supplementary information

Data · December 2015

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Data Analysis

Loading data

```
library(data.table)
library(ggplot2)

#open file
DT <- fread("./dataPreparation//data_repaired_translitarated.csv", colClasses=rep("charac
ter", 977))
#dim(DT)

#choose coloumns needed
chosen.col <- c(184, 185, 186, 28, 16, 6)
print("Names of chosen coloumns are:")
```

```
## [1] "Names of chosen coloumns are:"
```

```
names(DT)[chosen.col]
```

```
## [1] "T4"                "TTT"               "AT TP"
## [4] "otlichija BI i MI" "vozrast god"       "pol"
```

```
#change names to readable
DTsub <- subset(DT, select=chosen.col)
setnames(DTsub, c("T4", "TSH", "ATPO", "group", "age", "gender"))
print("changed to :")
```

```
## [1] "changed to :"
```

```
names(DTsub)
```

```
## [1] "T4"     "TSH"    "ATPO"   "group"  "age"    "gender"
```

```
#variables for plots
variable.names <- c("T4", "TSH", "ATPO")
var.plot.notation <- c("T4, pm/l", "TSH, mIU/L", "Anti-TPO, IU/mL")
```

preparation of the data

```
#make data numeric
num.var <- c("T4", "TSH", "ATPO", "age")
DTsub[, eval(num.var):=lapply(.SD, as.numeric), .SDcols=num.var]

#make appropriate factor levels
group.values <- c("1", "2", "3", "90", "7")
new.group.values <- c("LS", "TS", "TIA", "control", "control")

library(plyr)
DTsub[, group:=mapvalues(group, from=group.values, to=new.group.values)]
DTsub <- DTsub[group %in% new.group.values, ]
DTsub[ , group := factor(group)]
DTsub[ , group := factor(group, levels(group)[c(1, 4, 2, 3)])]

#gender
DTsub[, gender:=mapvalues(gender, from=c("m", "zh"), to=c("male", "female"))]

#age
DTsub[age<=45, age.cohort:="young"]
DTsub[age>45, age.cohort:="elderly"]
DTsub <- DTsub[age.cohort %in% c("young", "elderly"), ]

#all data for young, for transient ischemic attack are NA except one that is 0. So we do
not use them
DTsub <- DTsub[! (group=="TIA" & age.cohort=="young"), ]

#use only the data where all records available and refactor
DTsub <- na.omit(DTsub)
DTsub[, gender:=factor(gender)]
DTsub[, age.cohort:=factor(age.cohort)]
```

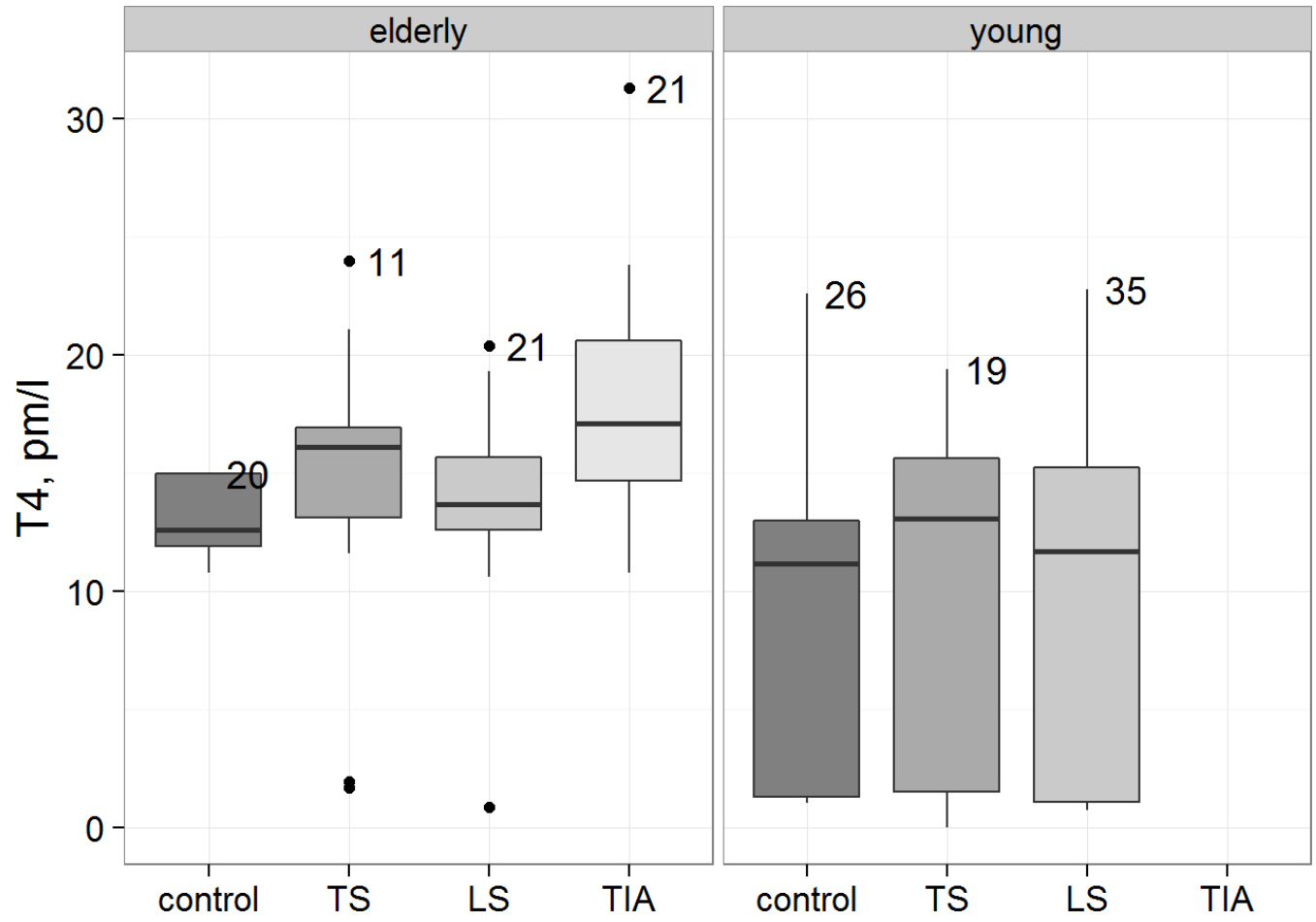
```
print.overview <- function(dt){#function to print overview of data
    #summary(DTsub)
    print("data summary---")
    print( summary(dt) )

    print("Male and Female separatelly---")
    data.overview <- dt[, list(.N, mean(age), sd(age)), by=list(age.cohort, group, gende
r)]
    setnames(data.overview, c("group", "V2", "V3"), c("group", "age.mean", "age.sd"))
    setkey(data.overview, age.cohort, group, gender)
    print( unique( data.overview ) )

    print("Male and Female together---")
    data.overview <- dt[, list(.N, mean(age), sd(age)), by=list(age.cohort, group)]
    setnames(data.overview, c("group", "V2", "V3"), c("group", "age.mean", "age.sd"))
    setkey(data.overview, age.cohort, group)
    print( unique( data.overview ) )

    }

print.overview(DTsub)
```

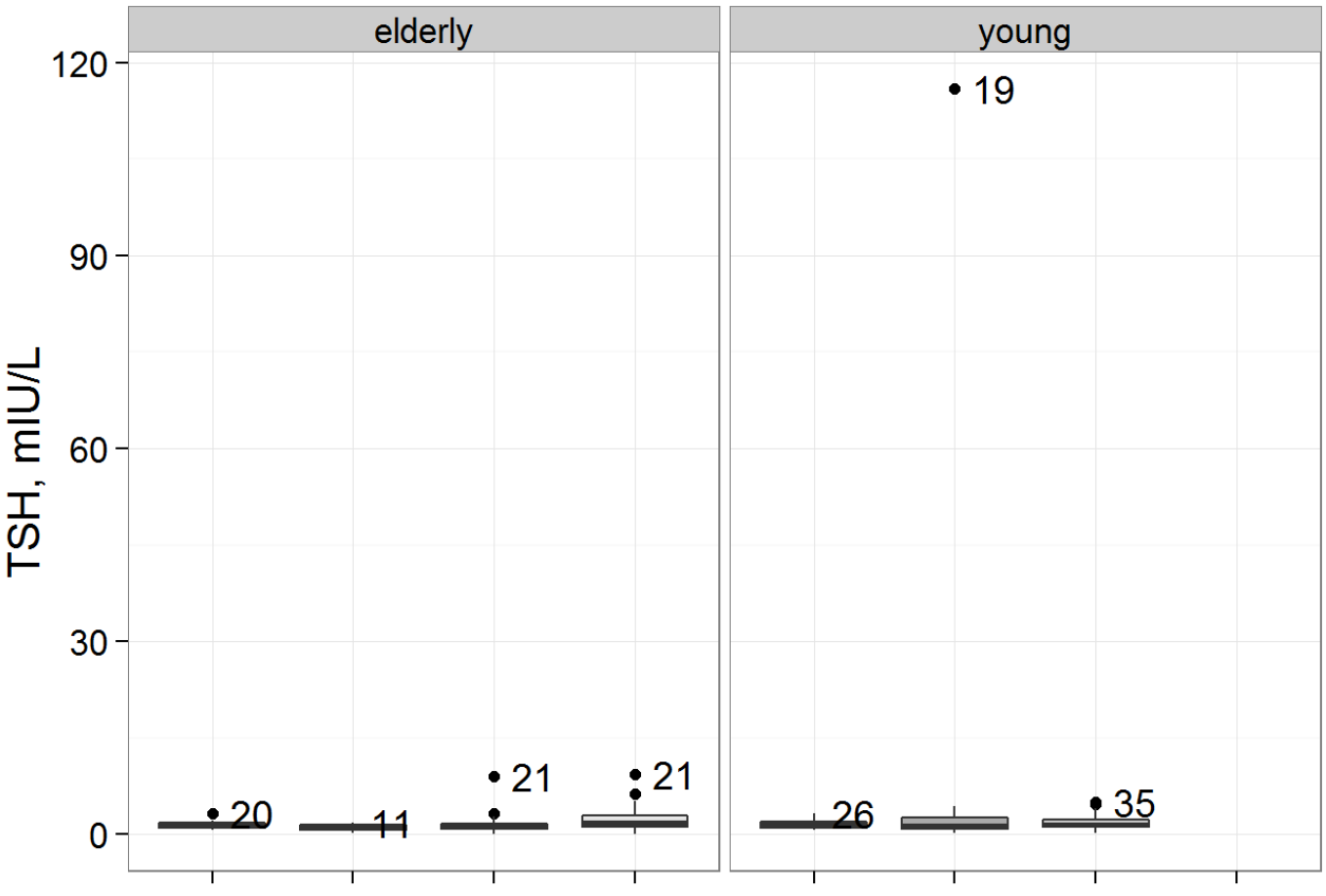
```
## [1] "data summary---"
##        T4              TSH               ATPO             group
##  Min.   : 0.01   Min.   :  0.054   Min.   :   0.00   control:46
##  1st Qu.:10.80   1st Qu.:  0.980   1st Qu.:   0.24   TS     :30
##  Median :13.20   Median :  1.450   Median :   1.30   LS     :56
##  Mean   :12.34   Mean   :  2.555   Mean   :  41.56   TIA    :21
##  3rd Qu.:16.00   3rd Qu.:  2.210   3rd Qu.:   5.00
##  Max.   :31.30   Max.   :115.900   Max.   :1000.00
##       age           gender     age.cohort
##  Min.   :17.00   female:85   elderly:73
##  1st Qu.:37.00   male  :68   young  :80
##  Median :45.00
##  Mean   :48.62
##  3rd Qu.:59.00
##  Max.   :90.00
## [1] "Male and Female separatelly---"
##     age.cohort   group gender  N age.mean    age.sd
##  1:    elderly control female  8 56.00000  4.000000
##  2:    elderly control   male 12 54.00000  5.009083
##  3:    elderly      TS female  5 60.80000  9.909591
##  4:    elderly      TS   male  6 61.33333 12.420413
##  5:    elderly      LS female 11 68.00000 11.575837
##  6:    elderly      LS   male 10 56.80000  7.969386
##  7:    elderly     TIA female 18 67.83333 14.805603
##  8:    elderly     TIA   male  3 74.33333  6.429101
##  9:      young control female 18 30.44444  5.382130
## 10:      young control   male  8 31.25000  5.873670
## 11:      young      TS female  4 36.25000  9.569918
## 12:      young      TS   male 15 39.73333  5.133457
## 13:      young      LS female 21 38.95238  6.719198
## 14:      young      LS   male 14 39.57143  7.324504
## [1] "Male and Female together---"
##    age.cohort   group  N age.mean    age.sd
## 1:    elderly control 20 54.80000  4.629425
## 2:    elderly      TS 11 61.09091 10.793095
## 3:    elderly      LS 21 62.66667 11.332843
## 4:    elderly     TIA 21 68.76190 13.996088
## 5:      young control 26 30.69231  5.431532
## 6:      young      TS 19 39.00000  6.155395
## 7:      young      LS 35 39.20000  6.867657
```

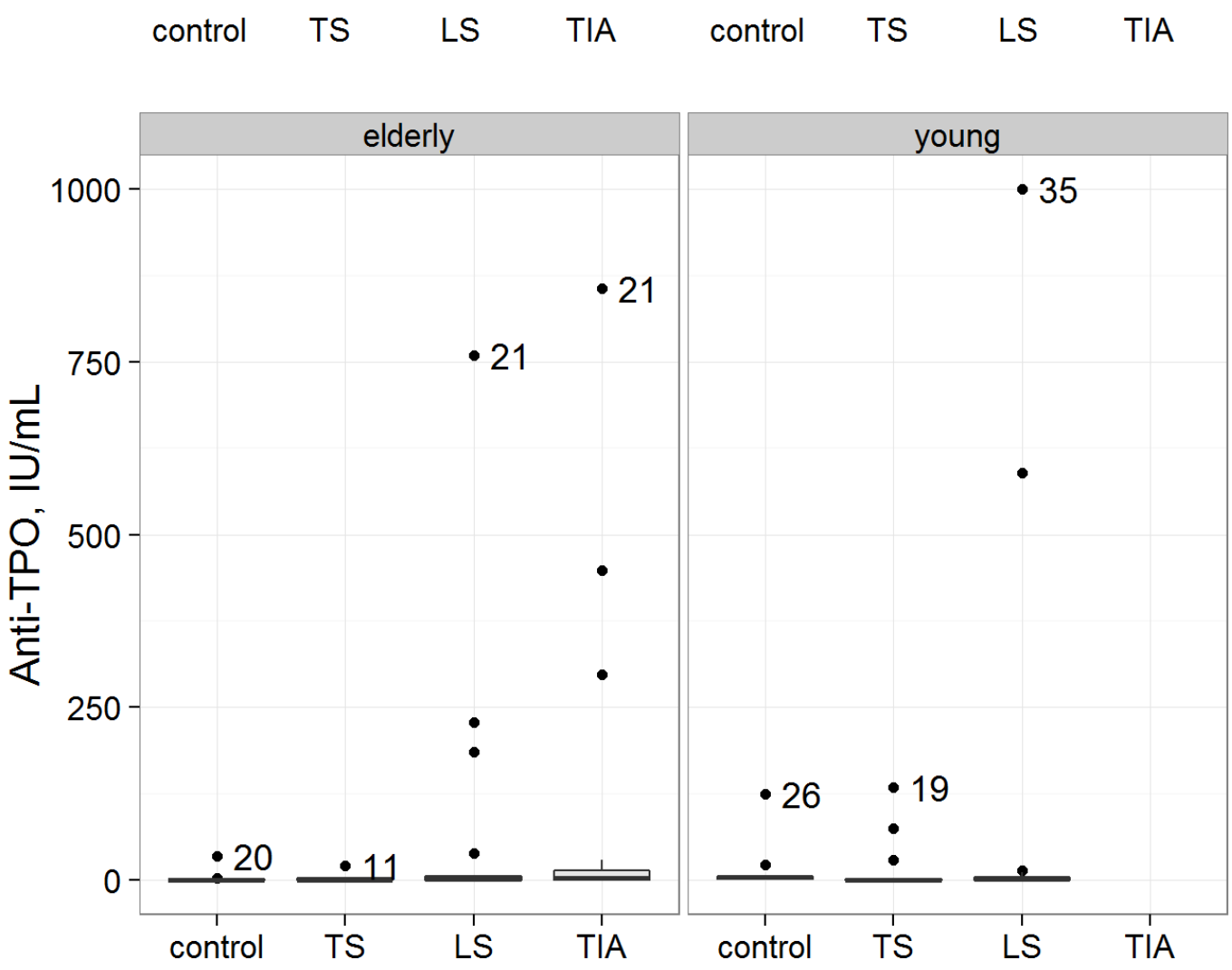
Plot

With outliers

```
give.n <- function(x){ #function for positioning numbers on plots
  return(data.frame(y = max(x), label = paste0("       ",length(x))))
}

for(variable in variable.names){
    plot.var <- ggplot(data=DTsub,
                       aes(x=group, y =get(variable), fill=group)) + geom_boxplot() +
        facet_grid(.~ age.cohort, drop=T, space="free_x") +
        theme_bw(16) +
        theme(legend.position="none", axis.title.x = element_blank()) +
        ylab(var.plot.notation[variable==variable.names]) +
        stat_summary(fun.data = give.n, geom = "text") +
        scale_fill_grey(start = 0.5, end = .9)

    print(plot.var)

    tiff(paste0("outfile",variable,"wo.out.tiff"), res=300, height=5, width=6.80, unit
s="in")
    print(plot.var)
    dev.off()
}
```


elderly
young
T4, pm/l
30
20
10
0
21
11
21
20
26
19
35
control
TS
LS
TIA
control
TS
LS
TIA


elderly
young
TSH, mIU/L
120
90
60
30
0
19
20
11
21
21
26
35



Without outliers

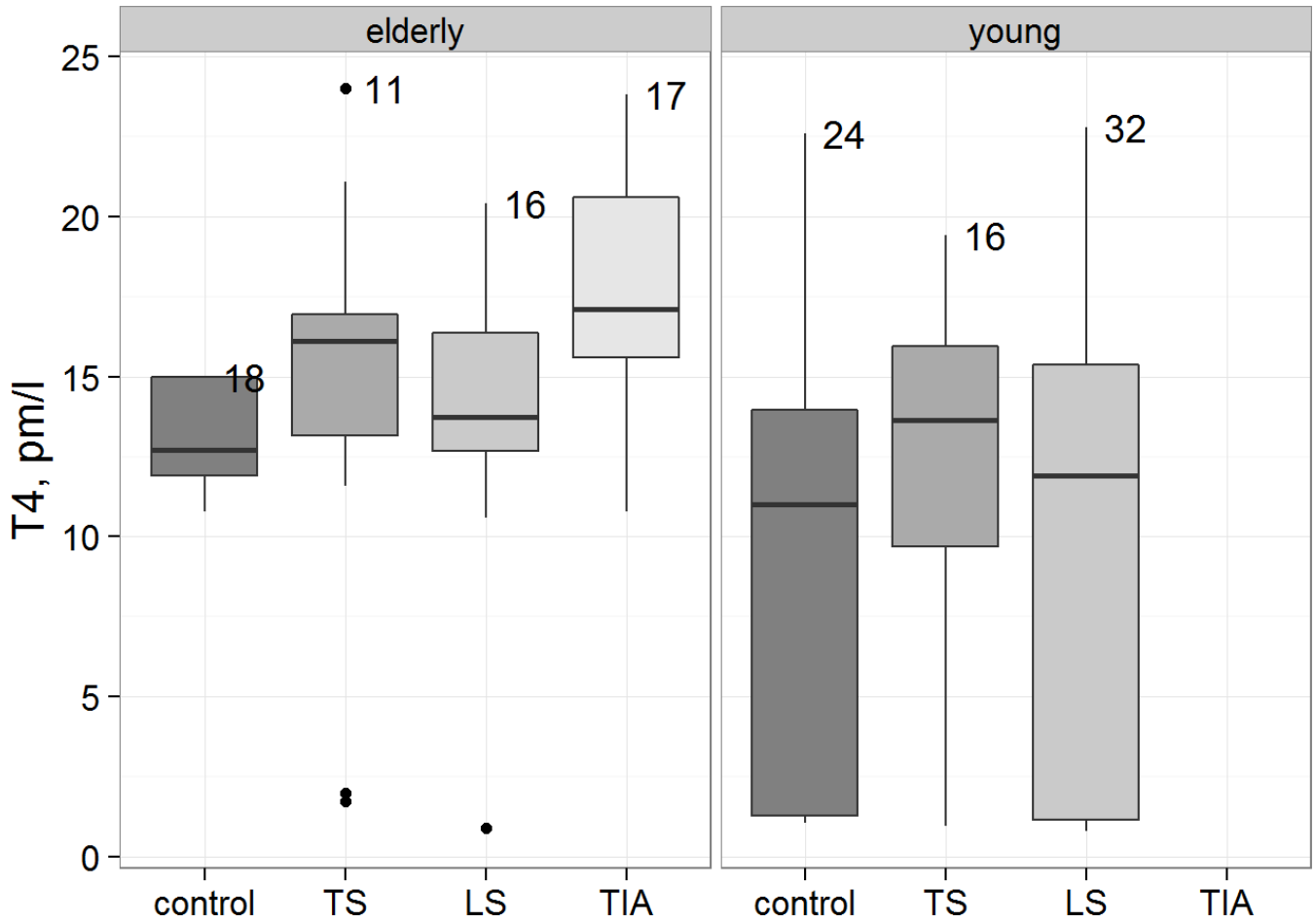
Here we are cuttiong out records for which values of variables are 5 IQR (Interquartile ranges) lower or higher relative to 1st and 3rd quartiles correspondingly.

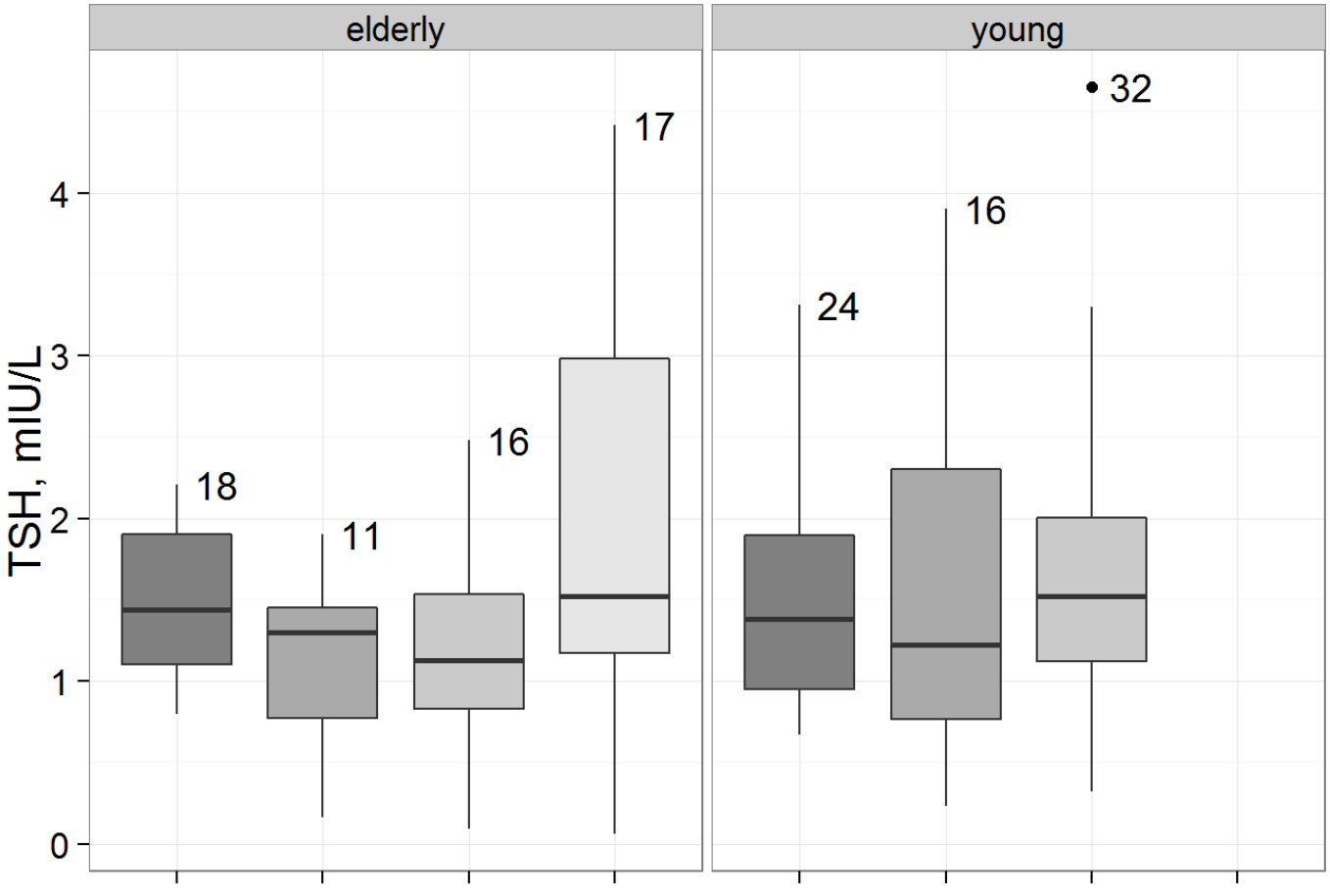
```
remove_outliers <- function(x, na.rm = TRUE, countIQR = 5, ...) {
    H <- countIQR * IQR(x, na.rm = na.rm)
    qrts <- quantile(x, probs=c(.25, .75), na.rm = na.rm, ...)
    y <- x
    y[x < (qrts[1] - H)] <- NA
    y[x > (qrts[2] + H)] <- NA
    y
}

DTsub.wout <- data.table(DTsub)

DTsub.wout[age.cohort=="young", eval(variable.names):=lapply(.SD, remove_outliers), .SDco
ls=variable.names]
DTsub.wout[age.cohort=="elderly", eval(variable.names):=lapply(.SD, remove_outliers), .SD
cols=variable.names]

DTsub.wout <- na.omit(DTsub.wout)
```

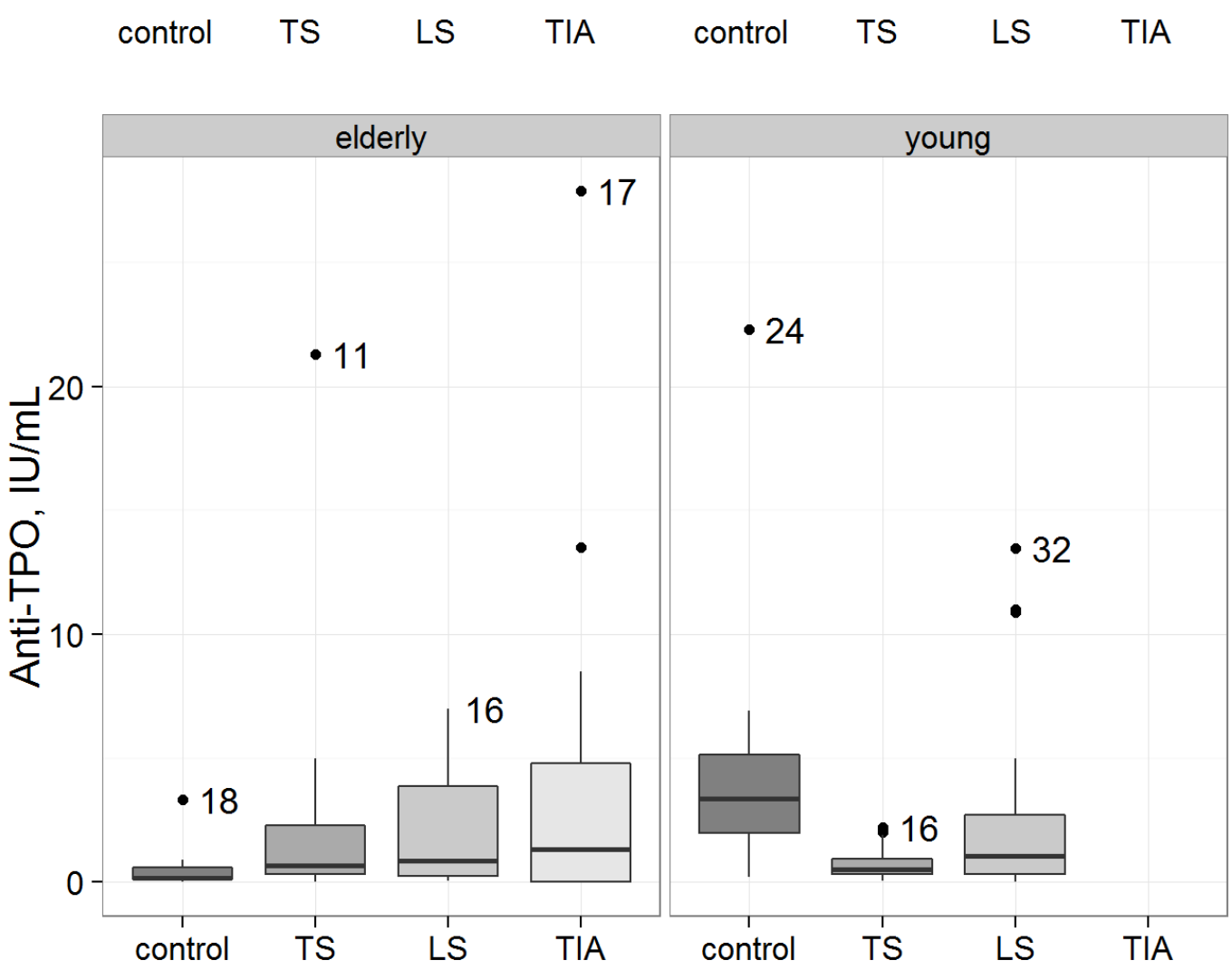
```
print.overview(DTsub.wout)
```

```
## [1] "data summary---"
##        T4              TSH              ATPO            group
##  Min.   : 0.80   Min.   :0.060   Min.   : 0.000   control:42
##  1st Qu.:10.80   1st Qu.:0.930   1st Qu.: 0.200   TS     :27
##  Median :13.50   Median :1.380   Median : 0.985   LS     :48
##  Mean   :12.40   Mean   :1.544   Mean   : 2.749   TIA    :17
##  3rd Qu.:16.25   3rd Qu.:1.920   3rd Qu.: 3.280
##  Max.   :24.00   Max.   :4.651   Max.   :27.900
##       age           gender     age.cohort
##  Min.   :17.00   female:71   elderly:62
##  1st Qu.:36.00   male  :63   young  :72
##  Median :45.00
##  Mean   :47.88
##  3rd Qu.:57.00
##  Max.   :90.00
## [1] "Male and Female separatelly---"
##     age.cohort   group gender  N age.mean    age.sd
##  1:    elderly control female  6 56.00000  4.732864
##  2:    elderly control   male 12 54.00000  5.009083
##  3:    elderly      TS female  5 60.80000  9.909591
##  4:    elderly      TS   male  6 61.33333 12.420413
##  5:    elderly      LS female  8 68.62500 12.557724
##  6:    elderly      LS   male  8 56.25000  8.795291
##  7:    elderly     TIA female 15 68.00000 15.487322
##  8:    elderly     TIA   male  2 73.00000  8.485281
##  9:      young control female 16 30.12500  5.643580
## 10:      young control   male  8 31.25000  5.873670
## 11:      young      TS female  2 28.00000  1.414214
## 12:      young      TS   male 14 39.35714  5.108171
## 13:      young      LS female 19 39.31579  6.633690
## 14:      young      LS   male 13 39.15385  7.448111
## [1] "Male and Female together---"
##    age.cohort   group  N age.mean    age.sd
## 1:    elderly control 18 54.66667  4.874906
## 2:    elderly      TS 11 61.09091 10.793095
## 3:    elderly      LS 16 62.43750 12.269033
## 4:    elderly     TIA 17 68.58824 14.735412
## 5:      young control 24 30.50000  5.618293
## 6:      young      TS 16 37.93750  6.147832
## 7:      young      LS 32 39.25000  6.858007
```

```
theme_set(theme_grey(base_size = 18))
for(variable in variable.names){

    plot.var <- ggplot(data=DTsub.wout,
                       aes(x=group, y =get(variable), fill=group)) + geom_boxplot() +
        theme_bw(16) +
        facet_grid(.~ age.cohort, drop=T, space="free_x") +
        theme(legend.position="none", axis.title.x = element_blank()) +
        ylab(var.plot.notation[variable==variable.names]) +
        stat_summary(fun.data = give.n, geom = "text")+
        scale_fill_grey(start = 0.5, end = .9)

    print(plot.var)

    tiff(paste0("outfile",variable,"wo.out.tiff"), res=300, height=5, width=6.80, unit
s="in")
    print(plot.var)
    dev.off()
}
```


elderly
young
T4, pm/l
25
20
15
10
5
0
11
17
16
18
24
32
16
control
TS
LS
TIA
control
TS
LS
TIA


elderly
young
TSH, mIU/L
4
3
2
1
0
17
32
16
24
16
18
11



Statistics (outliers removed)

First let us see if all groups are identical. In order to do this we could use ANOVA test if

- the data would be independent (yes in this case)
- normally distributed (no in this case). ANOVA can be robust to not normal data if sample sizes are equal (no in this case).
- have a identical variances (no for elderly patients - checked bellow with Levene's Test).

```
print.test <- function(dt, age.cohort.v, variable, statistics.v, test="kruskal"){
        print(age.cohort.v)
        print(variable)
        DTtemp <- na.omit(dt[age.cohort==age.cohort.v, list(get(variable), group)])
        setnames(DTtemp, "V1", eval(variable))

        DTstat <- DTtemp[, list(.N, mean(get(variable)), var(get(variable)), median(get(v
ariable))),
                         by=group]
        setnames(DTstat, c("N", "V2", "V3", "V4"), c("# of observations", "mean", "varian
ce", "median"))

        if(test == "levene"){
            library(car)
            print( leveneTest(get(variable) ~ group, data=DTtemp) )
        }
        if(test == "kruskal"){
            print( DTstat  )
            print(  kruskal.test(get(variable) ~ group, data=DTtemp)  )
        }
}
for(age.cohort.v in c("elderly", "young")){
    for(variable in variable.names){
        print.test(DTsub.wout, age.cohort.v, variable, test="levene")
    }
}
```

```
## [1] "elderly"
## [1] "T4"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  3  2.4886 0.06931 .
##       58
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## [1] "elderly"
## [1] "TSH"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value   Pr(>F)
## group  3  5.3346 0.002582 **
##       58
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## [1] "elderly"
## [1] "ATPO"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.8894 0.1414
##       58
## [1] "young"
## [1] "T4"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2  0.5839 0.5604
##       69
## [1] "young"
## [1] "TSH"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2  0.5837 0.5606
##       69
## [1] "young"
## [1] "ATPO"
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2  2.3095 0.1069
##       69
```

Thus, in the case we should use Kruskal-Wallis test, which does not require from data to satisfy those conditions.

```
for(age.cohort.v in c("elderly", "young")){
    for(variable in variable.names){
        print.test(DTsub.wout, age.cohort.v, variable, test="kruskal")
    }
}
```

```
## [1] "elderly"
## [1] "T4"
##      group # of observations     mean  variance median
## 1:     TIA                17 18.00000 12.671250  17.10
## 2:      LS                16 14.11250 20.373167  13.75
## 3:      TS                11 14.30000 48.166880  16.10
## 4: control                18 13.08889  2.443399  12.70
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 17.1701, df = 3, p-value = 0.000652
##
## [1] "elderly"
## [1] "TSH"
##      group # of observations     mean  variance median
## 1:     TIA                17 1.907647 1.7693191   1.52
## 2:      LS                16 1.210000 0.3495333   1.13
## 3:      TS                11 1.129091 0.3487291   1.30
## 4: control                18 1.481111 0.2355634   1.44
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 4.5738, df = 3, p-value = 0.2058
##
## [1] "elderly"
## [1] "ATPO"
##      group # of observations      mean  variance median
## 1:     TIA                17 4.3094118 51.184731   1.30
## 2:      LS                16 2.1712500  6.275238   0.87
## 3:      TS                11 3.0600000 38.907600   0.66
## 4: control                18 0.6166667  1.056118   0.16
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 5.8821, df = 3, p-value = 0.1175
##
## [1] "young"
## [1] "T4"
##      group # of observations     mean variance median
## 1:      TS                16 11.78937 43.96039  13.65
## 2:      LS                32  9.26375 51.46428  11.90
## 3: control                24 10.48750 61.91223  11.00
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 1.6173, df = 2, p-value = 0.4455
```

```
##
## [1] "young"
## [1] "TSH"
##      group # of observations     mean  variance median
## 1:      TS                16 1.529375 1.1537662   1.22
## 2:      LS                32 1.677375 0.8206844   1.52
## 3: control                24 1.576667 0.6283536   1.38
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 1.0398, df = 2, p-value = 0.5946
##
## [1] "young"
## [1] "ATPO"
##      group # of observations    mean   variance median
## 1:      TS                16 0.75375  0.4963183   0.50
## 2:      LS                32 2.64250 13.9706129   1.03
## 3: control                24 4.95750 32.1251152   3.35
##
##  Kruskal-Wallis rank sum test
##
## data:  get(variable) by group
## Kruskal-Wallis chi-squared = 18.5842, df = 2, p-value = 9.215e-05
```

We see there is at least one coulomn is different (p value < 0.05) for

- elderly and T4
- young and ATPO

Now we can use paired comparassion to find what exactly is statistically different inside these groups. In order to do this we use post-hoc comparassion following Siegel and Castellan procedure.

Elderly cohort

```
library(pgirmess)
kruskalmc(T4 ~ group, data=DTsub.wout[age.cohort=="elderly", ])
```

```
## Multiple comparison test after Kruskal-Wallis
## p.value: 0.05
## Comparisons
##               obs.dif critical.dif difference
## control-TS  14.398990     18.21625      FALSE
## control-LS   8.256944     16.35445      FALSE
## control-TIA 24.650327     16.09778       TRUE
## TS-LS        6.142045     18.64310      FALSE
## TS-TIA      10.251337     18.41836      FALSE
## LS-TIA      16.393382     16.57926      FALSE
```

```
#kruskalmc(T4 ~ group, data=DTsub.wout[age.cohort=="elderly", ], cont='two-tailed')
```

We see strong evidence of a difference between

- TIA and control

Young cohort

```
kruskalmc(ATPO ~ factor(group), data=DTsub.wout[age.cohort=="young", ])
```

```
## Multiple comparison test after Kruskal-Wallis
## p.value: 0.05
## Comparisons
##             obs.dif critical.dif difference
## control-TS 28.06250     16.17044       TRUE
## control-LS 16.90625     13.52916       TRUE
## TS-LS      11.15625     15.34063      FALSE
```

```
#kruskalmc(ATPO ~ factor(group), data=DTsub.wout[age.cohort=="young", ], cont='two-taile
d')
```

We see here strong evidence of a difference between

- TS and control
- LS and control