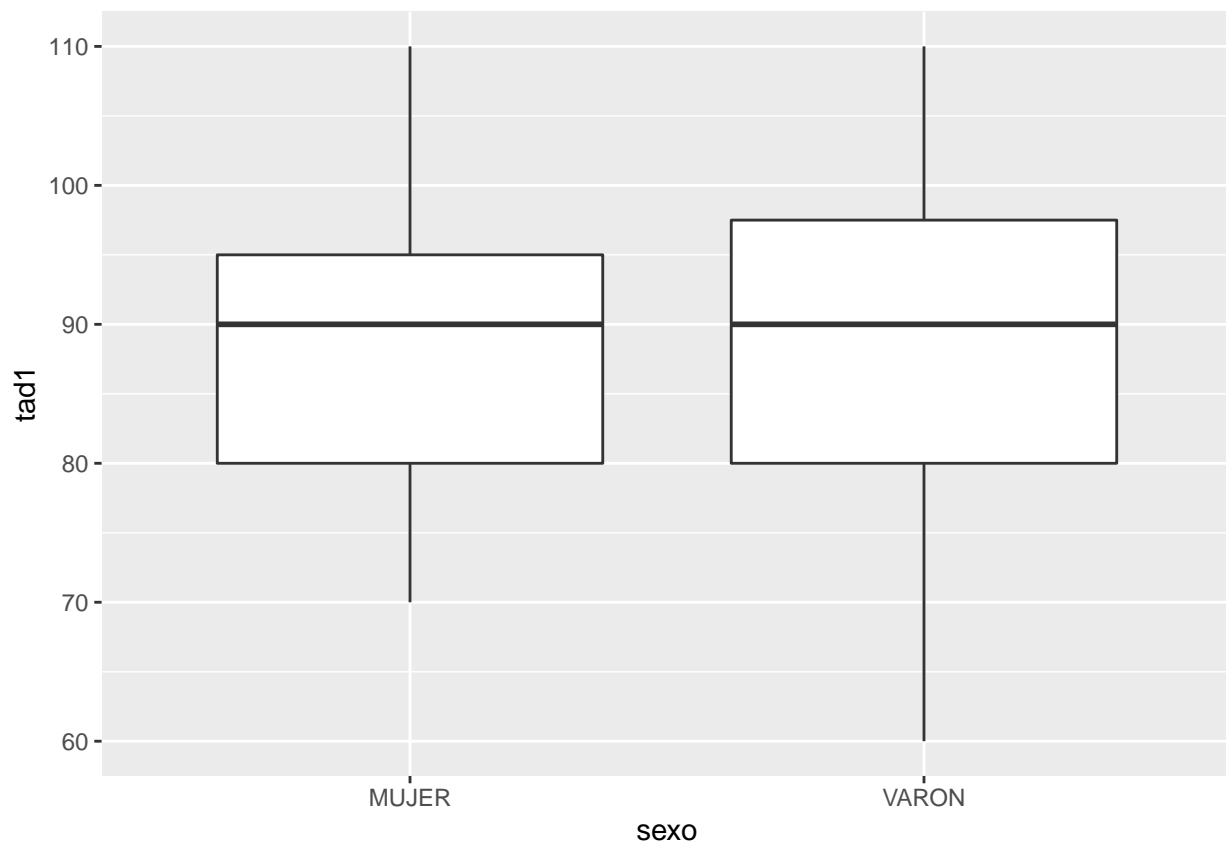
```
with(hta,t.test(tad1,mu=90) ) # One sample T.test
```

```
##  
##  One Sample t-test  
##  
## data:  tad1  
## t = -1.2137, df = 59, p-value = 0.2297  
## alternative hypothesis: true mean is not equal to 90  
## 95 percent confidence interval:  
##  85.80626 91.02707  
## sample estimates:
```

```
## mean of x
## 88.41667
```

Two groups comparison in independent samples

```
ggplot(hta, aes(x=sexo, y=tad1)) +  
  geom_boxplot()
```



Homogeneity variance Test

```
library(car)  
hta%>%  
  group_by(sexo) %>%  
  summarise(var = sd(tad1))
```

```
## # A tibble: 2 x 2  
##   sexo    var  
##   <chr> <dbl>  
## 1 MUJER  9.08  
## 2 VARON 11.8
```

```
with(hta, leveneTest(tad1~factor(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

T test when variances are equal

```
with(hta,t.test(tad1~factor(sexo),var.equal=TRUE ))
```

```
##
## Two Sample t-test
##
## data:  tad1 by factor(sexo)
## t = 0.35427, df = 58, p-value = 0.7244
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -4.453505  6.368899
## sample estimates:
## mean in group MUJER mean in group VARON
##      88.78378      87.82609
```

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

T test when variances are unequal

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

```
##
## Welch Two Sample t-test
##
## data:  tas1 by factor(sexo)
## t = -0.23436, df = 39.098, p-value = 0.8159
## alternative hypothesis: true difference in means is not equal to 0
## 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

U Mann-Whitney or Sum Rank non parametric test

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

```
##
## Wilcoxon rank sum test
```

```
##
## data: tad1 by factor(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

Two groups comparison in dependent samples

Paired T-test

```
with(hta,t.test(tad1,tad12,paired=TRUE))
```

```
##
## Paired t-test
##
## data: tad1 and tad12
## t = 1.8507, df = 51, p-value = 0.07001
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2364274 5.8133505
## sample estimates:
## mean of the differences
## 2.788462
```

```
summary(hta$tad1)
```

```
##   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  60.00  80.00   90.00   88.42  95.00  110.00
```

```
summary(hta$tad12)
```

```
##   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.   NA's
##  60.00  80.00   85.00   84.62  90.00  100.00      8
```

- P value is over 0.05

Paired Sign-Rank Wilcoxon Test

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

```
##
## Wilcoxon signed rank test with continuity correction
```

```
##
## data: tad1 and tad12
## V = 478.5, p-value = 0.05333
## alternative hypothesis: true location shift is not equal to 0
```

diabetes dataset

```
diabetes <- read_excel("datasets/diabetes.xls")
diabetes[1:5,1:9]
```

```
## # A tibble: 5 x 9
##   numpacie mort   tempsviu   edat   bmi edatdiag tabac      sbp   dbp
##   <dbl> <chr>   <dbl> <dbl> <dbl> <dbl> <chr>   <dbl> <dbl>
## 1     1 Vivo    12.4   44  34.2   41 No fumador  132   96
## 2     2 Vivo    12.4   49  32.6   48 Fumador    130   72
## 3     3 Vivo     9.6   49  22     35 Fumador    108   58
## 4     4 Vivo     7.2   47  37.9   45 No fumador  128   76
## 5     5 Vivo    14.1   43  42.2   42 Fumador    142   80
```

```
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"
```

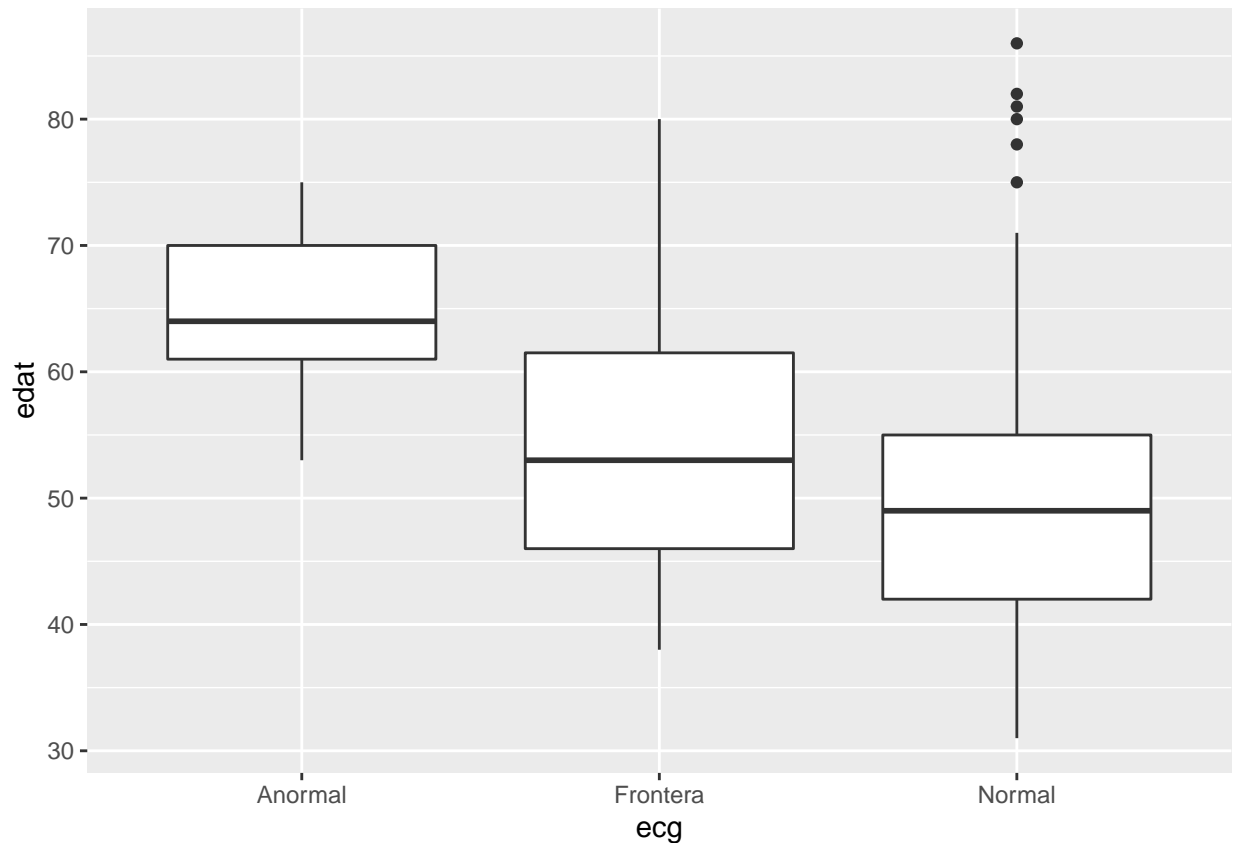
```
diabetes_factor <- diabetes %>%
  mutate_if(sapply(diabetes, is.character), as.factor) %>%
  select (-numpacie)
```

More than two groups comparison in independent samples

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

```
## # A tibble: 3 x 4
##   ecg      n mean   sd
##   <chr> <int> <dbl> <dbl>
## 1 Anormal    11  64.9  6.76
## 2 Frontera   27  53.8 11.4
## 3 Normal   111  50.5 11.5
```

```
ggplot(diabetes, aes(x=ecg, y=edat)) +
  geom_boxplot()
```



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
```

Tukey test for multiple comparison

```
# install.packages("multcomp")
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 0.016479 *
## Normal - Anormal == 0   -14.405      3.543  -4.065 0.000223 ***
## Normal - Frontera == 0   -3.310      2.405  -1.376 0.345712
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

Confidence intervals:

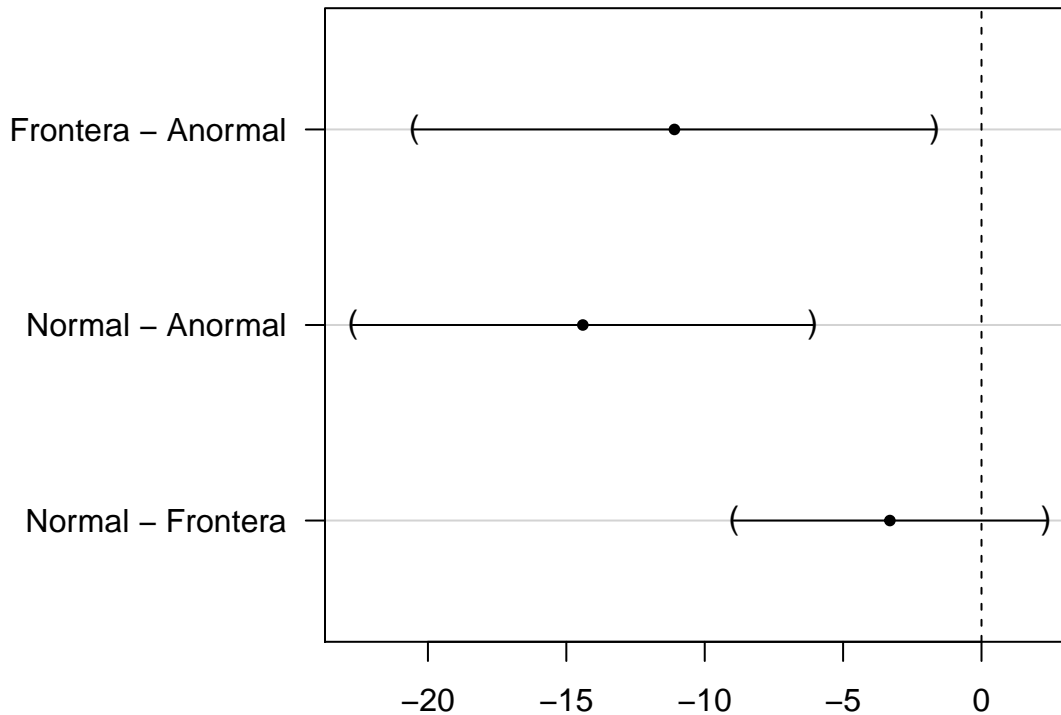
```
print(confint(tuk, level=0.95)) # confidence intervals
```

```
##
## Simultaneous Confidence Intervals
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: aov(formula = edat ~ ecg, data = diabetes_factor)
##
## Quantile = 2.346
## 95% family-wise confidence level
##
##
## Linear Hypotheses:
##               Estimate lwr      upr
## Frontera - Anormal == 0 -11.0943 -20.5010  -1.6876
## Normal - Anormal == 0  -14.4046 -22.7174  -6.0918
## Normal - Frontera == 0   -3.3103  -8.9534   2.3328
```

Multicomparison plot

```
par(mar=c(3,10,3,3))
plot(confint(tuk))
```

95% family-wise confidence level



Kruskal-Wallis Test (non-parametric)

```
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
```

Dunn Test for multiple comparison

```

# install.packages("dunn.test")
library(dunn.test)
with(diabetes_factor,dunn.test(edat,ecg,method="bonferroni"))

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

```

Exercise solutions

Exercise 1

a) Check the normality of tas1 variable in hta dataset

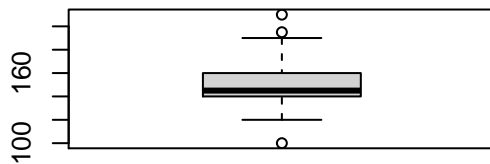
```
oldpar<-par(mfrow=c(1,1)) # Guarda los parámetros para el dibujo
par(mfrow=c(2,2)) # Dibuja cuatro gráficos por grafico
with(hta, boxplot(tas1, main="Box-plot") )

with(hta, hist(tas1) )

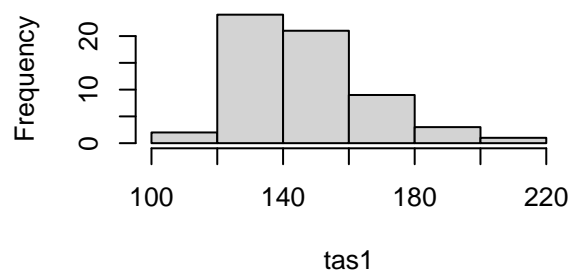
with(hta, qqnorm(tas1, main="Normal QQplot") );with(hta, qqline(tas1) )

par(oldpar) # Vuelve a los parámetros de dibujo originales
```

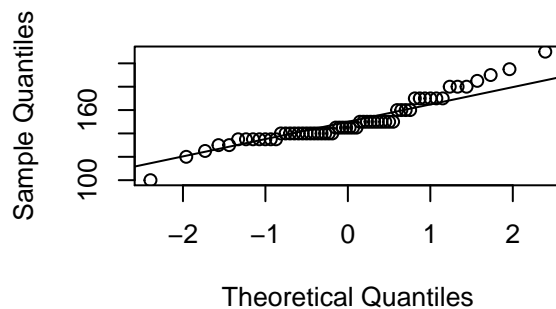
Box-plot



Histogram of tas1



Normal QQplot



```
with(hta,shapiro.test(tas1) ) # Shapiro Wilk test
```

```
##
##  Shapiro-Wilk normality test
##
## data:  tas1
## W = 0.91869, p-value = 0.0006796
```

b) Can it be accepted that the initial TAS is 120 in Hipertensive patients?

```
with(hta,t.test(tas1,mu=120)) # One sample T.test
```

```
##  
## One Sample t-test  
##  
## data: tas1  
## t = 12.088, df = 59, p-value < 2.2e-16  
## alternative hypothesis: true mean is not equal to 120  
## 95 percent confidence interval:  
## 145.1036 155.0631  
## sample estimates:  
## mean of x  
## 150.0833
```

c) Find the 95% confidence interval for the mean of tas1 variable

From the test before we can find the confidence interval for the mean.

d) Extra: Can it be accepted that the initial TAS is higher than 120 in Hipertensive women?

```
hta_w <- subset(hta, sexo=="MUJER")  
with(hta_w,t.test(tas1,mu=120, alternative="greater")) # One sample T.test
```

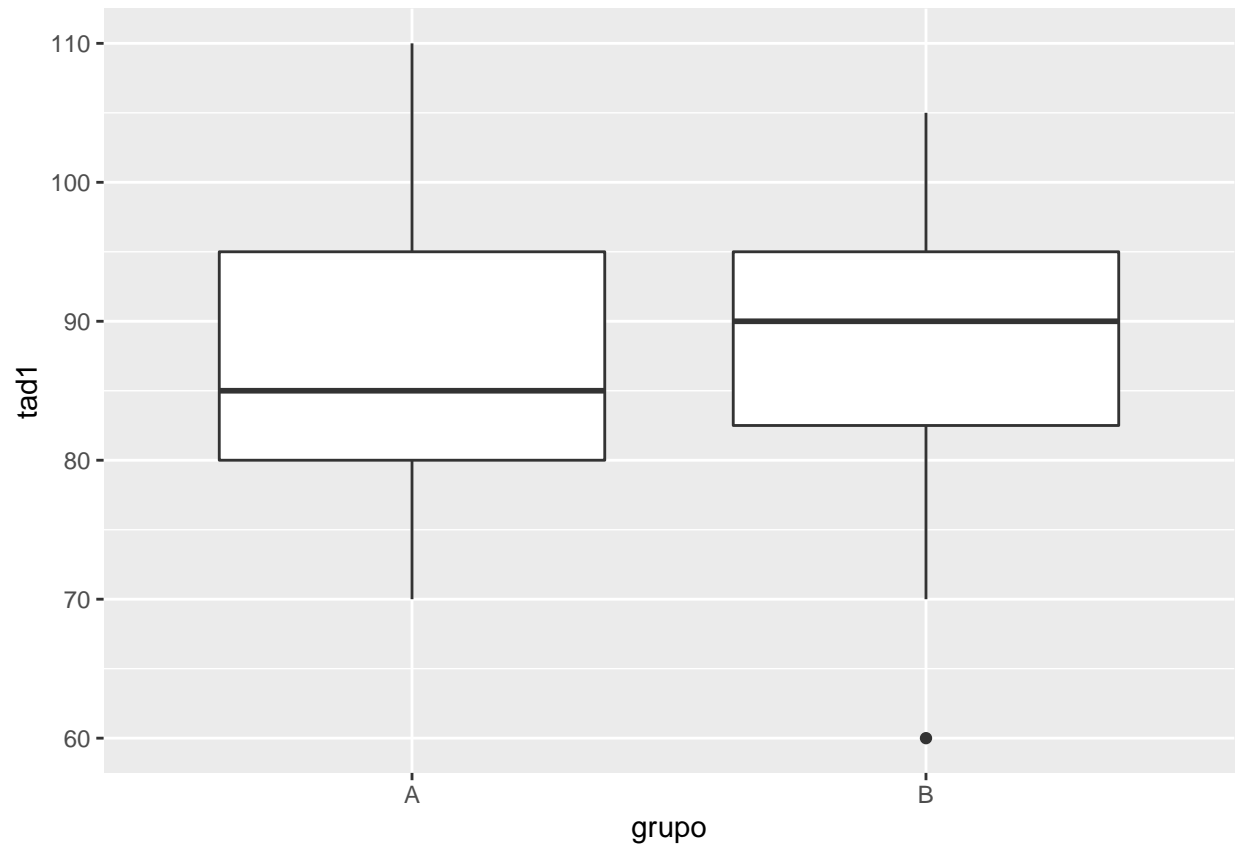
```
##  
## One Sample t-test  
##  
## data: tas1  
## t = 10.22, df = 36, p-value = 1.73e-12  
## alternative hypothesis: true mean is greater than 120  
## 95 percent confidence interval:  
## 144.7058 Inf  
## sample estimates:  
## mean of x  
## 149.5946
```

Exercise 2

Is TAD comparable at baseline time between groups?

We first plot the distribution of tad1 variables in each group

```
ggplot(hta, aes(x=grupo, y=tad1)) +  
  geom_boxplot()
```



Test for homogeneity of variances between the two groups

```
hta%>%
  group_by(grupo) %>%
  summarise(var = sd(tad1))
```

```
## # A tibble: 2 x 2
##   grupo   var
##   <chr> <dbl>
## 1 A     10.6
## 2 B      9.79
```

```
with(hta, leveneTest(tad1~factor(grupo), center="median"))
```

```
## Levene's Test for Homogeneity of Variance (center = "median")
##      Df F value Pr(>F)
## group 1  1.0839 0.3021
##      58
```

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

We apply the t.test for equal variances

```
with(hta, t.test(tad1~factor(grupo), var.equal=TRUE ))
```

```
##
## Two Sample t-test
##
```

```
## data: tad1 by factor(grupo)
## t = 0.15001, df = 58, p-value = 0.8813
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -4.874549 5.664316
## sample estimates:
## mean in group A mean in group B
##      88.62069      88.22581
```

- Differences between groups are not significant.

Using a non-parametric test:

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

```
##
## Wilcoxon rank sum test
##
## data: tad1 by factor(grupo)
## W = 432, p-value = 0.7926
## alternative hypothesis: true location shift is not equal to 0
```

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

```
## # A tibble: 2 x 2
##   grupo median
##   <chr>   <dbl>
## 1 A       85
## 2 B       90
```

- We obtain similar results.

Exercise 3

Is systolic blood pressure (TAS) comparable between first and 12th measures?

We can use the paired t.test since sample size is big and

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

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

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.     NA's  
##    110.0   130.0   139.0   137.2   150.0   175.0         8
```

- P value is below 0.05

With the non-parametric test Paired Sign-Rank Wilcoxon Test

```
with(hta,wilcox.test(tas1,tas12,  
  exact=TRUE, paired=TRUE))
```

```
##  
## Wilcoxon signed rank test with continuity correction  
##  
## data: tas1 and tas12  
## V = 1046, p-value = 2.398e-06  
## alternative hypothesis: true location shift is not equal to 0
```

- We obtain similar results

Exercise 4

- a) Se efectúa un estudio de seguimiento a 1018 sujetos atendidos en una clínica de obesidad. Se mide el Índice de Masa Corporal(IMC) y el perfil lipídico. Al cabo de 12 meses se evalúa de nuevo el IMC y el colesterol estando interesados en cuantificar la disminución de ambos parámetros.

Disponemos de dos variables cuantitativas medidas en dos momentos distintos (muestras apareadas). Se quiere analizar si los valores pre y post sufren algún cambio, para ello debemos: en primer lugar evaluar la normalidad de las variables. En caso de que se ajusten a una distribución normal, miramos la homogeneidad de las variables y realizamos un test t de Student para muestras apareadas. En el caso de que no sigan una distribución normal, realizaremos el test no paramétrico de Wilcoxon para muestras apareadas.

- b) Se analizan un grupo de variables inmunológicas (leucocitos totales, linfocitos B, natural Killer, etc) en una muestra de 102 hombres y 147 mujeres mayores de 65 años. Se está interesado en ver la existencia de diferencias por sexo.

Disponemos de distintas variables cuantitativas (leucocitos,linfocitos, etc.) y una variable cualitativa, sexo. Queremos ver si existen diferencias de las variables cuantitativas según el sexo, para ello debemos: en primer lugar evaluar la normalidad de las variables. En caso de que se ajusten a una distribución normal, miramos la homogeneidad de las variables y realizamos un test t de Student. En el caso de que no sigan una distribución normal, realizaremos el test no paramétrico U de Mann-Whitney.

Exercise 5

Are there differences between systolic pressure (sbp) and ECG in diabetic patients?

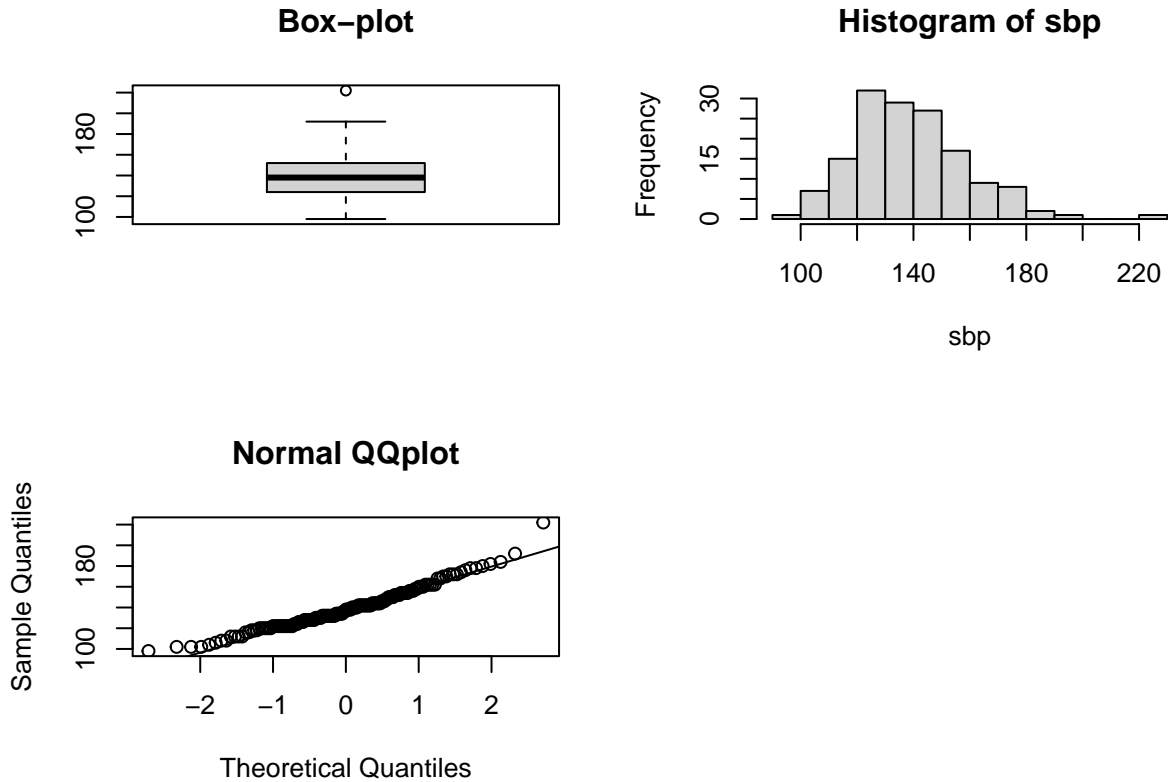
Extra: And between the follow-up time (tempsviu) and the ECG?

- a) For each variable, perform some descriptives and check normality assumptions
- Check normality

```
oldpar<-par(mfrow=c(1,1)) # Guarda los parámetros para el dibujo  
par(mfrow=c(2,2)) # Dibuja cuatro gráficos por grafico  
with(diabetes, boxplot(sbp, main="Box-plot" ) )  
  
with(diabetes, hist(sbp) )
```



```
with(diabetes, qqnorm(sbp, main="Normal QQplot") );with(diabetes, qqline(sbp) )
par(oldpar) # Vuelve a los parámetros de dibujo originales
```



```
with(diabetes, shapiro.test(sbp) ) # Shapiro Wilk test
```

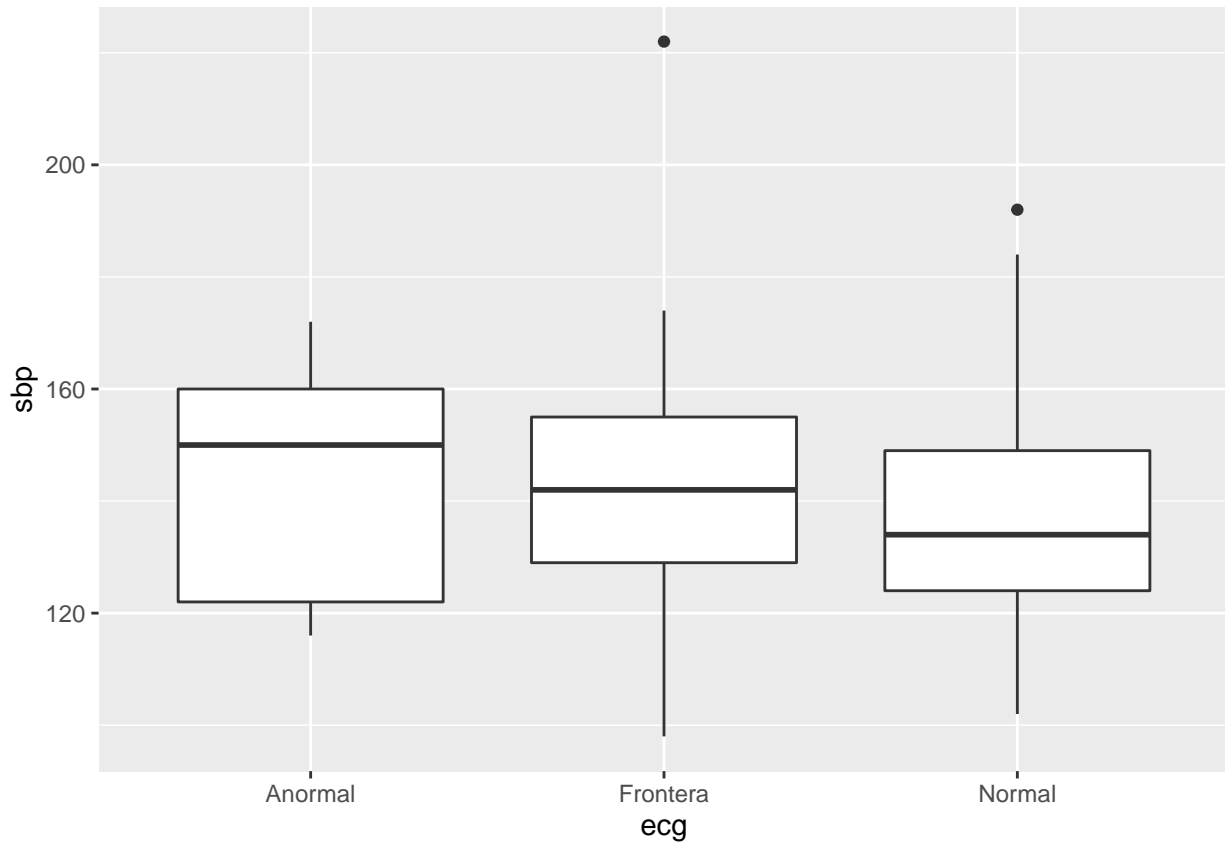
```
##
##  Shapiro-Wilk normality test
##
## data:  sbp
## W = 0.96785, p-value = 0.001455
```

- Summarise variable by ECG group

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

```
## # A tibble: 3 x 5
##   ecg          n mean median   sd
##   <chr>    <int> <dbl>  <dbl> <dbl>
## 1 Anormal     11  144    150  21.1
## 2 Frontera    27  144.    142  23.8
## 3 Normal    111  138.    134  19.1
```

```
ggplot(diabetes, aes(x=ecg, y=sbp)) +  
  geom_boxplot()
```



b) Set the hypothesis contrast and perform the test

H0: The means of all the groups are equal H1: Not all the means are equal. At least there are two different means

```
anova_sbp <- aov(sbp~ecg, data=diabetes_factor)  
summary(anova_sbp)
```

```
##           Df Sum Sq Mean Sq F value Pr(>F)  
## ecg         2   1061    530.3   1.301  0.275  
## Residuals 146   59518    407.7
```

c) If needed, apply a post-hoc test for multiple comparisons between groups to test which groups are different.

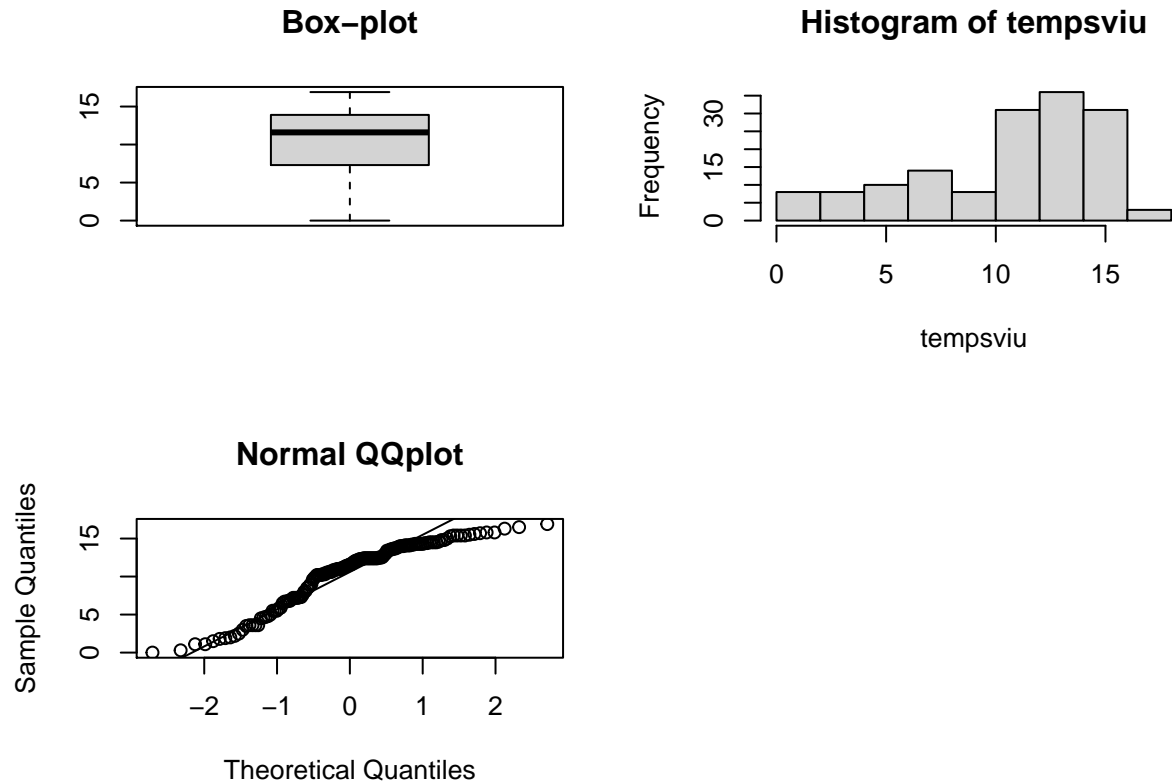
Not needed here since we cannot reject the null hypothesis.

d) Extra: Analyze the relation between the follow-up time (tempsviu) and the ECG.

- Check normality of tempsviu

```
oldpar<-par(mfrow=c(1,1)) # Guarda los parámetros para el dibujo  
par(mfrow=c(2,2)) # Dibuja cuatro gráficos por grafico  
with(diabetes, boxplot(tempsviu, main="Box-plot" ) )  
  
with(diabetes, hist(tempsviu) )
```

```
with(diabetes, qqnorm(tempsviu, main="Normal QQplot" ));with(diabetes, qqline(tempsviu) )
par(oldpar) # Vuelve a los parámetros de dibujo originales
```



```
with(diabetes, shapiro.test(tempsviu) ) # Shapiro Wilk test
```

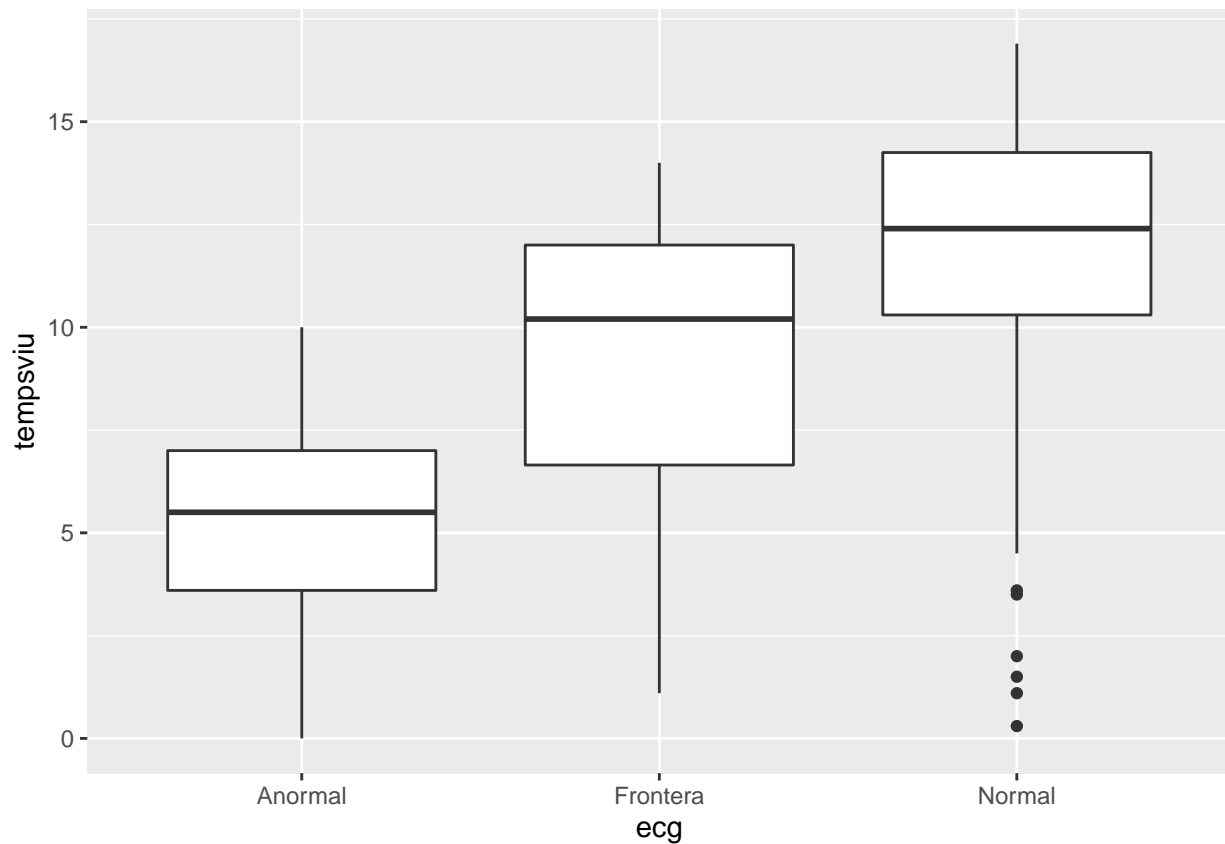
```
##
## Shapiro-Wilk normality test
##
## data: tempsviu
## W = 0.92048, p-value = 2.463e-07
```

- Summarise variable by ECG group

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

```
## # A tibble: 3 x 5
##   ecg      n mean median  sd
##   <chr> <int> <dbl> <dbl> <dbl>
## 1 Anormal    11  5.24   5.5  2.86
## 2 Frontera   27  8.64  10.2  4.04
## 3 Normal   111 11.5   12.4  3.68
```

```
ggplot(diabetes, aes(x=ecg, y=tempsviu)) +  
  geom_boxplot()
```



b) Set the hypothesis contrast and perform the test

H0: The means of all the groups are equal H1: Not all the means are equal. At least there are two different means

We will perform a non-parametric test since we can't assume normality of the data

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

```
## # A tibble: 3 x 2  
##   ecg      median  
##   <fct>    <dbl>  
## 1 Anormal     5.5  
## 2 Frontera    10.2  
## 3 Normal     12.4
```

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

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: tempsviu by ecg  
## Kruskal-Wallis chi-squared = 28.977, df = 2, p-value = 5.103e-07
```

- We can reject the null hypothesis (p-value < 0.05)
- c) If needed, apply a post-hoc test for multiple comparisons between groups to test which groups are different.

Non-parametric Dunn Test for multiple comparison

```
# install.packages("dunn.test")
library(dunn.test)
with(diabetes_factor,dunn.test(tempsviu,ecg,method="bonferroni"))
```

```
##    Kruskal-Wallis rank sum test
##
## data: tempsviu and ecg
## Kruskal-Wallis chi-squared = 28.9766, df = 2, p-value = 0
##
##
##                               Comparison of tempsviu by ecg
##                               (Bonferroni)
## Col Mean-|
## Row Mean |      Anormal   Frontera
## -----+-----
## Frontera |   -1.917822
##           |      0.0827
##           |
## Normal   |  -4.532635  -3.480052
##           |      0.0000*   0.0008*
##
## alpha = 0.05
## Reject Ho if p <= alpha/2
```

- Differences between Normal-Anormal groups and between Normal-Frontera groups are significant (adjusted p-value < 0.05).

Exercise 6

Se estudia un grupo de 33 pacientes afectados de Carcinoma hepatocelular, un grupo de 22 afectados únicamente de cirrosis y un grupo control de 31 donantes de sangre. Se determina la actividad celular NK y el número de células CK en los tres grupos. (Nota: la media de actividad celular es de 39 unidades líticas / 10^7 de linfocitos y la mediana es de 28 y la media del número de células es de 178 y la mediana de 163)

Solución: Se dispone de tres grupos de pacientes independientes y dos variables cuantitativas a comparar en los tres grupos. Aunque desconocemos el comportamiento de las variables, por las pistas vemos que las medias y las medianas son bastante diferentes con lo cual la distribución no debe de ser simétrica. Por otra parte el tamaño muestral de los grupos no es muy grande. Optaríamos por efectuar un contraste no paramétrico de igualdad de distribuciones en los tres grupos. Utilizaríamos la prueba de Kruskal-Wallis. Si hay diferencias, para identificar entre qué dos grupos están usaríamos la corrección de Bonferroni del ejercicio anterior o el Dunn-test que ya efectúa los contrastes con la p corregida.