25-VanLeijsen

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Reference

Van Leijsen et al. (2017). Plasma AB (Amyloid-B) Levels and Severity and Progression of Small Vessel Disease. Stroke, 16(5), 351–359. https://doi.org/10.1161/STROKEAHA.117.019810

We first load the appropriate packages

```
## Loading required package: survival
```

```
## Loading required package: carData
```

```
##
## Attaching package: 'car'
```

```
## The following object is masked from 'package:biostatUZH':
##
##     logit
```

Notes from reading methods section

- Dependant variable: AB38
- The independant variables
- 1 or more microbleeds (n = 81)
- no microbleed (n=405)
- 1 or more lacunes (n = 132)
- no lacunes (n = 355)
- Covariate: age, sex and hypertension

```
stats.orig.IV = data.frame(
  Fvalue =  NA,
  df1 = NA,
  df2 = NA,
  pvalue = "<0.01", # for microbleed presence and for lacune presence groups
  MD = NA,
  lowerCI = NA,
  upperCI = NA)

stats.orig.allCV =  data.frame(
  Fvalue =  NA,
  df1 = NA,
  df2 = NA,
  pvalue = NA,
  MD = NA,
  lowerCI = NA,
  upperCI = NA)
```

Reading data

Data is loaded, reshaped if necessary, and factors are specified.

```
data = read_excel("../results/data/25-VanLeijsen/VanLeijsen-1.xlsx")
data = data.frame(data)
str(data)
```

```
## 'data.frame':    487 obs. of  9 variables:
##  $ mb_presence_b : num  1 0 0 0 1 1 1 0 1 0 ...
##  $ lac_presence_b: num  1 1 1 1 1 1 1 1 1 0 ...
##  $ AB38          : num  23.7 29.7 26.7 33.5 16.6 38.4 27.1 31.8 24.4 30.1 ...
##  $ AB40          : num  224 225 195 243 131 ...
##  $ AB42          : num  55.8 63.6 63.9 62.3 57.4 72.1 64.8 62.7 60.2 64 ...
##  $ age           : num  73.8 69 74.3 64 71.7 ...
##  $ sex           : num  1 2 1 1 1 1 1 1 1 1 ...
##  $ hypertension  : num  1 1 1 1 1 1 1 1 0 1 ...
##  $ tbv_b         : num  955 1111 1069 1053 1025 ...
```

```
data$mb_presence_b = data$mb_presence_b == 1
data$lac_presence_b = data$lac_presence_b == 1
data$sex.factor = NA
data$sex.factor[data$sex == 1] = "male"
data$sex.factor[data$sex == 2] = "female"
data$sex.factor[data$sex.factor == "male" ] = 1
data$sex.factor[data$sex.factor == "female" ] = 0
data$sex.factor = as.factor(data$sex.factor)
data$hypertension.factor = NA
data$hypertension  = data$hypertension == 1

data$Group.factor = NA # only used for table
data[data$mb_presence_b == TRUE,]$Group.factor = "microbleed"
data[data$mb_presence_b == FALSE,]$Group.factor = "no microbleed"
data[data$lac_presence_b == TRUE,]$Group.factor = "lacunes"
data[data$lac_presence_b == FALSE,]$Group.factor = "no lacunes"
unique(data$Group.factor)
```

```
## [1] "lacunes"    "no lacunes"
```

```
data$Group.factor = factor(data$Group.factor,
                           levels = c("no microbleed", "microbleed",
                                      "no lacunes", "lacunes"))
```

Descriptives

Number of samples and mean (SD) in levels of the independant variables. We reproduce the mean and sd values of Table 2 of this study

```
a = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 0]), sd(data$AB38[data$mb_presence_b ==
b = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 1]), sd(data$AB38[data$mb_presence_b ==

c = sprintf("%.1f (%.1f)", mean(data$AB38[data$lac_presence_b == 0]), sd(data$AB38[data$lac_presence_b =
d = sprintf("%.1f (%.1f)", mean(data$AB38[data$lac_presence_b == 1]), sd(data$AB38[data$lac_presence_b =
```

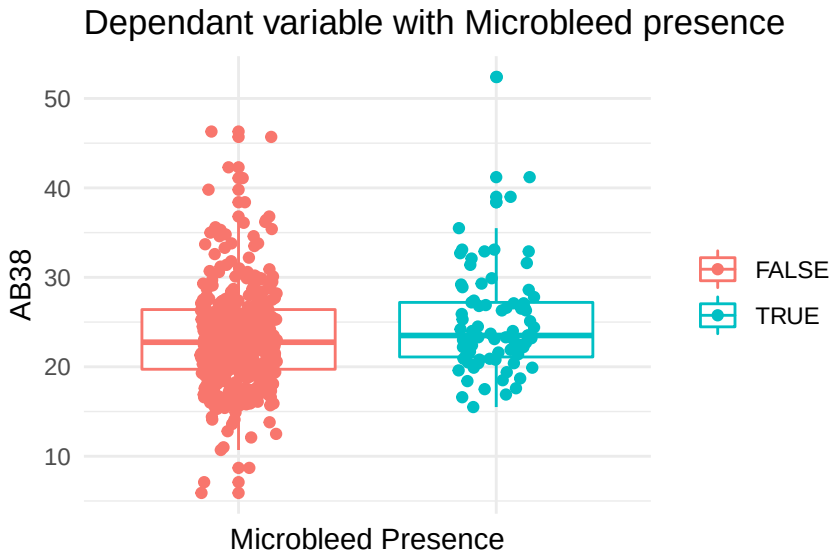
```r
idx = c(1, 3, 2, 4) # sorting as in publication
tab.dv = array(NA, dim=c(4,3))
tab.dv[,1] = levels(data$Group.factor)
tab.dv[,2] = c("405", "81", "355", "132")
tab.dv[,3] = c(a, b, c, d)
colnames(tab.dv) = c("group", "n", "mean (SD)")
print(tab.dv)
```

```
##      group           n     mean (SD)
## [1,] "no microbleed" "405" "23.2 (5.5)"
## [2,] "microbleed"    "81"  "25.2 (6.1)"
## [3,] "no lacunes"    "355" "23.1 (5.5)"
## [4,] "lacunes"       "132" "24.7 (5.9)"
```

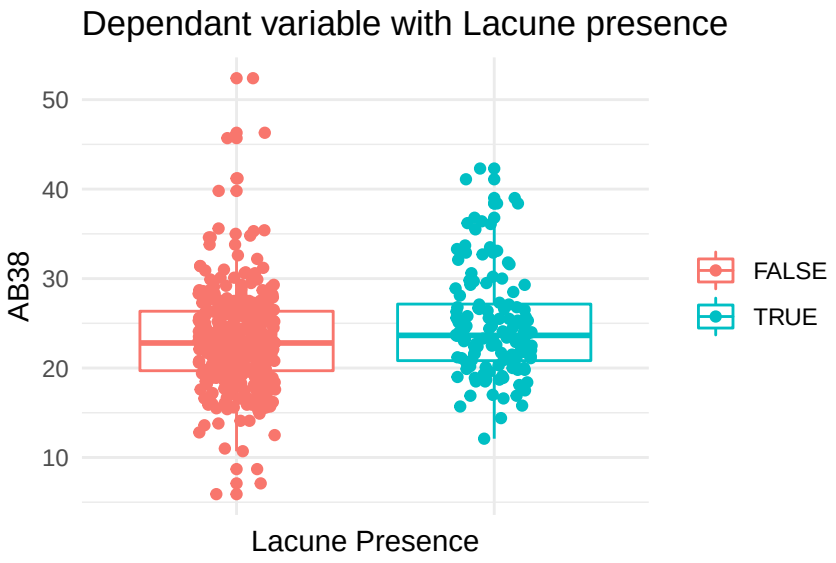
boxplot with DV

- Upon visual inspection, each level of each independant group seem to have a similar outcome effect.

```r
#IV : mb_presence_b
ggplot(data, aes(y=AB38, x = mb_presence_b, color =mb_presence_b )) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("Microbleed Presence") + ylab("AB38") +
  ggtitle("Dependant variable with Microbleed presence")
```




```r
#IV : lac_presence_b
ggplot(data, aes(y=AB38, x = lac_presence_b, color =lac_presence_b )) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("Lacune Presence") + ylab("AB38") +
  ggtitle("Dependant variable with Lacune presence")
```



Descriptives

COV with boxplot

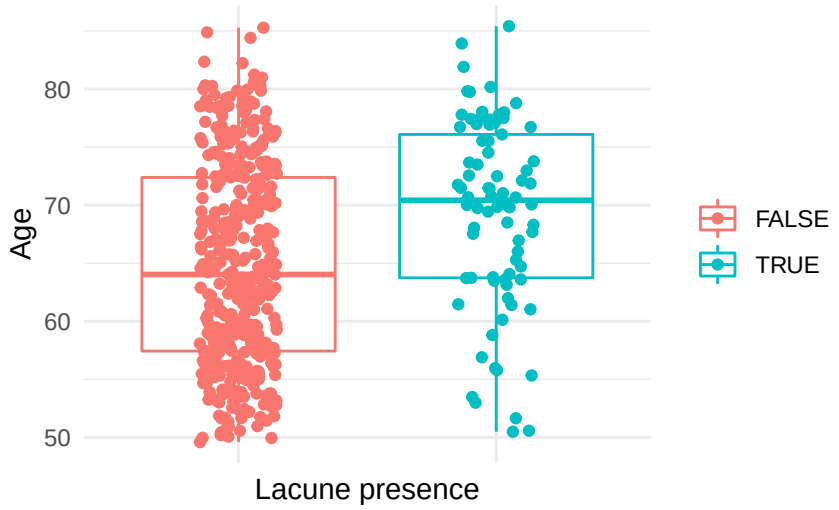
```
e = sprintf("%.1f (%.1f)", mean(data$age[data$mb_presence_b == 0]),
            sd(data$age[data$mb_presence_b == 0]))
f = sprintf("%.1f (%.1f)", mean(data$age[data$mb_presence_b == 1]),
            sd(data$age[data$mb_presence_b == 1]))
g = sprintf("%.1f (%.1f)", mean(data$age[data$lac_presence_b == 0]), sd(data$age[data$lac_presence_b ==
h = sprintf("%.1f (%.1f)", mean(data$age[data$lac_presence_b == 1]), sd(data$age[data$lac_presence_b ==

idx = c(1, 3, 2, 4) # sorting as in publication
tab.cv = array(NA, dim=c(4,3))
tab.cv[,1] = levels(data$Group.factor)
tab.cv[,2] = c("405", "81", "355" , "132")
tab.cv[,3] = c(e,f,g,h)
colnames(tab.cv) = c("group", "n", "mean (SD)")
print(tab.cv)
```

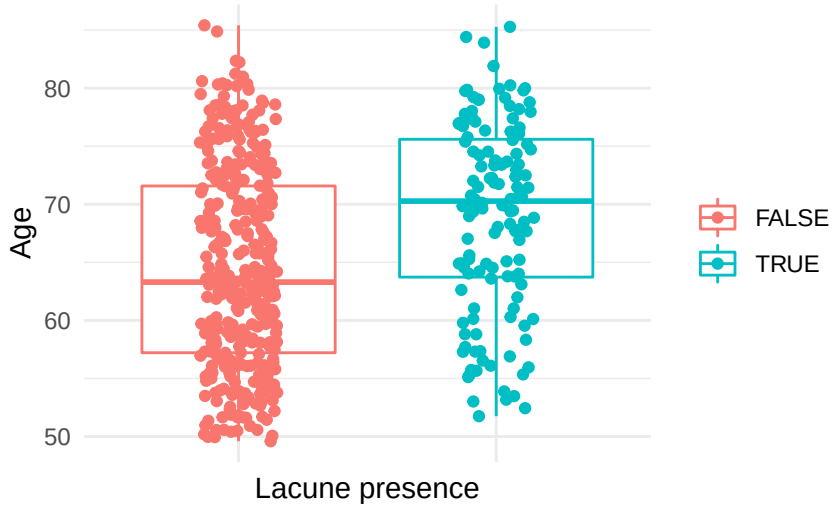
```
##      group           n     mean (SD)
## [1,] "no microbleed" "405" "64.9 (8.7)"
## [2,] "microbleed"    "81"  "69.4 (8.1)"
## [3,] "no lacunes"    "355" "64.4 (8.6)"
## [4,] "lacunes"       "132" "69.0 (8.2)"
```

- Age upon visual inspection is similar between groups

```
ggplot(data,
       aes(y=age, x=mb_presence_b, color=mb_presence_b)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme(legend.title = element_blank()) + theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  labs(x = "Lacune presence", y = "Age", title = "" )
```




```
ggplot(data,
       aes(y=age, x=lac_presence_b, color=lac_presence_b)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme(legend.title = element_blank()) + theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  labs(x = "Lacune presence", y = "Age", title = "" )
```



Main analysis ANCOVA

```
stats.orig.IVmicrobleed = data.frame(
  Fvalue =  NA,
  df1 = NA,
  df2 = NA,
  pvalue = "<0.01",
  MD = NA,
  lowerCI = NA,
  upperCI = NA)
```

```
stats.orig.IVlacunes = data.frame(
  Fvalue =  NA,
  df1 = NA,
  df2 = NA,
  pvalue = "<0.01",
  MD = NA,
  lowerCI = NA,
  upperCI = NA)
```

We verify the p values stated in Table 2 with respect to variables.There is evidence of significance that Age as a covariate contribute to variance in outcome (p = 3.825e-13)

```
# Orthogonal contrasts
contrasts(data$mb_presence_b) = contr.helmert(2)
contrasts(data$lac_presence_b) = contr.helmert(2)

#contrasts(data$IV)<-cbind(c(-2,1,1), c(0,-1,1))
fit <- aov(AB38 ~ age + sex.factor + hypertension + mb_presence_b*lac_presence_b , data = data)
summary(fit) # we use interaction term because we have lacunes yes microbleeds no
```

```
##                               Df Sum Sq Mean Sq F value   Pr(>F)
## age                            1    887   887.0  30.123 6.58e-08 ***
## sex.factor                     1      0     0.5   0.016   0.8982
## hypertension                   1    147   146.9   4.990   0.0260 *
## mb_presence_b                  1    100    99.8   3.388   0.0663 .
## lac_presence_b                 1     32    31.9   1.084   0.2983
## mb_presence_b:lac_presence_b   1     24    23.8   0.809   0.3688
## Residuals                    480  14134    29.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result = Anova(fit, type = 3) # this is not a balanced study, since ordering of variables matter, we us
print(result)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                               Sum Sq  Df F value    Pr(>F)
## (Intercept)                   1675.1   1 56.8892 2.327e-13 ***
## age                            463.6   1 15.7452 8.354e-05 ***
## sex.factor                       0.0   1  0.0005   0.98161
## hypertension                   124.8   1  4.2371   0.04009 *
## mb_presence_b                   64.9   1  2.2049   0.13823
## lac_presence_b                   4.5   1  0.1521   0.69673
## mb_presence_b:lac_presence_b    23.8   1  0.8091   0.36883
## Residuals                    14133.9 480
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
stats.rep.IVmicrobleeds = data.frame(Fvalue = sprintf("%.2f",result$`F value`[5]),
                          df1 = result$Df[5],
                          df2 = result$Df[8],
                          pvalue = formatPval(result$`Pr(>F)`[5]),
                          MD = NA,
                          lowerCI = NA,
                          upperCI = NA
```

```
)
stats.rep.IVlacunes = data.frame(Fvalue = sprintf("%.2f",result$`F value`[6]),
                           df1 = result$Df[6],
                           df2 = result$Df[8],
                           pvalue = formatPval(result$`Pr(>F)`[6]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
stats.rep.CVage = data.frame(Fvalue = sprintf("%.2f",result$`F value`[2]),
                           df1 = result$Df[2],
                           df2 = result$Df[8],
                           pvalue = formatPval(result$`Pr(>F)`[2]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
stats.rep.CVsex = data.frame(Fvalue = sprintf("%.2f",result$`F value`[3]),
                           df1 = result$Df[3],
                           df2 = result$Df[8],
                           pvalue = formatPval(result$`Pr(>F)`[3]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
stats.rep.CVhypertension = data.frame(Fvalue = sprintf("%.2f",result$`F value`[4]),
                           df1 = result$Df[4],
                           df2 = result$Df[8],
                           pvalue = formatPval(result$`Pr(>F)`[4]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
```

Comparing ANCOVA in original study with reanalysis

Independant variable

```
tab.IV = rbind(stats.orig.IVmicrobleed, stats.orig.IVlacunes, stats.rep.IVmicrobleeds, stats.rep.IVlacu
rownames(tab.IV) = c("original study microbleed", "original Study lacunes", "reanalysis for IV microble
print(t(tab.IV))
```

```
##         original study microbleed original Study lacunes
## Fvalue  NA                        NA
## df1     NA                        NA
## df2     NA                        NA
## pvalue  "<0.01"                   "<0.01"
## MD      NA                        NA
## lowerCI NA                        NA
## upperCI NA                        NA
##         reanalysis for IV microbleeds presence
```

```
## Fvalue  "2.20"
## df1     " 1"
## df2     "480"
## pvalue  "0.14"
## MD      NA
## lowerCI NA
## upperCI NA
##         reanalysis for IV lacunes presence
## Fvalue  "0.15"
## df1     " 1"
## df2     "480"
## pvalue  "0.70"
## MD      NA
## lowerCI NA
## upperCI NA
```

Covariate

- The study does not report results from covariate of age

```
tab.CV = rbind(stats.orig.allCV, stats.rep.CVage, stats.rep.CVsex, stats.rep.CVhypertension)
rownames(tab.CV) = c("original CV age,sex,hypertension", "reanalysis age", "reanalysis sex", "reanalysis
print(t(tab.CV))
```

```
##         original CV age,sex,hypertension reanalysis age reanalysis sex
## Fvalue  NA                               "15.75"        "0.00"
## df1     NA                               " 1"           " 1"
## df2     NA                               "480"          "480"
## pvalue  NA                               "< 0.0001"     "0.98"
## MD      NA                               NA             NA
## lowerCI NA                               NA             NA
## upperCI NA                               NA             NA
##         reanalysis hypertension
## Fvalue  "4.24"
## df1     " 1"
## df2     "480"
## pvalue  "0.04"
## MD      NA
## lowerCI NA
## upperCI NA
```

Assumptions

1. Homogeneity of variance

- ANOVA/ANCOVA is fairly robust in terms of the error rate when sample sizes are equal.
- When groups with larger sample sizes have larger variances than the groups with smaller sample sizes, the resulting F-ratio tends to be conservative. That is, it's more likely to produce a non-significant result when a genuine difference does exist in the population.
- Conversely, when the groups with larger sample sizes have smaller variances than the groups with smaller samples sizes, the resulting F-ratio tends to be liberal and can inflate the false positive rate.

- In this study, statistical descriptives show that the highest and lowest variances seem close. Also, there is homogeneity of variance as p values exceed 0.05 for the Levene's test

```
tapply(data$AB38, data$mb_presence_b, sd)
```

```
##    FALSE     TRUE
## 5.471873 6.069102
```

```
tapply(data$AB38, data$lac_presence_b, sd)
```

```
##    FALSE     TRUE
## 5.459909 5.870429
```

```
leveneTest(AB38 ~ lac_presence_b, data = data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group   1  1.3772 0.2412
##       485
```

```
leveneTest(AB38 ~ mb_presence_b, data = data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##        Df F value Pr(>F)
## group   1  0.0912 0.7628
##       485
```

2. Independence between covariate and IV

- The Independant variable of this study have evidence of significant effect to the covariate Age and Hypertension (for Lacune presence only). The assumption thus does not hold.
- As a way of comparison, we perform a two way anova to assess the main effect of two independant variables without covariates and find that there is evidence of significant of influence of Independant variables to Dependant variable.

```
fit.cvage = aov(age ~ lac_presence_b + mb_presence_b, data = data)
summary(fit.cvage)
```

```
##                 Df Sum Sq Mean Sq F value   Pr(>F)
## lac_presence_b   1   2039  2039.3  28.649 1.34e-07 ***
## mb_presence_b    1    638   637.9   8.962   0.0029 **
## Residuals      484  34453    71.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fit.cvsex = glm(sex.factor ~ lac_presence_b + mb_presence_b, family = binomial, data = data)
summary(fit.cvsex)
```

```
##
## Call:
## glm(formula = sex.factor ~ lac_presence_b + mb_presence_b, family = binomial,
##     data = data)
##
## Deviance Residuals:
##    Min      1Q  Median      3Q     Max
## -1.438  -1.261   0.995   1.096   1.096
##
```

```
## Coefficients:
##                 Estimate Std. Error z value Pr(>|z|)
## (Intercept)      0.39432    0.12815   3.077  0.00209 **
## lac_presence_b1  0.12588    0.10835   1.162  0.24532
## mb_presence_b1   0.07466    0.12967   0.576  0.56476
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 665.32  on 486  degrees of freedom
## Residual deviance: 663.09  on 484  degrees of freedom
## AIC: 669.09
##
## Number of Fisher Scoring iterations: 4
```

```
fit.cvhypertension = glm(hypertension ~ lac_presence_b + mb_presence_b, family = binomial, data = data)
summary(fit.cvhypertension )
```

```
##
## Call:
## glm(formula = hypertension ~ lac_presence_b + mb_presence_b,
##     family = binomial, data = data)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -2.0765  -1.5009   0.5632   0.8853   0.8853
##
## Coefficients:
##                 Estimate Std. Error z value Pr(>|z|)
## (Intercept)       1.3837     0.1705   8.118 4.75e-16 ***
## lac_presence_b1   0.5133     0.1426   3.599  0.00032 ***
## mb_presence_b1    0.1359     0.1600   0.849  0.39573
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 567.12  on 486  degrees of freedom
## Residual deviance: 548.58  on 484  degrees of freedom
## AIC: 554.58
##
## Number of Fisher Scoring iterations: 4
```

```
fit.main <- aov(AB38 ~ mb_presence_b + lac_presence_b, data = data) #checking DV and IV alone
summary(fit.main)
```

```
##                 Df Sum Sq Mean Sq F value  Pr(>F)
## mb_presence_b    1    251  250.81   8.133 0.00453 **
## lac_presence_b   1    147  147.43   4.781 0.02926 *
## Residuals      484  14926   30.84
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3. Homogeneity of regression slopes

- There is no evidence of significant interaction between covariate and independant variable. Thus we can assume homogeneity of regression slope.

```
fit.hrs = aov(AB38 ~ age*mb_presence_b, data = data)
Anova(fit.hrs, type=3) # no evidence of interaction, there is homogeneity of IV levels across age
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                     Sum Sq  Df F value    Pr(>F)
## (Intercept)          581.2   1 19.6293 1.164e-05 ***
## age                  528.9   1 17.8617 2.841e-05 ***
## mb_presence_b         16.5   1  0.5560    0.4562
## age:mb_presence_b     28.4   1  0.9603    0.3276
## Residuals          14301.0 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

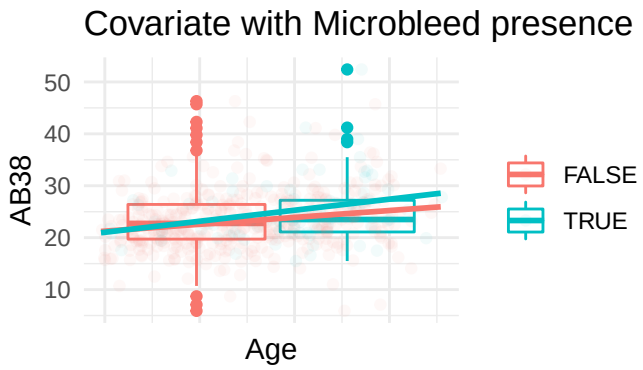
```
fit.hrs = aov(AB38 ~ age*lac_presence_b, data = data)
Anova(fit.hrs, type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                      Sum Sq  Df F value    Pr(>F)
## (Intercept)          1056.2   1 35.5735 4.750e-09 ***
## age                   616.0   1 20.7488 6.639e-06 ***
## lac_presence_b          4.1   1  0.1398    0.7087
## age:lac_presence_b     10.4   1  0.3490    0.5550
## Residuals           14340.2 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

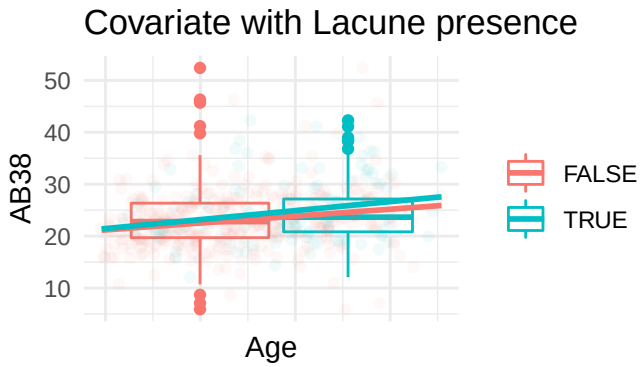
Independance of Covariate with Independant Variables (Visual inspection of Homogeneity of Regression slopes)

- Visually, the two levels of each independant variable follows the same pattern - there seems to be independance of Covariate versus independant variable.

```
ggplot(data, aes(y=AB38, x= age, color= mb_presence_b)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha = 0.05) +
  geom_smooth(formula = y ~ x,method=lm, se=FALSE, fullrange=TRUE) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("Age") + ylab("AB38") + ggtitle("Covariate with Microbleed presence")
```




```
ggplot(data, aes(y=AB38, x= age, color= lac_presence_b)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha = 0.05) +
  geom_smooth(formula = y ~ x,method=lm, se=FALSE, fullrange=TRUE) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("Age") + ylab("AB38") + ggtitle("Covariate with Lacune presence")
```



Reference : Field, Miles & Miles (2012), Discovering Statistics, Published by Sage Pub, UK.