25-VanLeijsen

Audrey Yeo & Simon Schwab*

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Contents

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

Notes from reading methods section

- Dependant variable: AB38

*University of Zurich, simon.schwab@uzh.ch

- The independant variables
  - presence of microbleeds (1 or more microbleeds (n = 81) vs no microbleed (n=406)
  - presence of lacunes (1 or more lacunes (n = 132) vs no lacunes (n = 355)
- Covariate: age, sex and hypertension, total brain volume

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


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

Reading data

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

```
PATH = file.path(path.expand("~"), "Data", "ancova") # ancova project folder
data = read_excel(file.path(PATH, "dataPrimaryStudies", "25-VanLeijsen", "25-VanLeijsen-1.xlsx"))
data = data.frame(data)

data$mb_presence_b.factor = NA
data$mb_presence_b.factor[data$mb_presence_b == 1] = "microbleeds"
data$mb_presence_b.factor[data$mb_presence_b == 0] = "no microbleeds"
data$mb_presence_b.factor = as.factor(data$mb_presence_b.factor)

data$lac_presence_b.factor = NA
data$lac_presence_b.factor[data$lac_presence_b == 1] = "lacunes"
data$lac_presence_b.factor[data$lac_presence_b == 0] = "no lacunes"
data$lac_presence_b.factor = as.factor(data$lac_presence_b.factor)

data$sex.factor = NA
data$sex.factor[data$sex == 1] = "male"
data$sex.factor[data$sex == 2] = "female"
data$sex.factor = as.factor(data$sex.factor)

data$hypertension.factor  = data$hypertension == 1

# data$groups = as.factor(paste(data$mb_presence_b.factor, data$lac_presence_b.factor))
```

Descriptives

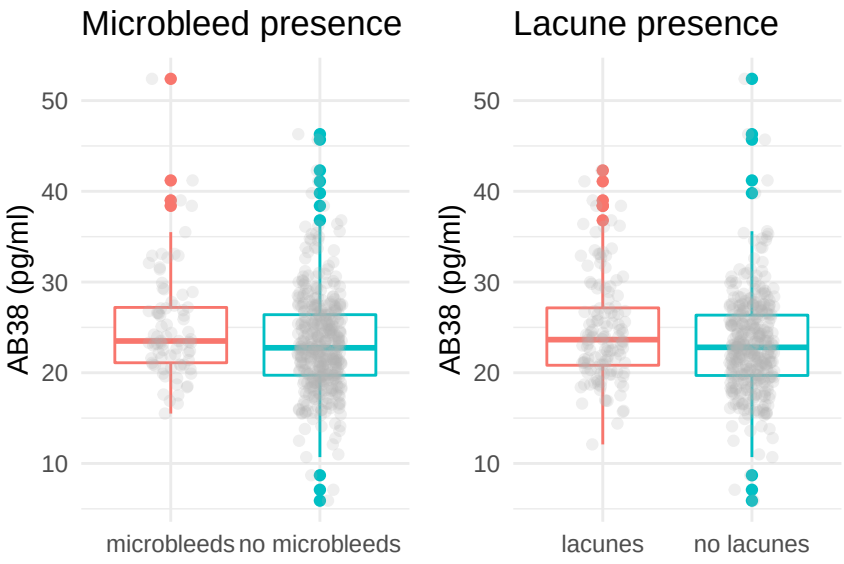
Dependant variable

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

```
tab.dv = array(NA, dim=c(4,2))
rownames(tab.dv) = c(rev(levels(data$mb_presence_b.factor)), rev(levels(data$lac_presence_b.factor)))
colnames(tab.dv) = c("n", "mean (SD)")

tab.dv[,1] = c(rev(summary(data$mb_presence_b.factor)),
               rev(summary(data$lac_presence_b.factor)))

tab.dv[1,2] = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 0]),
                      sd(data$AB38[data$mb_presence_b == 0]))
tab.dv[2,2] = sprintf("%.1f (%.1f)", mean(data$AB38[data$mb_presence_b == 1]),
                      sd(data$AB38[data$mb_presence_b == 1]))

tab.dv[3,2] = sprintf("%.1f (%.1f)", mean(data$AB38[data$lac_presence_b == 0]),
                      sd(data$AB38[data$lac_presence_b == 0]))
tab.dv[4,2] = sprintf("%.1f (%.1f)", mean(data$AB38[data$lac_presence_b == 1]),
                      sd(data$AB38[data$lac_presence_b == 1]))

print(tab.dv)
```

```
##                n     mean (SD)
## no microbleeds "406" "23.2 (5.5)"
## microbleeds    "81"  "25.2 (6.1)"
## no lacunes     "355" "23.1 (5.5)"
## lacunes        "132" "24.7 (5.9)"
```

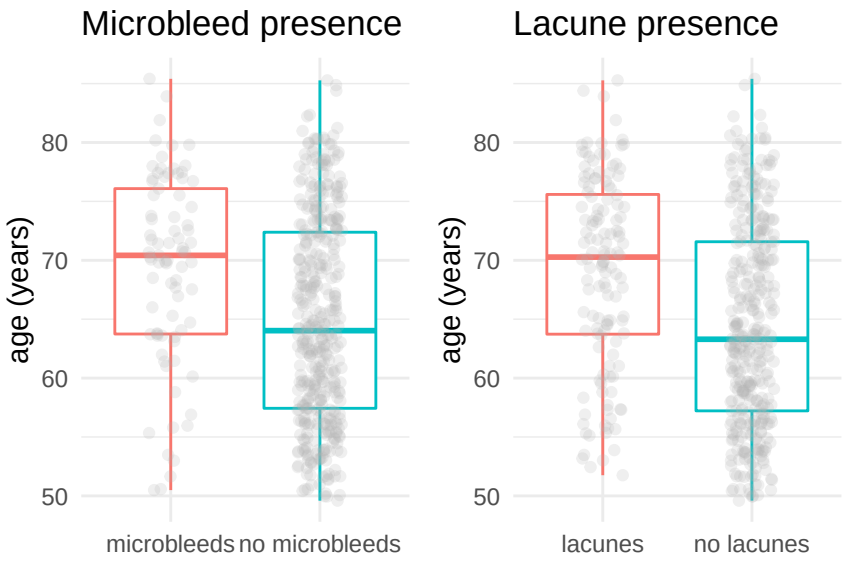
```
p1 = ggplot(data, aes(y=AB38, x = mb_presence_b.factor, color = mb_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Microbleed presence") + ylab("AB38 (pg/ml)")

p2 = ggplot(data, aes(y=AB38, x = lac_presence_b.factor, color =lac_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Lacune presence") + ylab("AB38 (pg/ml)")

plot_grid(p1, p2, nrow = 1, ncol = 2)
```



Covariates(s)

Age

```
p1 = ggplot(data, aes(y=age, x = mb_presence_b.factor, color = mb_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Microbleed presence") + ylab("age (years)")

p2 = ggplot(data, aes(y=age, x = lac_presence_b.factor, color =lac_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Lacune presence") + ylab("age (years)")
plot_grid(p1, p2, nrow = 1, ncol = 2)
```



Sex

```
tab = table(data$mb_presence_b.factor, data$sex.factor)
prc = tab/rowSums(tab)
tab[,1] = sprintf("%.1f%%", prc*100)[1:2]
tab[,2] = sprintf("%.1f%%", prc*100)[3:4]
print(tab)
```

```
##
##                   female male
##   microbleeds     38.3%  61.7%
##   no microbleeds 43.8%  56.2%
```

```
tab = table(data$lac_presence_b.factor, data$sex.factor)
prc = tab/rowSums(tab)
tab[,1] = sprintf("%.1f%%", prc*100)[1:2]
tab[,2] = sprintf("%.1f%%", prc*100)[3:4]
print(tab)
```

```
##
##              female male
##   lacunes    37.9%  62.1%
##   no lacunes 44.8%  55.2%
```

Hypertension

```
tab = table(data$mb_presence_b.factor, data$hypertension.factor)
prc = tab/rowSums(tab)
tab[,1] = sprintf("%.1f%%", prc*100)[1:2]
tab[,2] = sprintf("%.1f%%", prc*100)[3:4]
print(tab)
```

```
##
##                   FALSE TRUE
```

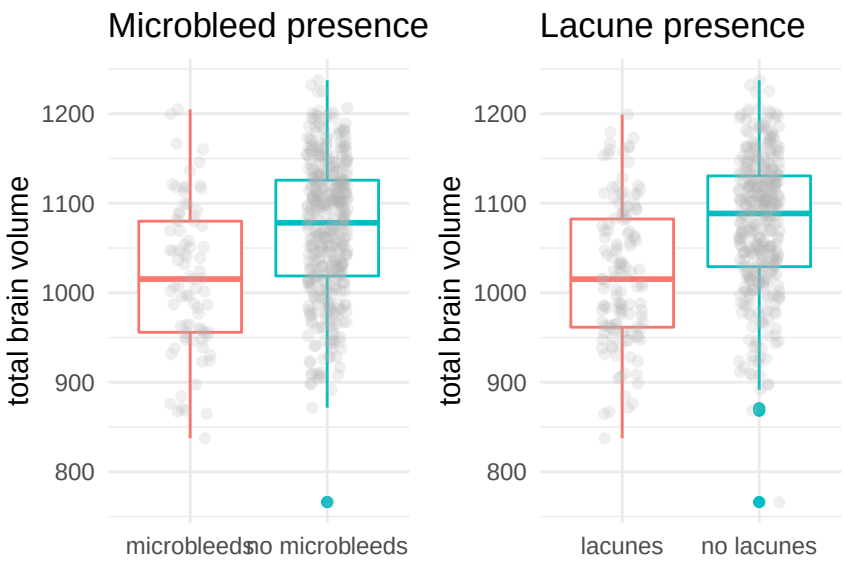
```
##   microbleeds    18.5% 81.5%
##   no microbleeds 28.6% 71.4%
```

```
tab = table(data$lac_presence_b.factor, data$hypertension.factor)
prc = tab/rowSums(tab)
tab[,1] = sprintf("%.1f%%", prc*100)[1:2]
tab[,2] = sprintf("%.1f%%", prc*100)[3:4]
print(tab)
```

```
##
##               FALSE TRUE
##   lacunes     13.6% 86.4%
##   no lacunes 31.8% 68.2%
```

Total brain volume

```
p1 = ggplot(data, aes(y=tbv_b, x = mb_presence_b.factor, color = mb_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Microbleed presence") + ylab("total brain volume")

p2 = ggplot(data, aes(y=tbv_b, x = lac_presence_b.factor, color =lac_presence_b.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0), alpha=0.2, col="gray70") +
  theme_minimal() +
  theme(legend.title = element_blank(),
        axis.title.x = element_blank(),
        legend.position = "none") +
  ggtitle("Lacune presence") + ylab("total brain volume")
plot_grid(p1, p2, nrow = 1, ncol = 2)
```



Main analysis ANCOVA

What has been done in the paper (one-way analyses)

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

# unadjusted one-way (not appropriate but thats what has been done in the paper)
fit.ancova= aov(AB38 ~ mb_presence_b.factor, data = data)
Anova(fit.ancova, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                       Sum Sq  Df   F value    Pr(>F)
## (Intercept)           158082   1 5086.5812 < 2.2e-16 ***
## mb_presence_b.factor    251   1    8.0703  0.004689 **
## Residuals             15073 485
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fit.ancova= aov(AB38 ~ lac_presence_b.factor, data = data)
Anova(fit.ancova, type = 3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                       Sum Sq  Df   F value    Pr(>F)
## (Intercept)           220267   1 7090.0613 < 2.2e-16 ***
## lac_presence_b.factor    256   1    8.2509  0.004252 **
## Residuals              15067 485
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# adjusted for all covariates
fit.ancova = aov(AB40 ~ age + sex.factor + hypertension.factor +
                   tbv_b + mb_presence_b.factor, data = data)
Anova(fit.ancova, type = 3) # Type III
```

```
## Anova Table (Type III tests)
##
## Response: AB40
##                       Sum Sq  Df F value    Pr(>F)
## (Intercept)            18918   1 14.4710 0.0001607 ***
## age                    16602   1 12.6993 0.0004023 ***
## sex.factor               174   1  0.1333 0.7152018
## hypertension.factor     2391   1  1.8288 0.1769004
## tbv_b                   2714   1  2.0758 0.1503066
## mb_presence_b.factor    9232   1  7.0623 0.0081342 **
## Residuals             628799 481
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
fit.ancova = aov(AB40 ~ age + sex.factor + hypertension.factor +
                   tbv_b + lac_presence_b.factor, data = data)
Anova(fit.ancova, type = 3) # Type III
```

```
## Anova Table (Type III tests)
##
## Response: AB40
##                        Sum Sq  Df F value    Pr(>F)
## (Intercept)             18824   1 14.2755 0.0001777 ***
## age                     16978   1 12.8754 0.0003670 ***
## sex.factor                196   1  0.1489 0.6997526
## hypertension.factor      1894   1  1.4360 0.2313727
## tbv_b                    3023   1  2.2926 0.1306519
## lac_presence_b.factor    3778   1  2.8652 0.0911619 .
## Residuals              634253 481
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

What should have been done in the paper (two-way analysis)

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

# unadjusted
fit.ancova= aov(AB38 ~ mb_presence_b.factor*lac_presence_b.factor, data = data)
result = Anova(fit.ancova, type = 3) # Type III
print(result)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                                           Sum Sq  Df   F value Pr(>F)
## (Intercept)                               148296   1 4806.2581 <2e-16 ***
## mb_presence_b.factor                         135   1    4.3608 0.0373 *
## lac_presence_b.factor                         57   1    1.8414 0.1754
## mb_presence_b.factor:lac_presence_b.factor    23   1    0.7368 0.3911
## Residuals                                  14903 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# adjusted for all covariates
fit.ancova = aov(AB40 ~ age + sex.factor + hypertension.factor +
                   tbv_b + mb_presence_b.factor*lac_presence_b.factor, data = data)
result = Anova(fit.ancova, type = 3) # Type III
print(result)
```

```
## Anova Table (Type III tests)
##
## Response: AB40
##                                           Sum Sq  Df F value    Pr(>F)
## (Intercept)                                17807   1 13.6054 0.0002514 ***
## age                                        16434   1 12.5570 0.0004334 ***
```

```
## sex.factor                                     195   1  0.1493 0.6993628
## hypertension.factor                           1963   1  1.4997 0.2213140
## tbv_b                                         2023   1  1.5454 0.2144299
## mb_presence_b.factor                          7304   1  5.5811 0.0185545 *
## lac_presence_b.factor                         1730   1  1.3216 0.2508822
## mb_presence_b.factor:lac_presence_b.factor     129   1  0.0983 0.7540404
## Residuals                                   626906 479
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Comparing ANCOVA in original study with reanalysis

We skip this section as no p-values or test statistics are reported.

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.factor, sd)
```

```
##    microbleeds no microbleeds
##       6.069102       5.471873
```

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

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

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

```
##    lacunes no lacunes
##   5.870429   5.459909
```

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

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

2. Independence between covariate and IV

When the covariate and the experimental effect (independent variable) are not independent the treatment effect is obscured, spurious treatment effects can arise and the interpretation of the ANCOVA is seriously compromised.

We test whether our groups differ on the CV. If the groups do not significantly differ then is appropriate to use the covariate.

```
# Age
fit.cv.age = aov(age ~ lac_presence_b + mb_presence_b, data = data)
Anova(fit.cv.age, type=3)
```

```
## Anova Table (Type III tests)
##
## Response: age
##                  Sum Sq  Df    F value    Pr(>F)
## (Intercept)    1372627   1 19282.9584 < 2.2e-16 ***
## lac_presence_b    1334   1    18.7464 1.816e-05 ***
## mb_presence_b      638   1     8.9616  0.002898 **
## Residuals        34453 484
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Sex
fit.cv.sex = glm(sex.factor~ lac_presence_b + mb_presence_b, family = binomial, data = data)
Anova(fit.cv.sex, type=3)
```

```
## Analysis of Deviance Table (Type III tests)
##
## Response: sex.factor
##                LR Chisq Df Pr(>Chisq)
## lac_presence_b  1.36028  1     0.2435
## mb_presence_b   0.33341  1     0.5637
```

```
# Hypertension
fit.cv.hypertension = glm(hypertension.factor ~ lac_presence_b + mb_presence_b, family = binomial, data
Anova(fit.cv.hypertension, type=3)
```

```
## Analysis of Deviance Table (Type III tests)
##
## Response: hypertension.factor
##                LR Chisq Df Pr(>Chisq)
## lac_presence_b  14.8425  1  0.0001169 ***
## mb_presence_b    0.7439  1  0.3884022
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Total brain volume
fit.cv.tbw = aov(tbv_b ~ lac_presence_b + mb_presence_b, data = data)
Anova(fit.cv.tbw, type=3)
```

```
## Anova Table (Type III tests)
##
## Response: tbv_b
##                   Sum Sq  Df   F value    Pr(>F)
## (Intercept)    390620282   1 68918.966 < 2.2e-16 ***
## lac_presence_b    202364   1    35.704 4.457e-09 ***
```

```
## mb_presence_b      89885   1    15.859 7.873e-05 ***
## Residuals        2743225 484
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3. Homogeneity of regression slopes

- We test the interaction between the IV and the CV

```
# Age
Anova(aov(AB38 ~ age*mb_presence_b.factor, data = data), type=3)
```

```
## 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.factor         16.5   1  0.5560    0.4562
## age:mb_presence_b.factor     28.4   1  0.9603    0.3276
## Residuals                14301.0 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova(aov(AB38 ~ age*lac_presence_b.factor, data = data), 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.factor          4.1   1  0.1398    0.7087
## age:lac_presence_b.factor     10.4   1  0.3490    0.5550
## Residuals                 14340.2 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Sex
Anova(aov(AB38 ~ sex.factor*mb_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                                 Sum Sq  Df   F value  Pr(>F)
## (Intercept)                      59156   1 1906.9045 < 2e-16 ***
## sex.factor                          45   1    1.4411 0.23055
## mb_presence_b.factor                 6   1    0.2052 0.65078
## sex.factor:mb_presence_b.factor     89   1    2.8782 0.09043 .
## Residuals                        14984 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova(aov(AB38 ~ sex.factor*lac_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
```

```
##
## Response: AB38
##                                  Sum Sq  Df   F value Pr(>F)
## (Intercept)                       85711   1 2753.4007 <2e-16 ***
## sex.factor                            8   1    0.2410 0.6237
## lac_presence_b.factor                32   1    1.0324 0.3101
## sex.factor:lac_presence_b.factor     32   1    1.0291 0.3109
## Residuals                         15035 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Hypertenson
Anova(aov(AB38 ~ hypertension.factor*mb_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                                        Sum Sq  Df  F value    Pr(>F)
## (Intercept)                           26794.2   1 880.3884 < 2.2e-16 ***
## hypertension.factor                     214.6   1   7.0523  0.008178 **
## mb_presence_b.factor                     16.8   1   0.5534  0.457306
## hypertension.factor:mb_presence_b.factor  6.0   1   0.1983  0.656270
## Residuals                             14699.9 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova(aov(AB38 ~ hypertension.factor*lac_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                                        Sum Sq  Df   F value    Pr(>F)
## (Intercept)                           30776.7   1 1008.2994 < 2.2e-16 ***
## hypertension.factor                     229.4   1    7.5146  0.006347 **
## lac_presence_b.factor                     6.7   1    0.2190  0.640013
## hypertension.factor:lac_presence_b.factor 7.6   1    0.2495  0.617620
## Residuals                             14742.8 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Total brain volume
Anova(aov(AB38 ~ tbv_b*mb_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                             Sum Sq  Df F value    Pr(>F)
## (Intercept)                 2705.7   1 90.1924 < 2.2e-16 ***
## tbv_b                        412.9   1 13.7621 0.0002316 ***
## mb_presence_b.factor          10.3   1  0.3448 0.5573285
## tbv_b:mb_presence_b.factor     6.0   1  0.2013 0.6538747
## Residuals                  14489.6 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Anova(aov(AB38 ~ tbv_b*lac_presence_b.factor, data = data), type=3)
```

```
## Anova Table (Type III tests)
##
## Response: AB38
##                               Sum Sq  Df  F value    Pr(>F)
## (Intercept)                   3154.9   1 104.9384 < 2.2e-16 ***
## tbv_b                          440.8   1  14.6613 0.0001456 ***
## lac_presence_b.factor            0.1   1   0.0035 0.9525567
## tbv_b:lac_presence_b.factor      0.1   1   0.0029 0.9570343
## Residuals                    14520.9 483
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Notes

- The authors performed both unadjusted (using ANOVA) and adjusted analyses (using ANCOVA).
- We reproduced the effect of microbleeds and lacunes on ABeta38 using two one-ways ANOVAs.
- However, the appropriate analysis would be a 2-way ANOVA and here only an effect of microbleeds was statistically significant.
- We could not reproduce the result with ANCOVA with 4 covariates (named model 4). There was again an effect of microbleeds, but the paper reported it was not significant.
- No exact p-values and no test statistics were reported.
- Some assumptions were not met: three covariates age, hypertension and total brain volume were not independent from the group variable.

Data was analyzed according to recommendations by Field, Miles, & Field (2012).