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

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.CV = stats.orig.IV
```

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[data$sex == 2] = "0"
data$sex.factor[data$sex == 0] = "female"
data$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

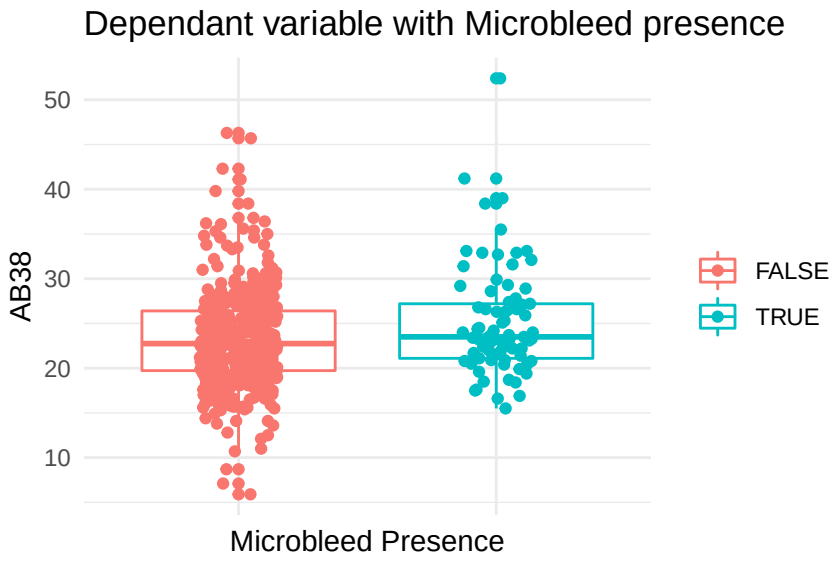
```
a = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 0]), sd(data$AB38[data$mb_presence_b ==
b = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 1]), sd(data$AB38[data$mb_presence_b ==

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 =

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)"
```

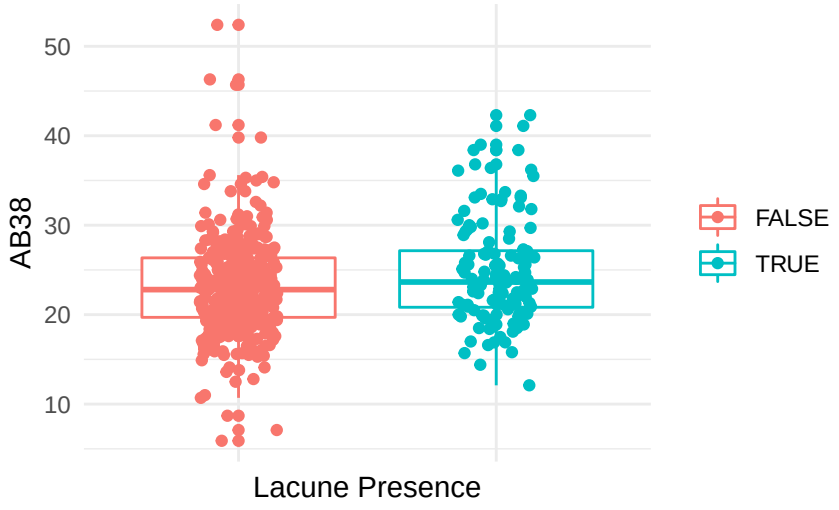
boxplot with DV

- 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

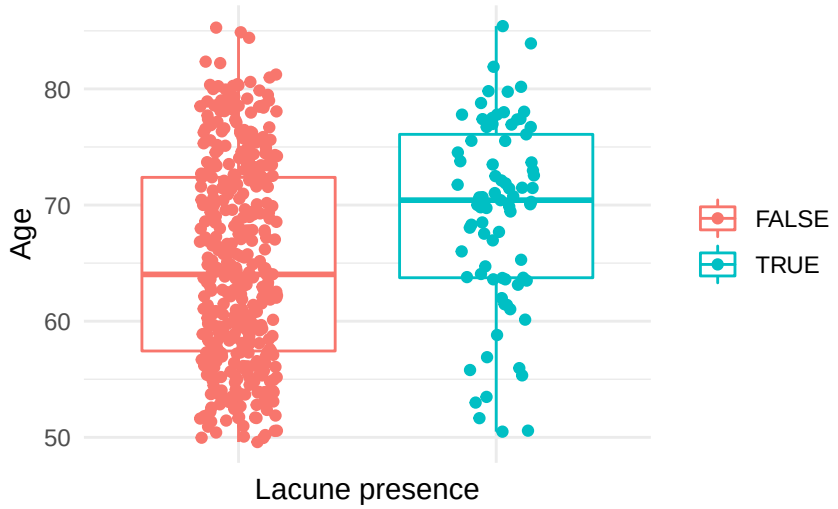
COV with boxplot

```
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)"
```

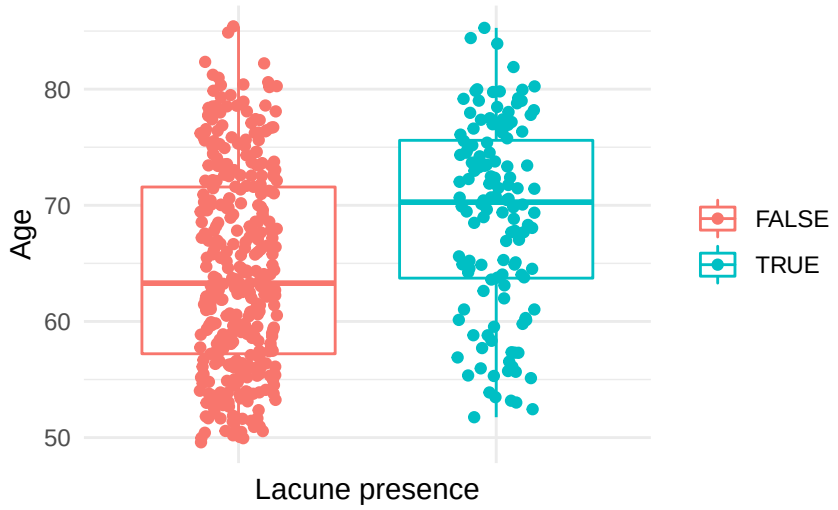
- Age upon visual inspection is similar between groups

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

stats.orig.CV = stats.orig.IV

# enter all results from primary study here
stats.orig.IV$pvalue = 0.01
```

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 = 8.037e-05)

```
# 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 ~ mb_presence_b*lac_presence_b + age + sex.factor + hypertension, data = data)
summary(fit) # we use interaction term because we have lacunes yes microbleeds no
```

```
##                              Df Sum Sq Mean Sq F value   Pr(>F)
## mb_presence_b                 1    251   250.8   8.518  0.00368 **
## lac_presence_b                1    147   147.4   5.007  0.02571 *
## age                           1    645   645.2  21.913 3.72e-06 ***
```

```
## sex.factor                     1      0     0.0   0.000  0.98588
## hypertension                   1    123   122.6   4.164  0.04185 *
## mb_presence_b:lac_presence_b   1     24    23.8   0.809  0.36883
## 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
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 ***
## mb_presence_b                   64.9   1  2.2049   0.13823
## lac_presence_b                   4.5   1  0.1521   0.69673
## 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: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$`F value`[2]),
                           df1 = result$Df[2],
                           df2 = result$Df[3],
                           pvalue = formatPval(result$`Pr(>F)`[2]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
stats.rep.IVlacunes = data.frame(Fvalue = sprintf("%.2f",result$`F value`[3]),
                           df1 = result$Df[2],
                           df2 = result$Df[3],
                           pvalue = formatPval(result$`Pr(>F)`[3]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
stats.rep.CV = data.frame(Fvalue = sprintf("%.2f",result$`F value`[2]),
                           df1 = result$Df[2],
                           df2 = result$Df[4],
                           pvalue = formatPval(result$`Pr(>F)`[2]),
                           MD = NA,
                           lowerCI = NA,
                           upperCI = NA
)
```

Comparing ANCOVA in original study with reanalysis

Independant variable

```
tab.IV = rbind(stats.orig.IV, stats.rep.IVmicrobleeds, stats.rep.IVlacunes)
rownames(tab.IV) = c("original Study", "reanalysis for IV microbleeds presence", "reanalysis for IV lacu
print(t(tab.IV))
```

```
##         original Study reanalysis for IV microbleeds presence
## Fvalue  NA             "2.20"
## df1     NA             " 1"
## df2     NA             " 1"
## pvalue  "0.01"         "0.14"
## MD      NA             NA
## lowerCI NA             NA
## upperCI NA             NA
##         reanalysis for IV lacunes presence
## Fvalue  "0.15"
## df1     " 1"
## df2     " 1"
## pvalue  "0.70"
## MD      NA
## lowerCI NA
## upperCI NA
```

Covariate

- The study does not report results from covariate

```
tab.CV = rbind(stats.orig.CV, stats.rep.CV)
rownames(tab.CV) = c("original Study", "reanalysis")
print(t(tab.CV))
```

```
##         original Study reanalysis
## Fvalue  NA             "2.20"
## df1     NA             " 1"
## df2     NA             " 1"
## pvalue  NA             "0.14"
## MD      NA             NA
## lowerCI NA             NA
## upperCI NA             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, there is homogeneity of variance

```
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. 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 (of two levels FALSE and TRUE) 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
```

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

```
##                 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
```

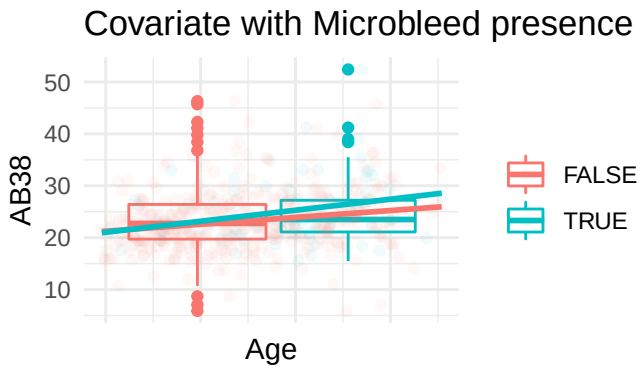
```
fit.hrs = aov(AB38 ~ age*lac_presence_b, data = data)
Anova(fit.hrs, type=3)
```

```
## 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 Lacunes presence")
```