Profil_4cer_Statistical_analysis

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```r
#Import and set cv death and cv complications to factors
data <- vroom::vroom(here::here("data/0033_profil_4cer_data_preprocessed.csv"))
```

```
## Rows: 721 Columns: 74
## -- Column specification ---------------------------------------------------
## Delimiter: "\t"
## dbl  (72): Sample_ID, Target_16, Target_18, Target_20, Target_22, Target_24_...
## date  (2): pro_date_index, pro_date_end
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
#Convert factors
data <- data %>%
  mutate(across(which(
    apply(., 2, function(x) {
    tmp <- unique(x)
    length(tmp) - sum(is.na(tmp)) == 2L})),
  ~ factor(.))) %>%
    mutate_at(c("Sample_ID", "Albuminuri_3_groups", "Retinopathy"), ~ factor(.)) %>%
    mutate(Gender = factor(Gender, levels = c(1, 0), labels = c("Male", "Female")))

#Split curated (Complete data with 662 observations) and
#full data set (721 observations but many missing values)
data_full <- data
data_curated <- data %>%
  filter(Curated == 1)

#Scaled ceramides
data_scale <- data_curated %>%
    mutate(across(starts_with("Target")|starts_with("Ratio"), ~scale(.)))

#Go with curated
data <- data_curated


#Vector of all variables between "Age" and "Spiron" used for clinical characteristics table
CCtable_vars <- colnames(data)[which(colnames(data) == "Age"):
                                which(colnames(data) == "Spiron")]

#Clinical characteristics table, stratified by previous CV complications
CCtable <- data %>%
```

```r
  mutate(RAAS = factor(RAAS,levels = c(2, 1))) %>%
    mutate(HbA1c_percent = (HbA1C_mmol_mol/10.929)+2.15) %>%
    mutate(Retinopathy_v2 = if_else(Retinopathy == 0, "No", "Other")) %>%
    mutate(Retinopathy_v2 = if_else(Retinopathy == 1 | Retinopathy == 2,
                                    "Mild-moderate", Retinopathy_v2)) %>%
    mutate(Retinopathy_v2 = if_else(Retinopathy == 3, "Proliferative", Retinopathy_v2)) %>%
    mutate(Retinopathy = Retinopathy_v2) %>%
    mutate(CB = if_else(CB > 1, 1, CB)) %>%
    mutate(CB = factor(CB)) %>%
      CreateTableOne(vars = c(CCtable_vars, "HbA1c_percent"),
                           strata = "cv_komb_profil",
                           #strata = "ESRD_profil",
                           #strata = "doed_profil",
                           addOverall = TRUE)

CCtable
```

```
##                              Stratified by cv_komb_profil
##                               Overall         0             1
##   n                              662            568            94
##   Age (mean (SD))              54.61 (12.66)  53.61 (12.85)  60.70 (9.49)
##   Duration_DM (mean (SD))      32.74 (15.85)  31.31 (15.83)  41.33 (13.05)
##   Gender = Female (%)            296 (44.8)     264 (46.6)     32 (34.4)
##   Smoking = 1 (%)                137 (20.7)     116 (20.4)     21 (22.3)
##   Weight (mean (SD))           76.37 (15.05)  76.22 (14.90)  77.29 (15.94)
##   Height (mean (SD))          173.40 (9.99)  173.48 (10.08) 172.94 (9.51)
##   BMI (mean (SD))              25.42 (5.79)   25.37 (6.02)   25.70 (4.15)
##   Previous_CVD = 1 (%)           139 (21.0)      85 (15.0)     54 (57.4)
##   Retinopathy (%)
##      Mild-moderate               275 (41.5)     246 (43.3)     29 (30.9)
##      No                          140 (21.1)     133 (23.4)      7 ( 7.4)
##      Other                       127 (19.2)      91 (16.0)     36 (38.3)
##      Proliferative               120 (18.1)      98 (17.3)     22 (23.4)
##   logUAER (mean (SD))           1.43 (0.70)    1.37 (0.68)    1.77 (0.71)
##   HbA1C_mmol_mol (mean (SD))   64.31 (12.65)  63.76 (12.60)  67.65 (12.49)
##   Blood_HgB (mean (SD))         8.45 (0.86)    8.49 (0.83)    8.21 (0.99)
##   Total_cholesterol (mean (SD)) 4.68 (0.86)    4.66 (0.83)    4.80 (1.04)
##   Blood_HDL (mean (SD))         1.70 (0.54)    1.71 (0.54)    1.61 (0.55)
##   Blood_LDL (mean (SD))         2.47 (0.75)    2.44 (0.71)    2.60 (0.94)
##   Blood_VLDL (mean (SD))        0.51 (0.27)    0.50 (0.27)    0.57 (0.30)
##   Blood_CREAE (mean (SD))      90.78 (45.59)  87.06 (40.43) 112.88 (64.69)
##   Blood_TGA (mean (SD))         1.13 (0.66)    1.09 (0.58)    1.35 (1.01)
##   GFRepi (mean (SD))           81.53 (25.51)  83.95 (24.69)  67.00 (25.68)
##   hsCRP (mean (SD))             3.41 (7.02)    3.36 (7.20)    3.70 (5.83)
##   CALSBP (mean (SD))          131.77 (17.41) 130.89 (16.90) 137.10 (19.48)
##   Cal_DIA (mean (SD))          74.25 (9.31)   74.52 (9.15)   72.62 (10.08)
##   Albuminuri_3_groups (%)
##      1                           308 (46.5)     290 (51.1)     18 (19.1)
##      3                           165 (24.9)     133 (23.4)     32 (34.0)
##      4                           189 (28.5)     145 (25.5)     44 (46.8)
##   RAAS = 1 (%)                   445 (67.3)     358 (63.1)     87 (92.6)
##   AHT = 1 (%)                    475 (71.9)     382 (67.4)     93 (98.9)
##   BB = 1 (%)                      85 (12.8)      58 (10.2)     27 (28.7)
```

```
##    CB = 1 (%)                        202 (30.5)     155 (27.3)      47 (50.0)
##    Pump = 1 (%)                        57 ( 8.6)      52 ( 9.2)       5 ( 5.3)
##    Insulin_day_dose (mean (SD))     48.66 (34.97)  48.78 (36.22)  47.91 (26.29)
##    Statin = 1 (%)                     397 (60.1)     321 (56.6)      76 (80.9)
##    ASA_plavix = 1 (%)                 349 (52.9)     273 (48.2)      76 (80.9)
##    Diuretics = 1 (%)                  334 (50.5)     260 (45.8)      74 (78.7)
##    Thiazide = 1 (%)                   186 (28.1)     159 (28.0)      27 (28.7)
##    Furosemide = 1 (%)                 149 (22.5)     105 (18.5)      44 (46.8)
##    Spiron = 1 (%)                      28 ( 4.2)      17 ( 3.0)      11 (11.7)
##    HbA1c_percent (mean (SD))         8.03 (1.16)    7.98 (1.15)    8.34 (1.14)
##                              Stratified by cv_komb_profil
##                               p      test
##    n
##    Age (mean (SD))            <0.001
##    Duration_DM (mean (SD))    <0.001
##    Gender = Female (%)         0.038
##    Smoking = 1 (%)             0.774
##    Weight (mean (SD))          0.524
##    Height (mean (SD))          0.627
##    BMI (mean (SD))             0.619
##    Previous_CVD = 1 (%)       <0.001
##    Retinopathy (%)            <0.001
##       Mild-moderate
##       No
##       Other
##       Proliferative
##    logUAER (mean (SD))        <0.001
##    HbA1C_mmol_mol (mean (SD))  0.006
##    Blood_HgB (mean (SD))       0.004
##    Total_cholesterol (mean (SD))  0.126
##    Blood_HDL (mean (SD))       0.083
##    Blood_LDL (mean (SD))       0.069
##    Blood_VLDL (mean (SD))      0.017
##    Blood_CREAE (mean (SD))    <0.001
##    Blood_TGA (mean (SD))      <0.001
##    GFRepi (mean (SD))         <0.001
##    hsCRP (mean (SD))           0.666
##    CALSBP (mean (SD))          0.001
##    Cal_DIA (mean (SD))         0.067
##    Albuminuri_3_groups (%)    <0.001
##       1
##       3
##       4
##    RAAS = 1 (%)               <0.001
##    AHT = 1 (%)                <0.001
##    BB = 1 (%)                 <0.001
##    CB = 1 (%)                 <0.001
##    Pump = 1 (%)                0.303
##    Insulin_day_dose (mean (SD))  0.824
##    Statin = 1 (%)             <0.001
##    ASA_plavix = 1 (%)         <0.001
##    Diuretics = 1 (%)          <0.001
##    Thiazide = 1 (%)            0.982
##    Furosemide = 1 (%)         <0.001
```

```
##   Spiron = 1 (%)                  <0.001
##   HbA1c_percent (mean (SD))        0.006
```

```r
#write.csv(print(CCtable, printToggle = FALSE), here("data/CCtable.csv"))

#Median days followed
data %>%
  mutate(followed = pro_date_end - pro_date_index) %>%
  summarise(
    Median = median(followed),
    IQR = IQR(followed),
    Median_year = (median(followed)) / 365,
    IQR_year = (IQR(followed)) / 365)
```

```
## # A tibble: 1 x 4
##   Median      IQR Median_year    IQR_year
##   <drtn>    <dbl> <drtn>            <dbl>
## 1 2312 days   293 6.334247 days     0.803
```

```r
#UAER interquartile range
quantile(exp(data$logUAER), na.rm = TRUE)
```

```
##        0%       25%       50%       75%       100%
##   1.197217  2.459603  3.455613  6.110447 50.400445
```

```r
quantile(exp(data$logUAER[data$cv_komb_profil == 0]), na.rm = TRUE)
```

```
##        0%       25%       50%       75%       100%
##   1.491825  2.410900  3.189933  5.584528 50.400445
```

```r
quantile(exp(data$logUAER[data$cv_komb_profil == 1]), na.rm = TRUE)
```

```
##        0%       25%       50%       75%       100%
##   1.197217  3.678516  5.365824 10.381237 33.784428
```

```r
rm(CCtable, CCtable_vars)
```

```r
#Note: measures are changed from ug/ml to ng/ml
#Table of mean, startified by CVE
CerTable_CVE <- data %>%
    select(c(starts_with("Target"), starts_with("Ratio"), cv_komb_profil)) %>%
    mutate(across(starts_with("Target"), ~.*1000)) %>%
    rename_with( ~gsub("Target_", "Cer", .)) %>%
    rename_with( ~gsub("Ratio ", "Ratio Cer", .)) %>%
    rename_with( ~gsub("24_", "24:", .)) %>%
    rename_with( ~gsub("/24", "/Cer24", .)) %>%
        CreateTableOne(data = .,
                    strata = "cv_komb_profil",
                            #strata = "ESRD_profil",
                            #strata = "doed_profil",
```

```
                             addOverall = TRUE)

#Table of quartiles
CerTable_quartiles <- data %>%
    select(c(starts_with("Target"), starts_with("Ratio"))) %>%
    mutate(across(starts_with("Target"), ~.*1000)) %>%
    rename_with( ~gsub("Target_", "Cer", .)) %>%
    rename_with( ~gsub("Ratio ", "Ratio Cer", .)) %>%
    rename_with( ~gsub("24_", "24:", .)) %>%
    rename_with( ~gsub("/24", "/Cer24", .)) %>%
    summarise(across(everything(),  ~quantile(.))) %>%
    t() %>%
    round(., digits = 2) %>%
    as.data.frame()

colnames(CerTable_quartiles) <- c("Min", "Q1", "Median", "Q3", "Max")

CerTable_CVE
```

```
##                                Stratified by cv_komb_profil
##                                 Overall            0
##    n                                662              568
##    Cer16 (mean (SD))            123.64 (24.86)   122.62 (25.01)
##    Cer18 (mean (SD))             70.53 (20.43)    69.55 (19.80)
##    Cer20 (mean (SD))             99.89 (37.17)    99.49 (36.71)
##    Cer22 (mean (SD))            574.74 (239.69)  573.21 (236.25)
##    Cer24:0 (mean (SD))         2239.64 (912.47) 2236.76 (895.99)
##    Cer24:1 (mean (SD))         1094.60 (416.64) 1081.78 (403.31)
##    Ratio Cer16/Cer24:0 (mean (SD))   0.06 (0.02)      0.06 (0.02)
##    Ratio Cer18/Cer24:0 (mean (SD))   0.03 (0.01)      0.03 (0.01)
##    Ratio Cer20/Cer24:0 (mean (SD))   0.05 (0.01)      0.05 (0.01)
##    Ratio Cer22/Cer24:0 (mean (SD))   0.26 (0.04)      0.26 (0.04)
##    Ratio Cer24:1/Cer24:0 (mean (SD)) 0.51 (0.11)      0.50 (0.11)
##    cv_komb_profil = 1 (%)            94 (14.2)         0 (0.0)
##                                Stratified by cv_komb_profil
##                                 1                  p      test
##    n                                 94
##    Cer16 (mean (SD))            129.78 (23.07)    0.010
##    Cer18 (mean (SD))             76.43 (23.16)    0.002
##    Cer20 (mean (SD))            102.31 (39.97)    0.496
##    Cer22 (mean (SD))            584.00 (260.68)   0.686
##    Cer24:0 (mean (SD))         2257.03 (1011.39) 0.842
##    Cer24:1 (mean (SD))         1172.05 (484.82)  0.052
##    Ratio Cer16/Cer24:0 (mean (SD))   0.07 (0.03)      0.102
##    Ratio Cer18/Cer24:0 (mean (SD))   0.04 (0.01)      0.009
##    Ratio Cer20/Cer24:0 (mean (SD))   0.05 (0.01)      0.218
##    Ratio Cer22/Cer24:0 (mean (SD))   0.26 (0.04)      0.437
##    Ratio Cer24:1/Cer24:0 (mean (SD)) 0.54 (0.12)      0.002
##    cv_komb_profil = 1 (%)            94 (100.0)       <0.001
```

CerTable_quartiles

```
##                                 Min      Q1  Median     Q3     Max
```

```
## Cer16                    48.41  106.78  122.16  138.71  200.74
## Cer18                    25.08   55.54   67.14   81.38  133.20
## Cer20                    23.69   73.15   94.80  119.71  209.82
## Cer22                   121.78  397.82  537.63  704.07 1293.32
## Cer24:0                 435.60 1567.73 2114.09 2739.99 5027.83
## Cer24:1                 301.39  788.45 1036.21 1299.02 2342.05
## Ratio Cer16/Cer24:0       0.01    0.05    0.06    0.07    0.14
## Ratio Cer18/Cer24:0       0.01    0.03    0.03    0.04    0.07
## Ratio Cer20/Cer24:0       0.02    0.04    0.05    0.05    0.08
## Ratio Cer22/Cer24:0       0.15    0.23    0.26    0.28    0.37
## Ratio Cer24:1/Cer24:0     0.27    0.43    0.49    0.57    0.83
```

```r
# write.csv(print(CerTable_CVE, printToggle = FALSE), here("data/CerTable_CVE.csv"))
# write.csv(print(CerTable_quartiles, printToggle = FALSE), here("data/CerTable_quartiles.csv"))

rm(CerTable_CVE, CerTable_quartiles)


#Function for extracting Coefficient, upper and lower confidence interval and p-value
Cox_extract <- function(Data, Formula){

  #Extract explanatory variable
  expl_var <- word(as.character(c(Formula)), 3)
      #strsplit(as.character(Formula)[3], split = " ")[[1]][1]

  #Fit Cox regression model
  tmp_cox <- coxph(formula = as.formula(Formula), data = Data)

  #Summary object
  tmp_cox_sum <- summary(tmp_cox)

  #Vector to output
  out_df <- data.frame(expl_var = word(as.character(c(Formula)), 3),
              coeff = tmp_cox_sum$conf.int[expl_var, "exp(coef)"],
              conf_low = tmp_cox_sum$conf.int[expl_var, "lower .95"],
              conf_up = tmp_cox_sum$conf.int[expl_var, "upper .95"],
              pval = tmp_cox_sum$coefficients[expl_var, "Pr(>|z|)"])

  return(out_df)

}


#Survival object
surv_object <- Surv(data_scale$t_cv_komb_profil, as.numeric(as.character(data_scale$cv_komb_profil)))

## 1) CVE - Crude model
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_1 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
```

```r
    mutate("model" = "Crude")

Cox_overview_1
```

```
## # A tibble: 11 x 8
##    name           expl_var          coeff conf_low conf_up    pval     fdr model
##    <chr>          <chr>             <dbl>    <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16      Target_16          1.29     1.06    1.56 0.0101  0.0277 Crude
##  2 Target_18      Target_18          1.32     1.10    1.59 0.00263 0.0145 Crude
##  3 Target_20      Target_20          1.06     0.871   1.29 0.553   0.676  Crude
##  4 Target_22      Target_22          1.03     0.843   1.26 0.768   0.845  Crude
##  5 Target_24_0    Target_24_0        1.01     0.826   1.24 0.904   0.904  Crude
##  6 Target_24_1    Target_24_1        1.20     0.991   1.45 0.0619  0.124  Crude
##  7 Ratio_16/24:0  `Ratio_16/24:0`    1.19     0.987   1.44 0.0678  0.124  Crude
##  8 Ratio_18/24:0  `Ratio_18/24:0`    1.29     1.08    1.55 0.00588 0.0215 Crude
##  9 Ratio_20/24:0  `Ratio_20/24:0`    1.14     0.935   1.39 0.197   0.310  Crude
## 10 Ratio_22/24:0  `Ratio_22/24:0`    1.08     0.880   1.32 0.473   0.650  Crude
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.34     1.11    1.61 0.00188 0.0145 Crude
```

```r
## 2) CVE - Adjusted model
Cox_overview_2 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                   + Age
                   + BMI
                   + Blood_LDL
                   + Blood_TGA
                   + CALSBP
                   + Gender
                   + GFRepi
                   + HbA1C_mmol_mol
                   + logUAER
                   + Previous_CVD
                   + Smoking
                   + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Adjusted")

Cox_overview_2
```

```
## # A tibble: 11 x 8
##    name           expl_var         coeff conf_low conf_up   pval    fdr model
##    <chr>          <chr>            <dbl>    <dbl>   <dbl>  <dbl>  <dbl> <chr>
##  1 Target_16      Target_16         1.04    0.832   1.30  0.738  0.777 Adjust~
##  2 Target_18      Target_18         1.03    0.819   1.31  0.777  0.777 Adjust~
##  3 Target_20      Target_20         0.769   0.584   1.01  0.0614 0.135 Adjust~
##  4 Target_22      Target_22         0.722   0.537   0.971 0.0310 0.114 Adjust~
##  5 Target_24_0    Target_24_0       0.761   0.572   1.01  0.0606 0.135 Adjust~
##  6 Target_24_1    Target_24_1       0.832   0.630   1.10  0.195  0.357 Adjust~
##  7 Ratio_16/24:0  `Ratio_16/24:0`   1.32    1.04    1.67  0.0220 0.114 Adjust~
```

```
##  8 Ratio_18/24:0   `Ratio_18/24:0`  1.33      1.06     1.68  0.0140 0.114 Adjust~
##  9 Ratio_20/24:0   `Ratio_20/24:0`  1.08      0.862    1.36  0.491  0.675 Adjust~
## 10 Ratio_22/24:0   `Ratio_22/24:0`  0.963     0.767    1.21  0.743  0.777 Adjust~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.10     0.886    1.36  0.391  0.614 Adjust~
```

```r
## 3) CVE - Adjusted light model
Cox_overview_3 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                      + Age
                      + BMI
                      + Blood_LDL
                      + Blood_TGA
                      + CALSBP
                      + Gender
                      + HbA1C_mmol_mol
                      + Previous_CVD
                      + Smoking
                      + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr"))%>%
    mutate("model" = "Adj. light")

Cox_overview_3
```

```
## # A tibble: 11 x 8
##    name             expl_var          coeff conf_low conf_up    pval    fdr model
##    <chr>            <chr>             <dbl>    <dbl>   <dbl>   <dbl>  <dbl> <chr>
##  1 Target_16        Target_16         1.06    0.850    1.31  0.621   0.760 Adj.~
##  2 Target_18        Target_18         1.04    0.825    1.31  0.743   0.817 Adj.~
##  3 Target_20        Target_20         0.776   0.596    1.01  0.0602  0.133 Adj.~
##  4 Target_22        Target_22         0.734   0.553    0.975 0.0330  0.121 Adj.~
##  5 Target_24_0      Target_24_0       0.755   0.573    0.994 0.0451  0.124 Adj.~
##  6 Target_24_1      Target_24_1       0.835   0.639    1.09  0.186   0.340 Adj.~
##  7 Ratio_16/24:0    `Ratio_16/24:0`   1.37    1.09     1.73  0.00787 0.0433 Adj.~
##  8 Ratio_18/24:0    `Ratio_18/24:0`   1.37    1.10     1.72  0.00580 0.0433 Adj.~
##  9 Ratio_20/24:0    `Ratio_20/24:0`   1.12    0.894    1.40  0.327   0.449 Adj.~
## 10 Ratio_22/24:0    `Ratio_22/24:0`   1.02    0.818    1.27  0.869   0.869 Adj.~
## 11 Ratio_24:1/24:0  `Ratio_24:1/24:0` 1.14    0.923    1.40  0.228   0.358 Adj.~
```

```r
#Combine the 3 CVE models into a single table
Cox_overview_CVE <- Cox_overview_1 %>%
    rbind(., Cox_overview_2, Cox_overview_3) %>%
    mutate("outcome" = "CVE")

rm(Cox_overview_1, Cox_overview_2, Cox_overview_3, surv_object)
```

```r
#Survival object
surv_object <- Surv(data_scale$t_ESRD_profil, as.numeric(as.character(data_scale$ESRD_profil)))
# surv_object <- Surv(data_scale$t_komb_nyre_endepunkt_p, as.numeric(as.character(data_scale$komb_nyre_
```

```r
## 4) ESKD - Crude model
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_4 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Crude")

Cox_overview_4
```

```
## # A tibble: 11 x 8
##    name           expl_var         coeff conf_~1 conf_up    pval      fdr model
##    <chr>          <chr>            <dbl>   <dbl>   <dbl>   <dbl>    <dbl> <chr>
##  1 Target_16      Target_16         1.52   1.04     2.21 0.0299  0.0821  Crude
##  2 Target_18      Target_18         1.20   0.823    1.75 0.344   0.472   Crude
##  3 Target_20      Target_20         1.16   0.794    1.70 0.439   0.536   Crude
##  4 Target_22      Target_22         1.13   0.773    1.67 0.519   0.571   Crude
##  5 Target_24_0    Target_24_0       0.914  0.599    1.40 0.677   0.677   Crude
##  6 Target_24_1    Target_24_1       1.31   0.911    1.88 0.145   0.228   Crude
##  7 Ratio_16/24:0  `Ratio_16/24:0`   1.42   0.998    2.02 0.0513  0.113   Crude
##  8 Ratio_18/24:0  `Ratio_18/24:0`   1.34   0.929    1.92 0.118   0.216   Crude
##  9 Ratio_20/24:0  `Ratio_20/24:0`   1.51   1.04     2.20 0.0289  0.0821  Crude
## 10 Ratio_22/24:0  `Ratio_22/24:0`   1.87   1.27     2.76 0.00145 0.00796 Crude
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.79  1.26     2.53 0.00106 0.00796 Crude
## # ... with abbreviated variable name 1: conf_low
```

```r
## 5) ESKD - Adjusted model
Cox_overview_5 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                    + Age
                    + BMI
                    + Blood_LDL
                    + Blood_TGA
                    + CALSBP
                    + Gender
                    + GFRepi
                    + HbA1C_mmol_mol
                    + logUAER
                    + Previous_CVD
                    + Smoking
                    + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Adjusted")

Cox_overview_5
```

```
## # A tibble: 11 x 8
##    name            expl_var          coeff conf_low conf_up  pval   fdr model
##    <chr>           <chr>             <dbl>    <dbl>   <dbl> <dbl> <dbl> <chr>
##  1 Target_16       Target_16         0.786    0.426    1.45 0.440 0.726 Adjusted
##  2 Target_18       Target_18         0.764    0.415    1.41 0.387 0.726 Adjusted
##  3 Target_20       Target_20         0.783    0.412    1.49 0.456 0.726 Adjusted
##  4 Target_22       Target_22         0.882    0.442    1.76 0.722 0.795 Adjusted
##  5 Target_24_0     Target_24_0       0.747    0.392    1.42 0.374 0.726 Adjusted
##  6 Target_24_1     Target_24_1       1.31     0.605    2.85 0.490 0.726 Adjusted
##  7 Ratio_16/24:0   `Ratio_16/24:0`   1.23     0.640    2.37 0.533 0.726 Adjusted
##  8 Ratio_18/24:0   `Ratio_18/24:0`   1.03     0.590    1.79 0.923 0.923 Adjusted
##  9 Ratio_20/24:0   `Ratio_20/24:0`   1.18     0.647    2.14 0.594 0.726 Adjusted
## 10 Ratio_22/24:0   `Ratio_22/24:0`   1.52     0.841    2.74 0.166 0.726 Adjusted
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.52     0.902    2.55 0.116 0.726 Adjusted
```

```r
## 6) ESKD - Adjusted light model
Cox_overview_6 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                    + Age
                    + BMI
                    + Blood_LDL
                    + Blood_TGA
                    + CALSBP
                    + Gender
                    + HbA1C_mmol_mol
                    + Previous_CVD
                    + Smoking
                    + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Adj. light")

Cox_overview_6
```

```
## # A tibble: 11 x 8
##    name            expl_var          coeff conf_~1 conf_up    pval     fdr model
##    <chr>           <chr>             <dbl>   <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16       Target_16         1.42    0.910    2.22 1.22e-1 0.191   Adj.~
##  2 Target_18       Target_18         1.01    0.634    1.60 9.78e-1 0.978   Adj.~
##  3 Target_20       Target_20         0.909   0.560    1.47 6.99e-1 0.854   Adj.~
##  4 Target_22       Target_22         0.806   0.475    1.37 4.25e-1 0.584   Adj.~
##  5 Target_24_0     Target_24_0       0.591   0.342    1.02 5.85e-2 0.107   Adj.~
##  6 Target_24_1     Target_24_1       1.04    0.643    1.68 8.77e-1 0.965   Adj.~
##  7 Ratio_16/24:0   `Ratio_16/24:0`   2.10    1.37     3.22 6.56e-4 0.00361 Adj.~
##  8 Ratio_18/24:0   `Ratio_18/24:0`   1.69    1.11     2.58 1.50e-2 0.0330  Adj.~
##  9 Ratio_20/24:0   `Ratio_20/24:0`   1.73    1.14     2.62 9.93e-3 0.0273  Adj.~
## 10 Ratio_22/24:0   `Ratio_22/24:0`   1.90    1.23     2.94 3.83e-3 0.0141  Adj.~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 2.06    1.38     3.07 4.14e-4 0.00361 Adj.~
## # ... with abbreviated variable name 1: conf_low
```

```
#Combine the 3 ESKD models into a single table
Cox_overview_ESKD <- Cox_overview_4 %>%
    rbind(., Cox_overview_5, Cox_overview_6) %>%
    mutate("outcome" = "ESKD")

rm(Cox_overview_4, Cox_overview_5, Cox_overview_6, surv_object)


#Survival object
surv_object <- Surv(data_scale$t_doed_profil , as.numeric(as.character(data_scale$doed_profil)))

## 7) Mortality - Crude model
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_7 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Crude")

Cox_overview_7
```

```
## # A tibble: 11 x 8
##    name            expl_var         coeff conf_~1 conf_up    pval      fdr model
##    <chr>           <chr>            <dbl>   <dbl>   <dbl>   <dbl>    <dbl> <chr>
##  1 Target_16       Target_16         1.07   0.830   1.38  6.00e-1 6.60e-1 Crude
##  2 Target_18       Target_18         0.925  0.710   1.21  5.65e-1 6.60e-1 Crude
##  3 Target_20       Target_20         0.728  0.544   0.974 3.23e-2 5.08e-2 Crude
##  4 Target_22       Target_22         0.545  0.392   0.758 3.16e-4 6.95e-4 Crude
##  5 Target_24_0     Target_24_0       0.584  0.425   0.801 8.67e-4 1.59e-3 Crude
##  6 Target_24_1     Target_24_1       0.734  0.547   0.986 4.00e-2 5.50e-2 Crude
##  7 Ratio_16/24:0   `Ratio_16/24:0`   1.77   1.43    2.17  9.21e-8 1.01e-6 Crude
##  8 Ratio_18/24:0   `Ratio_18/24:0`   1.71   1.39    2.11  6.04e-7 3.32e-6 Crude
##  9 Ratio_20/24:0   `Ratio_20/24:0`   1.58   1.25    2.00  1.29e-4 3.54e-4 Crude
## 10 Ratio_22/24:0   `Ratio_22/24:0`   1.02   0.789   1.32  8.70e-1 8.70e-1 Crude
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.65   1.31    2.06  1.46e-5 5.36e-5 Crude
## # ... with abbreviated variable name 1: conf_low
```

```
## 8) ESKD - Adjusted model
Cox_overview_8 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                    + Age
                    + BMI
                    + Blood_LDL
                    + Blood_TGA
                    + CALSBP
                    + Gender
                    + GFRepi
                    + HbA1C_mmol_mol
                    + logUAER
```

```
                      + Previous_CVD
                      + Smoking
                      + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Adjusted")

Cox_overview_8
```

```
## # A tibble: 11 x 8
##    name            expl_var       coeff conf_~1 conf_up    pval     fdr model
##    <chr>           <chr>          <dbl>   <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16       Target_16      0.809   0.587    1.11 1.93e-1 2.12e-1 Adju~
##  2 Target_18       Target_18      0.711   0.509   0.994 4.59e-2 7.22e-2 Adju~
##  3 Target_20       Target_20      0.554   0.379   0.811 2.36e-3 5.20e-3 Adju~
##  4 Target_22       Target_22      0.376   0.237   0.597 3.26e-5 3.58e-4 Adju~
##  5 Target_24_0     Target_24_0    0.473   0.307   0.727 6.61e-4 2.42e-3 Adju~
##  6 Target_24_1     Target_24_1    0.480   0.310   0.743 9.99e-4 2.75e-3 Adju~
##  7 Ratio_16/24:0   `Ratio_16/24:0` 1.69   1.27     2.23 2.78e-4 1.53e-3 Adju~
##  8 Ratio_18/24:0   `Ratio_18/24:0` 1.48   1.12     1.94 5.03e-3 9.22e-3 Adju~
##  9 Ratio_20/24:0   `Ratio_20/24:0` 1.31   0.992    1.72 5.71e-2 7.86e-2 Adju~
## 10 Ratio_22/24:0   `Ratio_22/24:0` 0.899  0.669    1.21 4.83e-1 4.83e-1 Adju~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.21 0.935    1.57 1.46e-1 1.78e-1 Adju~
## # ... with abbreviated variable name 1: conf_low
```

```
## 9) ESKD - Adjusted light model
Cox_overview_9 <- data_scale %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(data_scale, Formula = surv_object ~ .
                      + Age
                      + BMI
                      + Blood_LDL
                      + Blood_TGA
                      + CALSBP
                      + Gender
                      + HbA1C_mmol_mol
                      + Previous_CVD
                      + Smoking
                      + Statin))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Adj. light")

Cox_overview_9
```

```
## # A tibble: 11 x 8
##    name            expl_var       coeff conf_~1 conf_up    pval     fdr model
##    <chr>           <chr>          <dbl>   <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16       Target_16      0.876   0.639    1.20 4.13e-1 4.55e-1 Adj.~
##  2 Target_18       Target_18      0.746   0.533    1.04 8.68e-2 1.06e-1 Adj.~
```

```
## 3 Target_20       Target_20        0.570   0.390   0.835 3.85e-3 7.06e-3 Adj.~
## 4 Target_22       Target_22        0.382   0.242   0.603 3.62e-5 2.34e-4 Adj.~
## 5 Target_24_0     Target_24_0      0.459   0.299   0.704 3.56e-4 1.31e-3 Adj.~
## 6 Target_24_1     Target_24_1      0.521   0.344   0.790 2.15e-3 4.73e-3 Adj.~
## 7 Ratio_16/24:0   `Ratio_16/24:0`  1.76    1.34    2.31  4.25e-5 2.34e-4 Adj.~
## 8 Ratio_18/24:0   `Ratio_18/24:0`  1.58    1.21    2.05  7.04e-4 1.93e-3 Adj.~
## 9 Ratio_20/24:0   `Ratio_20/24:0`  1.41    1.08    1.84  1.13e-2 1.77e-2 Adj.~
## 10 Ratio_22/24:0  `Ratio_22/24:0`  0.983   0.737   1.31  9.05e-1 9.05e-1 Adj.~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.33  1.03    1.72  3.00e-2 4.12e-2 Adj.~
## # ... with abbreviated variable name 1: conf_low
```

```r
#Combine the 3 mortality models into a single table
Cox_overview_Mortality <- Cox_overview_7 %>%
    rbind(., Cox_overview_8, Cox_overview_9) %>%
    mutate("outcome" = "Mortality")


rm(Cox_overview_7, Cox_overview_8, Cox_overview_9, surv_object)
```

```r
#Combine the 3 outcome tables into a single table
Cox_overview <- Cox_overview_CVE %>%
    rbind(., Cox_overview_ESKD, Cox_overview_Mortality)

rm(Cox_overview_CVE, Cox_overview_ESKD, Cox_overview_Mortality)

#Plot overview table as a Forest Plot
Fig1_forest_plot <- Cox_overview %>%
    mutate(name = gsub("Target_", "Cer ", name)) %>%
    mutate(name = gsub("Ratio_", "Ratio Cer ", name)) %>%
    mutate(name = gsub("_", ":", name)) %>%
    mutate(name = factor(name, levels = rev(unique(name)))) %>%
    mutate(model = factor(model, levels = c("Crude", "Adj. light", "Adjusted"),
                        labels = c("Crude",
                                    "Level 1 Adjusted",
                                    "Level 2 Adjusted"))) %>%
    mutate(outcome = factor(outcome, levels = c("CVE", "ESKD", "Mortality"),
                        labels = c("Cardiovascular Events",
                                    "Kidney Failure",
                                    "All-Cause Mortality"))) %>%
    mutate(Significance = if_else(pval < 0.05, "p < 0.05", "None")) %>%
    mutate(Significance = if_else(pval < 0.001, "p < 0.001", Significance)) %>%
    mutate(Significance = factor(Significance, levels = c("None", "p < 0.05", "p < 0.001"))) %>%
    ggplot(aes(x = coeff, y = name, color = Significance)) +
        geom_errorbar(aes(xmin = conf_low, xmax = conf_up), width = 0.5, size = 0.8) +
        geom_pointrange(aes(xmin = conf_low, xmax = conf_up)) +
        geom_vline(xintercept = 1, linetype = "dashed") +
        scale_x_log10() +
        scale_color_manual(values = c("black", "#D9B54A", "#8C2336")) +
        facet_grid(outcome ~ model) +
        xlab(label = "Hazard Ratio") +
        theme_bw() +
        theme(axis.title.y = element_blank(),
                legend.position = "top",
                strip.background = element_rect(colour="black",
```

```
                                    fill="#F2EFE9"))
#Legend text
Fig1_legend_text <- ggparagraph(text = paste("Figure 1 - Forest plot of hazard ratios for ceramide and

ggarrange(Fig1_forest_plot, Fig1_legend_text,
          ncol = 1,
          heights = c(1, 0.2))
```
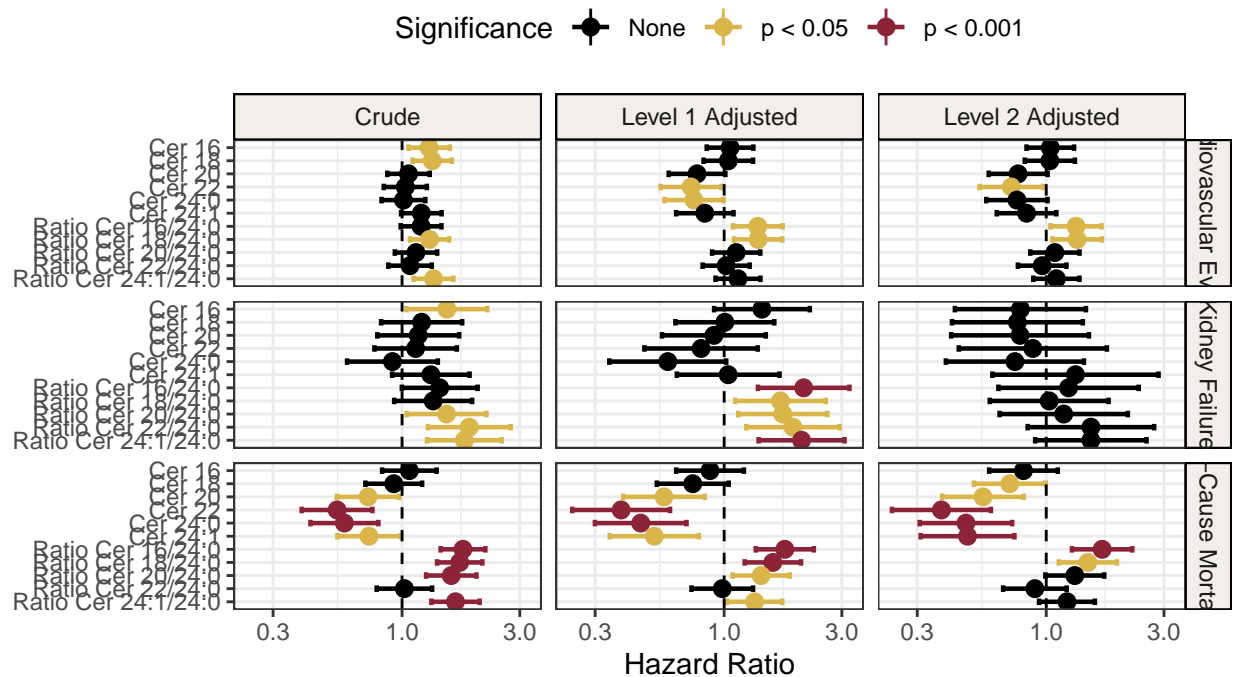

Figure 1 – Forest plot of hazard ratios for ceramide and ratios for outcomes of cardiovascular events, kidney failure and mortality. The crude models are unadjusted, level 1 adjusted for age, sex, BMI, LDL, triglycerides, systolic blood pressure, HbA1C, history of CVD, smoking status and statin use. Level 2 adjusted for all the same variables as level 1, but also included eGFR and UAER. Hazard ratios (HRs) are

```
#export at 8x7.1 inches with legend (7.1x7.1 without)

#Correct naming in Cox_overview
Cox_overview <- Cox_overview %>%
    mutate(model = gsub("Adjusted", "Level 2 Adjusted", model)) %>%
    mutate(model = gsub("Adj. light", "Level 1 Adjusted", model)) %>%
    mutate(name = gsub("Target_", "Cer", name)) %>%
    mutate(name = gsub("Ratio_", "Ratio Cer", name)) %>%
    mutate(name = gsub("/24", "/Cer24", name)) %>%
    mutate(name = gsub("_", ":", name)) %>%
    select(-"expl_var")
#write supplementary table
#write.csv(Cox_overview, here("data/Cox_overview.csv"))


# #Plot single model as a Forest Plot
# Cox_overview %>%
```

```r
#      mutate(name = gsub("Target_", "Cer ", name)) %>%
#      mutate(name = gsub("Ratio_", "Ratio Cer ", name)) %>%
#      mutate(name = gsub("_", ":", name)) %>%
#      #arrange(value$pval) %>%
#      mutate(name = factor(name, levels = rev(unique(name)))) %>%
#      filter(model == "Adj. light") %>%
#      filter(outcome == "ESKD") %>%
#      ggplot(aes(x = coeff, y = name)) +
#         geom_errorbar(aes(xmin = conf_low, xmax = conf_up), width = 0.5, size = 0.8) +
#         geom_pointrange(aes(xmin = conf_low, xmax = conf_up)) +
#         geom_vline(xintercept = 1, linetype = "dashed") +
#         scale_x_log10() +
#         xlab(label = "Hazard Ratio") +
#         theme_bw() +
#         theme(axis.title.y = element_blank())

rm(Fig1_forest_plot, Fig1_legend_text)


#Survival object - Cardiovascular events
surv_object <- Surv(data$t_cv_komb_profil, as.numeric(as.character(data$cv_komb_profil)))

#Vector of clinical factors to investigate
clin_num <- c("Age", "BMI", "Blood_LDL", "Blood_TGA", "CALSBP", "GFRepi", "HbA1C_mmol_mol", "logUAER")

clin_fac <- c("Gender", "Previous_CVD", "Smoking", "Statin")


## 10) CVE - Clinical Crude model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)

#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data, Formula = paste0("surv_object ~ ", clin_num[i]))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_tmp <-
        summary(coxph(formula = as.formula(paste0("surv_object ~ ", clin_fac[j])), data = data))

    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
```

```r
            Cox_clinical_tmp$conf.int[1, "lower .95"]
        Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
            Cox_clinical_tmp$conf.int[1, "upper .95"]
        Cox_clinical_overview[length(clin_num)+j, "pval"]     <-
            Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]


}

#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]

#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Crude"

#Rename data frame
Cox_clinical_overview_10 <- Cox_clinical_overview


## 11) CVE - Clinical adjusted model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)

#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_num[i])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data,
                    Formula = paste0("surv_object ~ ", clin_num[i], " + ", clin_tmp))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_fac[j])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model
    Cox_clinical_tmp <-
        summary(coxph(data = data,
```

```r
                        formula = as.formula(paste0("surv_object ~ ", clin_fac[j], " + ", clin_tmp)), ))

    #Extract the results and save into data frame
    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)[1]
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
        Cox_clinical_tmp$conf.int[1, "lower .95"]
    Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
        Cox_clinical_tmp$conf.int[1, "upper .95"]
    Cox_clinical_overview[length(clin_num)+j, "pval"]     <-
        Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]

}


#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]


#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Adjusted"

#Rename data frame
Cox_clinical_overview_11 <- Cox_clinical_overview



#Combine the 2 CVE models into a single table
Cox_clinical_overview_CVE <- Cox_clinical_overview_10 %>%
    rbind(., Cox_clinical_overview_11) %>%
    mutate("outcome" = "CVE")

#Remove temporary objects
rm(Cox_clinical_overview, Cox_clinical_overview_10, Cox_clinical_overview_11,
   Cox_clinical_tmp, clin_fac, clin_num, clin_tmp, i, j, surv_object)


#Survival object - kidney disease
surv_object <- Surv(data$t_ESRD_profil, as.numeric(as.character(data$ESRD_profil)))

#Vector of clinical factors to investigate
clin_num <- c("Age", "BMI", "Blood_LDL", "Blood_TGA", "CALSBP", "GFRepi", "HbA1C_mmol_mol", "logUAER")

clin_fac <- c("Gender", "Previous_CVD", "Smoking", "Statin")


## 12) ESKD - Clinical Crude model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)
```

```r
#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data, Formula = paste0("surv_object ~ ", clin_num[i]))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_tmp <-
        summary(coxph(formula = as.formula(paste0("surv_object ~ ", clin_fac[j])), data = data))

    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
        Cox_clinical_tmp$conf.int[1, "lower .95"]
    Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
        Cox_clinical_tmp$conf.int[1, "upper .95"]
    Cox_clinical_overview[length(clin_num)+j, "pval"]     <-
        Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]

}

#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]

#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Crude"

#Rename data frame
Cox_clinical_overview_12 <- Cox_clinical_overview


## 13) ESKD - Clinical adjusted model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)

#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
```

```r
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_num[i])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data,
                    Formula = paste0("surv_object ~ ", clin_num[i], " + ", clin_tmp))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_fac[j])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model
    Cox_clinical_tmp <-
        summary(coxph(data = data,
                      formula = as.formula(paste0("surv_object ~ ", clin_fac[j], " + ", clin_tmp)), ))

    #Extract the results and save into data frame
    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)[1]
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
        Cox_clinical_tmp$conf.int[1, "lower .95"]
    Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
        Cox_clinical_tmp$conf.int[1, "upper .95"]
    Cox_clinical_overview[length(clin_num)+j, "pval"]     <-
        Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]

}

#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]

#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Adjusted"

#Rename data frame
Cox_clinical_overview_13 <- Cox_clinical_overview




#Combine the 2 kidney models into a single table
```

```r
Cox_clinical_overview_ESKD <- Cox_clinical_overview_12 %>%
    rbind(., Cox_clinical_overview_13) %>%
    mutate("outcome" = "ESKD")

#Remove temporary objects
rm(Cox_clinical_overview, Cox_clinical_overview_12, Cox_clinical_overview_13,
   Cox_clinical_tmp, clin_fac, clin_num, clin_tmp, i, j, surv_object)


#Survival object - kidney disease
surv_object <- Surv(data$t_doed_profil, as.numeric(as.character(data$doed_profil)))

#Vector of clinical factors to investigate
clin_num <- c("Age", "BMI", "Blood_LDL", "Blood_TGA", "CALSBP", "GFRepi", "HbA1C_mmol_mol", "logUAER")

clin_fac <- c("Gender", "Previous_CVD", "Smoking", "Statin")


## 14) Mortality - Clinical Crude model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)

#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data, Formula = paste0("surv_object ~ ", clin_num[i]))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_tmp <-
        summary(coxph(formula = as.formula(paste0("surv_object ~ ", clin_fac[j])), data = data))

    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
        Cox_clinical_tmp$conf.int[1, "lower .95"]
    Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
        Cox_clinical_tmp$conf.int[1, "upper .95"]
    Cox_clinical_overview[length(clin_num)+j, "pval"]     <-
        Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]

}

#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
```

```r
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]

#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Crude"

#Rename data frame
Cox_clinical_overview_14 <- Cox_clinical_overview


## 15) Mortality - Clinical adjusted model
#Create an empty dataframe to populate
Cox_clinical_overview <- data.frame("expl_var" = NA, "coeff" = NA,
                                    "conf_low" = NA, "conf_up" = NA, "pval"= NA)

#Iterate over numeric clinical variables
for(i in 1:length(clin_num)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_num[i])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model, extract the results, save into data frame
    Cox_clinical_overview[i,] <-
        Cox_extract(Data = data,
                    Formula = paste0("surv_object ~ ", clin_num[i], " + ", clin_tmp))

}

#Iterate over factorized clinical variables
for(j in 1:length(clin_fac)){

    #String of adjustments
    clin_tmp <- c(clin_num, clin_fac)
    clin_tmp <- clin_tmp[!clin_tmp %in% c(clin_fac[j])]
    clin_tmp <- paste(clin_tmp, collapse = " + ")

    #Run Cox model
    Cox_clinical_tmp <-
        summary(coxph(data = data,
                      formula = as.formula(paste0("surv_object ~ ", clin_fac[j], " + ", clin_tmp)), ))

    #Extract the results and save into data frame
    Cox_clinical_overview[length(clin_num)+j, "expl_var"] <-
        rownames(Cox_clinical_tmp$conf.int)[1]
    Cox_clinical_overview[length(clin_num)+j, "coeff"]    <-
        Cox_clinical_tmp$conf.int[1, "exp(coef)"]
    Cox_clinical_overview[length(clin_num)+j, "conf_low"] <-
        Cox_clinical_tmp$conf.int[1, "lower .95"]
    Cox_clinical_overview[length(clin_num)+j, "conf_up"]  <-
```

```r
        Cox_clinical_tmp$conf.int[1, "upper .95"]
    Cox_clinical_overview[length(clin_num)+j, "pval"]    <-
        Cox_clinical_tmp$coefficients[1, "Pr(>|z|)"]


}


#Insert names of clinical variables
Cox_clinical_overview$"name" <- c(clin_num, clin_fac)
Cox_clinical_overview <- Cox_clinical_overview[, c(6, 1:5)]

#FDR
Cox_clinical_overview$fdr <- p.adjust(Cox_clinical_overview$pval, method = "fdr")

#Model
Cox_clinical_overview$model <- "Adjusted"

#Rename data frame
Cox_clinical_overview_15 <- Cox_clinical_overview




#Combine the 2 mortality models into a single table
Cox_clinical_overview_Mortality <- Cox_clinical_overview_14 %>%
    rbind(., Cox_clinical_overview_15) %>%
    mutate("outcome" = "Mortality")

#Remove temporary objects
rm(Cox_clinical_overview, Cox_clinical_overview_14, Cox_clinical_overview_15,
   Cox_clinical_tmp, clin_fac, clin_num, clin_tmp, i, j, surv_object)


#Combine the 3 outcome tables into a single table
Cox_clinical_overview <- Cox_clinical_overview_CVE %>%
    rbind(., Cox_clinical_overview_ESKD, Cox_clinical_overview_Mortality)

rm(Cox_clinical_overview_CVE, Cox_clinical_overview_ESKD, Cox_clinical_overview_Mortality)

#Clean names, !!NOTE VULNERABLE TO CHANGES!!
tmp_names <- c("Age", "BMI", "LDL", "Triglyceride","Systolic Blood Pressure",
               "eGFR", "HbA1C", "log(UAER)", "Female", "Previous CVD", "Smoking", "Statins")

Cox_clinical_overview$name <- tmp_names

#Plot cox clinical overview table as a Forest Plot
Cox_clinical_overview %>%
    mutate(name = factor(name, levels = rev(unique(name)))) %>%
    mutate(model = factor(model, levels = c("Crude", "Adjusted"))) %>%
    mutate(outcome = factor(outcome, levels = c("CVE", "ESKD", "Mortality"),
                      labels = c("Cardiovascular Events",
                                 "Kidney Failure",
                                 "All-Cause Mortality"))) %>%
    mutate(Significance = if_else(pval < 0.05, "p < 0.05", "None")) %>%
    mutate(Significance = if_else(pval < 0.001, "p < 0.001", Significance)) %>%
    mutate(Significance = factor(Significance, levels = c("None", "p < 0.05", "p < 0.001"))) %>%
```

```
ggplot(aes(x = coeff, y = name, color = Significance)) +
    geom_errorbar(aes(xmin = conf_low, xmax = conf_up), width = 0.5, size = 0.8) +
    geom_pointrange(aes(xmin = conf_low, xmax = conf_up)) +
    geom_vline(xintercept = 1, linetype = "dashed") +
    scale_x_log10() +
    scale_color_manual(values = c("black", "#D9B54A", "#8C2336")) +
    facet_grid(outcome ~ model) +
    xlab(label = "Hazard Ratio") +
    theme_bw() +
    theme(axis.title.y = element_blank(),
          legend.position = "top",
          strip.background = element_rect(colour="black",
                                          fill="#F2EFE9"))
```



```
#export at 8x8



#write supplementary table
#write.csv(Cox_clinical_overview, here("data/Cox_clinical_overview.csv"))

rm(tmp_names)

#Ceramide Ratio and CVE

#Survival object
```

```r
surv_object <- Surv((data$t_cv_komb_profil)/365, as.numeric(as.character(data$cv_komb_profil)))

#Create binary variable for targets and ratios
data_bin <- data %>%
  #rename(Ratio241_240 = `Ratio 24:1/24:0`) %>%
  rename(Ratio18_240 = `Ratio 18/24:0`) %>%
  mutate(across(starts_with("Target") | starts_with("Ratio"),
    ~ ifelse(. >= quantile(.)[["50%"]], "Above median", "Below median")))

#FitKaplan-Meier model
KM_fit <- survfit(formula = surv_object ~ data_bin$Ratio18_240, data = data_bin)

#Plot Model
p1 <- ggsurvplot(
  KM_fit,
  data = data_bin,
  conf.int = FALSE,
  pval = TRUE,
  xlab = "Years",
  ylab = "CVE rate",
  palette =  c("#5888A6", "#8C2336"),
  #legend = "bottom",
  legend.title = "Ceramide Ratio\nCer18/Cer24:0",
  legend.labs = c("Low", "High"),
  risk.table = TRUE)
  #ggtheme = theme_bw())

p1
```
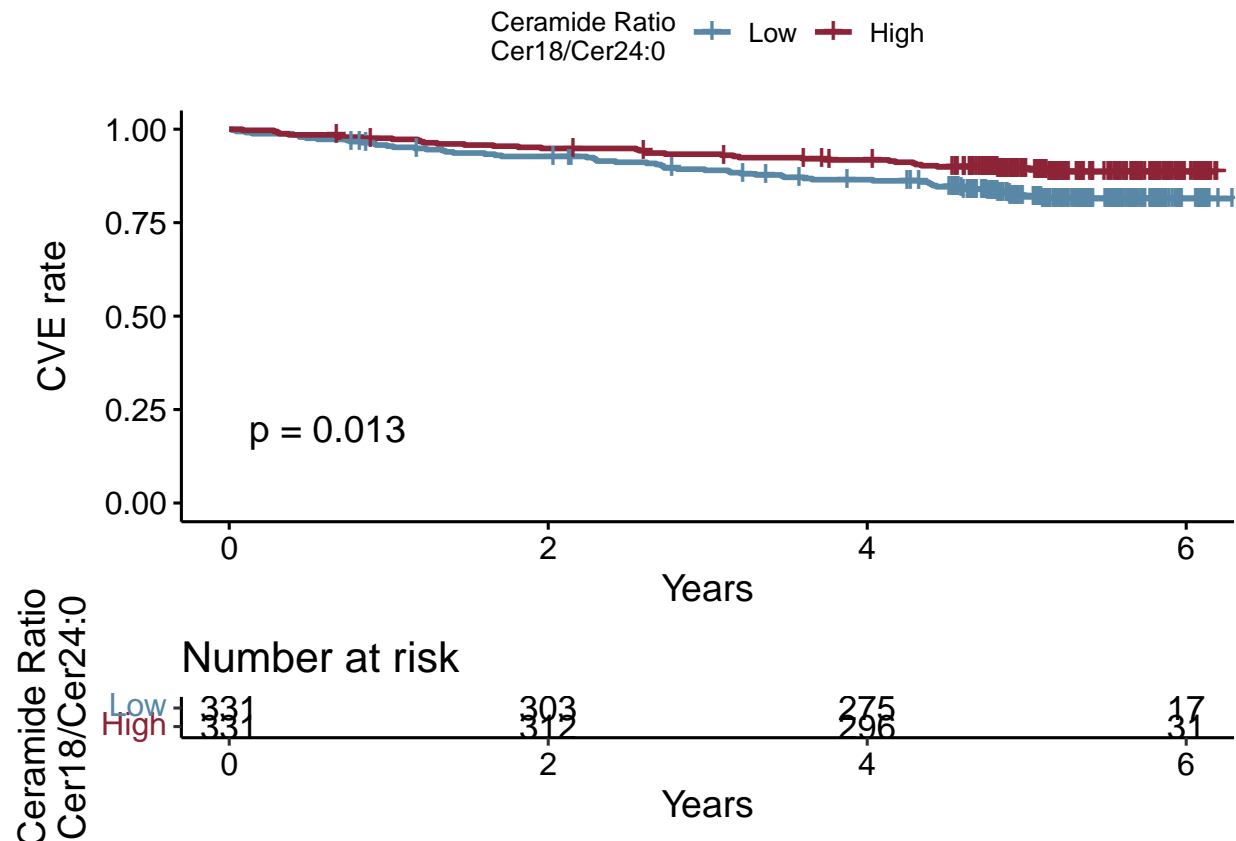
```
p1t <- p1$table + labs(x = "", y = "")


## LDL and CVE

#Create binary variable for targets and ratios
data_bin <- data %>%
  mutate(across(starts_with("Target") | starts_with("Ratio"),
    ~ ifelse(. >= quantile(.)[["50%"]], "Above median", "Below median"))) %>%
  filter(!is.na(Blood_LDL)) %>%
  mutate(Blood_LDL = ifelse(Blood_LDL >= quantile(Blood_LDL)[["50%"]], "Above median", "Below median"))

#Survival object
surv_object <- Surv((data_bin$t_cv_komb_profil)/365, as.numeric(as.character(data_bin$cv_komb_profil)))

#FitKaplan-Meier model
KM_fit <- survfit(formula = surv_object ~ data_bin$Blood_LDL, data = data_bin)

#Plot Model
p2 <- ggsurvplot(
  KM_fit,
  data = data_bin,
  conf.int = FALSE,
  pval = TRUE,
  xlab = "Years",
  palette =  c("#D9B54A", "#A6763C"),
```

```
  #legend = "bottom",
  legend.title = "LDL",
  legend.labs = c("Low", "High"),
  risk.table = TRUE)
  #ggtheme = theme_bw())

p2
```
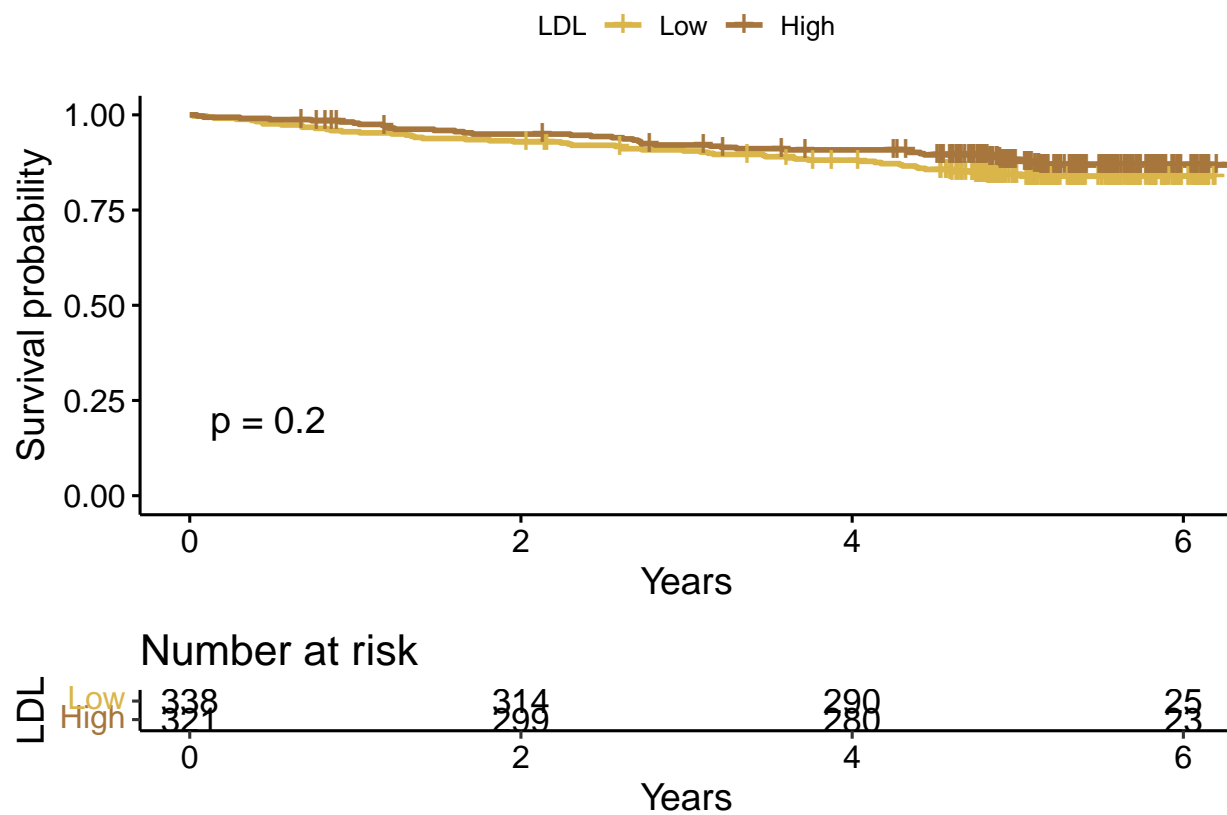


```
p2$plot <- p2$plot + labs(y = "")

p2t <- p2$table + labs(x = "", y = "", title = "")


## Join plots together
Fig2_km_plots <- ggarrange(p1$plot, p2$plot, p1t, p2t,
        labels = c("A", "B"),
        heights = c(1.5, 0.5),
        align = "v",
        ncol = 2, nrow = 2)

#Legend text
Fig2_legend_text <- ggparagraph(text = paste("Figure 2 - Kaplan Meier plot of cer18/cer24:0 ratio and LD

ggarrange(Fig2_km_plots, Fig2_legend_text,
        ncol = 1,
```

```
    heights = c(1, 0.2))
```

A

B
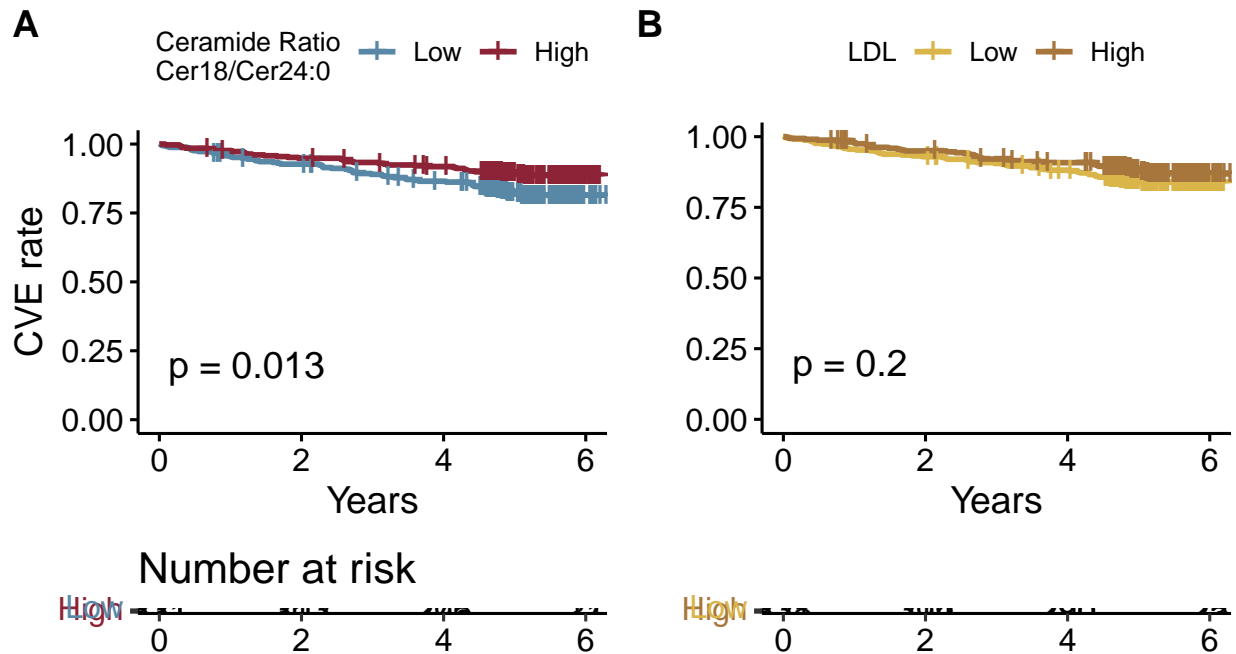
Figure 2 – Kaplan Meier plot of cer18/cer24:0 ratio and LDL against CVE. High is metabolite level greater than or equal to the median, low is individuals with a metabolite level below the median.

```
#Export ratio 6x10 with legend
#Export ratio 5x10 without

# #Extract Risks
# p1$data.survplot[p1$data.survplot$Ratio18_240 == "Below median" & p1$data.survplot$time == 2212,]
# p1$data.survplot[p1$data.survplot$Ratio18_240 == "Above median" & p1$data.survplot$time == 2212,]
#
# p2$data.survplot[p2$data.survplot$Blood_LDL == "Below median" & p2$data.survplot$time == 2212,]
# p2$data.survplot[p2$data.survplot$Blood_LDL == "Above median" & p2$data.survplot$time == 2212,]

rm(surv_object, data_bin, KM_fit, p1, p1t, p2, p2t, Fig2_km_plots, Fig2_legend_text)

library(ggcorrplot)
library(Hmisc)


## Warning: package 'Hmisc' was built under R version 4.2.1


## Loading required package: lattice


## Loading required package: Formula


##
## Attaching package: 'Hmisc'
```

```
## The following objects are masked from 'package:dplyr':
##
##     src, summarize


## The following objects are masked from 'package:base':
##
##     format.pval, units

#prepare data frame
tmp_cor <- data %>%
    select_if(., is.numeric) %>%
    select(!contains("censor")) %>%
    select(!contains("retino")) %>%
    select(-c(Height, Weight, t_komb_nyre_endepunkt_p, t_alb_prog, t_gfrfald30_p, CB)) %>%
    #select(-c(hsCRP, Cal_DIA, CB,)) %>%
    #select(-c(Target_20, Target_22, Target_24_1,
    #         `Ratio 16/24:0`, `Ratio 18/24:0`, `Ratio 20/24:0`, `Ratio 22/24:0`)) %>%
    rename_with( ~gsub("Target_", "Cer_", .)) %>%
    rename_with( ~gsub(" ", "_", .)) %>%
    rename_with( ~gsub(":", "_", .)) %>%
    rename_with( ~gsub("/", "_", .)) %>%
    mutate(across(everything(), ~ if_else(is.na(.x), median(., na.rm = TRUE), .)))

#Calculate correlation matrix
tmp_cor <- rcorr(as.matrix(tmp_cor), type = "pearson")

#extract correlation coeffiecints and p-values
tmp1 <- tmp_cor$r

#set NA p-values to 0
tmp_cor$P[is.na(tmp_cor$P)] <- 0
tmp2 <- tmp_cor$P

#Clean names, !!NOTE VULNERABLE TO CHANGES!!
tmp_names <- c("Cer16", "Cer18", "Cer20", "Cer22", "Cer24:0", "Cer24:1",
               "Ratio Cer16/Cer24:0", "Ratio Cer18/Cer24:0", "Ratio Cer20/Cer24:0",
               "Ratio Cer22/Cer24:0", "Ratio Cer24:1/Cer24:0", "Age",
               "Diabetes Duration", "BMI", "log(UAER)",
               "HbA1C", "Hemoglobin", "Cholesterol", "HDL", "LDL", "VLDL", "Creatinine",
               "Triglyceride", "eGFR", "hsCRP", "Systolic Blood Pressure",
               "Diastolic Blood Pressure", "Insulin Dose", "Mortality",
               "CVE", "ESRD")

colnames(tmp1) <- rownames(tmp1) <- rownames(tmp2) <- colnames(tmp2) <- tmp_names

#plot
ggcorrplot(tmp1,
           hc.order = FALSE,
           outline.col = "#F2EFE9",
           p.mat = tmp2,
           sig.level = 0.05,
           insig = "blank",
           lab = TRUE,
```

```r
        ggtheme = ggplot2::theme_minimal,
        colors = c("#5888A6", "white", "#8C2336"))
```



```r
#export size 15x15



#Subset plot for of Cer18/cer24:0 and CVD confounders only
subset_vars <- c("Age",
            "Diabetes Duration", "BMI", "log(UAER)",
            "HbA1C", "LDL",  "Triglyceride", "eGFR",
            "Systolic Blood Pressure")

tmp3 <- tmp1[subset_vars, grepl("Cer", colnames(tmp1))]
tmp3 <- tmp3[,rev(colnames(tmp3))]


tmp4 <- tmp2[subset_vars, grepl("Cer", colnames(tmp2))]
tmp4 <- tmp4[,rev(colnames(tmp4))]


#Ceramides vs. CVD confounders
Fig3_correlation_matrix <- ggcorrplot(tmp3,
        p.mat = tmp4, insig = "blank",
        colors = c("#5888A6", "white", "#8C2336"))


#Legend text
Fig3_legend_text <- ggparagraph(text = paste("Figure 3 - Heatmap of ceramides correlation to possible CV
```

```
ggarrange(Fig3_correlation_matrix, Fig3_legend_text,
          ncol = 1,
          heights = c(1, 0.2))
```
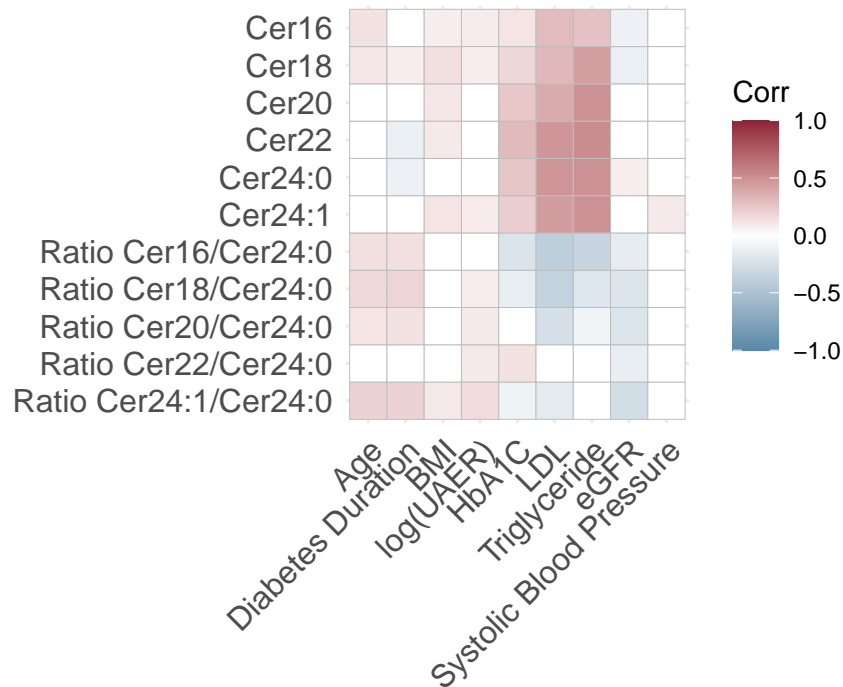

Figure 3 – Heatmap of ceramides correlation to possible CVE confounders, presented as correlations coefficients from Pearson's correlation.

```
#export 7.1x7.1 with legend
#export size 6x6 without

# #Ratio Cer18/Cer24:0 vs confounder
# tmp3 <- as.data.frame(tmp1[subset_vars, "Ratio Cer18/Cer24:0"])
# colnames(tmp3) <- "Ratio Cer18/Cer24:0"
#
# ggcorrplot(tmp3, method = "circle",
#            colors = c("#5888A6", "white", "#8C2336"))

#export size 6x6

rm(tmp_cor, tmp1, tmp2, tmp3, tmp4, subset_vars, tmp_names, Fig3_correlation_matrix, Fig3_legend_text)

## Normoalbuminuria - n = 308

#Subset normoalbuminuria group
data_scale_norm <- data_scale %>%
    filter(Albuminuri_3_groups == 1)
```

```
#Survival object
surv_object <- Surv(data_scale_norm$t_cv_komb_profil, as.numeric(as.character(data_scale_norm$cv_komb_p

## 16) CVE - Crude model Normoalbuminuria
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_norm <- data_scale_norm %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale_norm, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Normoalbuminuria")

Cox_overview_norm
```

```
## # A tibble: 11 x 8
##    name            expl_var        coeff conf_low conf_up    pval     fdr model
##    <chr>           <chr>           <dbl>    <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16       Target_16        1.02    0.649    1.60 0.939   0.939   Norm~
##  2 Target_18       Target_18        1.27    0.825    1.96 0.276   0.439   Norm~
##  3 Target_20       Target_20        0.892   0.555    1.44 0.639   0.781   Norm~
##  4 Target_22       Target_22        0.872   0.539    1.41 0.577   0.781   Norm~
##  5 Target_24_0     Target_24_0      0.757   0.457    1.25 0.279   0.439   Norm~
##  6 Target_24_1     Target_24_1      0.950   0.591    1.53 0.831   0.914   Norm~
##  7 Ratio_16/24:0   `Ratio_16/24:0`  1.44    0.948    2.18 0.0874  0.192   Norm~
##  8 Ratio_18/24:0   `Ratio_18/24:0`  1.78    1.23     2.58 0.00230 0.0253  Norm~
##  9 Ratio_20/24:0   `Ratio_20/24:0`  1.50    0.979    2.31 0.0625  0.172   Norm~
## 10 Ratio_22/24:0   `Ratio_22/24:0`  1.61    1.03     2.51 0.0363  0.133   Norm~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.72   1.12     2.65 0.0138  0.0760  Norm~
```

```
rm(data_scale_norm)
```

Microalbuminuria - n = 165

```
#Subset Microalbuminuria group
data_scale_micro <- data_scale %>%
    filter(Albuminuri_3_groups == 3)

#Survival object
surv_object <- Surv(data_scale_micro$t_cv_komb_profil, as.numeric(as.character(data_scale_micro$cv_komb

## 17) CVE - Crude model Microalbuminuria
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_micro <- data_scale_micro %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale_micro, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Microalbuminuria")
```

```
Cox_overview_micro
```

```
## # A tibble: 11 x 8
##    name            expl_var        coeff conf_~1 conf_up    pval     fdr model
##    <chr>           <chr>           <dbl>   <dbl>   <dbl>   <dbl>   <dbl> <chr>
##  1 Target_16       Target_16        1.54   1.08     2.21 1.82e-2 0.0667  Micr~
##  2 Target_18       Target_18        1.80   1.31     2.48 2.99e-4 0.00329 Micr~
##  3 Target_20       Target_20        1.30   0.923    1.84 1.33e-1 0.269   Micr~
##  4 Target_22       Target_22        1.26   0.887    1.79 1.97e-1 0.310   Micr~
##  5 Target_24_0     Target_24_0      1.30   0.928    1.82 1.27e-1 0.269   Micr~
##  6 Target_24_1     Target_24_1      1.54   1.14     2.08 4.73e-3 0.0260  Micr~
##  7 Ratio_16/24:0   `Ratio_16/24:0`  1.06   0.760    1.48 7.28e-1 0.801   Micr~
##  8 Ratio_18/24:0   `Ratio_18/24:0`  1.20   0.875    1.63 2.62e-1 0.360   Micr~
##  9 Ratio_20/24:0   `Ratio_20/24:0`  1.01   0.717    1.42 9.61e-1 0.961   Micr~
## 10 Ratio_22/24:0   `Ratio_22/24:0`  0.892  0.630    1.26 5.20e-1 0.635   Micr~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 1.25  0.926    1.68 1.47e-1 0.269   Micr~
## # ... with abbreviated variable name 1: conf_low
```

```
rm(data_scale_micro)
```

```
## Macroalbuminuria - n = 189

#Subset Microalbuminuria group
data_scale_macro <- data_scale %>%
    filter(Albuminuri_3_groups == 4)

#Survival object
surv_object <- Surv(data_scale_macro$t_cv_komb_profil, as.numeric(as.character(data_scale_macro$cv_komb

## 18) CVE - Crude model Macroalbuminuria
#Use Cox_extract function across data that starts with Target or Ratio, pivot into a table
Cox_overview_macro <- data_scale_macro %>%
    rename_with(~gsub("Ratio ", "Ratio_", .), starts_with("Ratio")) %>%
    summarise(across(starts_with("Target") | starts_with("Ratio"),
        ~ Cox_extract(Data = data_scale_macro, Formula = surv_object ~ .))) %>%
    pivot_longer(cols = everything()) %>%
    unnest(cols = everything()) %>%
    mutate("fdr" = p.adjust(pval, method = "fdr")) %>%
    mutate("model" = "Macroalbuminuria")

Cox_overview_macro
```

```
## # A tibble: 11 x 8
##    name          expl_var        coeff conf_low conf_up   pval    fdr model
##    <chr>         <chr>           <dbl>    <dbl>   <dbl>  <dbl>  <dbl> <chr>
##  1 Target_16     Target_16        1.30    0.996    1.68 0.0536 0.590 Macroa~
##  2 Target_18     Target_18        1.11    0.851    1.44 0.451  0.879 Macroa~
##  3 Target_20     Target_20        1.03    0.781    1.37 0.812  0.879 Macroa~
##  4 Target_22     Target_22        1.07    0.809    1.42 0.629  0.879 Macroa~
##  5 Target_24_0   Target_24_0      1.11    0.832    1.48 0.477  0.879 Macroa~
##  6 Target_24_1   Target_24_1      1.09    0.823    1.44 0.549  0.879 Macroa~
##  7 Ratio_16/24:0 `Ratio_16/24:0`  1.02    0.767    1.36 0.879  0.879 Macroa~
##  8 Ratio_18/24:0 `Ratio_18/24:0`  0.956   0.710    1.29 0.769  0.879 Macroa~
```

```
## 9 Ratio_20/24:0    `Ratio_20/24:0`   0.897    0.669     1.20 0.467  0.879 Macroa~
## 10 Ratio_22/24:0    `Ratio_22/24:0`   0.908    0.681     1.21 0.509  0.879 Macroa~
## 11 Ratio_24:1/24:0 `Ratio_24:1/24:0` 0.971    0.715     1.32 0.848  0.879 Macroa~
```

```r
rm(data_scale_macro)


#Combine the 3 albuminuria groups into a single table
Cox_overview_albuminuria <- Cox_overview_norm %>%
    rbind(., Cox_overview_micro, Cox_overview_macro)

rm(Cox_overview_norm, Cox_overview_micro, Cox_overview_macro)

#Plot overview table as a Forest Plot
Fig4_forest_plot_albuminuria <- Cox_overview_albuminuria %>%
    mutate(name = gsub("Target_", "Cer ", name)) %>%
    mutate(name = gsub("Ratio_", "Ratio Cer ", name)) %>%
    mutate(name = gsub("_", ":", name)) %>%
    mutate(name = factor(name, levels = rev(unique(name)))) %>%
    mutate(model = factor(model,
                          levels = c("Normoalbuminuria",
                                     "Microalbuminuria",
                                     "Macroalbuminuria"),
                          labels = c("Normoalbuminuria",
                                     "Moderately Increased",
                                     "Severely Increased"))) %>%
    # mutate(outcome = factor(outcome, levels = c("CVE", "ESKD", "Mortality"),
    #                  labels = c("Cardiovascular Events",
    #                             "Kidney Disease",
    #                             "All-Cause Mortality"))) %>%
    mutate(Significance = if_else(pval < 0.05, "p < 0.05", "None")) %>%
    mutate(Significance = if_else(pval < 0.001, "p < 0.001", Significance)) %>%
    mutate(Significance = factor(Significance, levels = c("None", "p < 0.05", "p < 0.001"))) %>%
    ggplot(aes(x = coeff, y = name, color = Significance)) +
        geom_errorbar(aes(xmin = conf_low, xmax = conf_up), width = 0.5, size = 0.8) +
        geom_pointrange(aes(xmin = conf_low, xmax = conf_up)) +
        geom_vline(xintercept = 1, linetype = "dashed") +
        scale_x_log10() +
        scale_color_manual(values = c("black", "#D9B54A", "#8C2336")) +
        facet_grid( ~ model) +
        xlab(label = "CVE Hazard Ratio") +
        theme_bw() +
        theme(axis.title.y = element_blank(),
              legend.position = "top",
              strip.background = element_rect(colour="black",
                                              fill="#F2EFE9"))

#Legend text
Fig4_legend_text <- ggparagraph(text = paste("Figure 4 - Forest plot of hazard ratios for ceramide and

ggarrange(Fig4_forest_plot_albuminuria, Fig4_legend_text,
          ncol = 1,
          heights = c(1, 0.2))
```
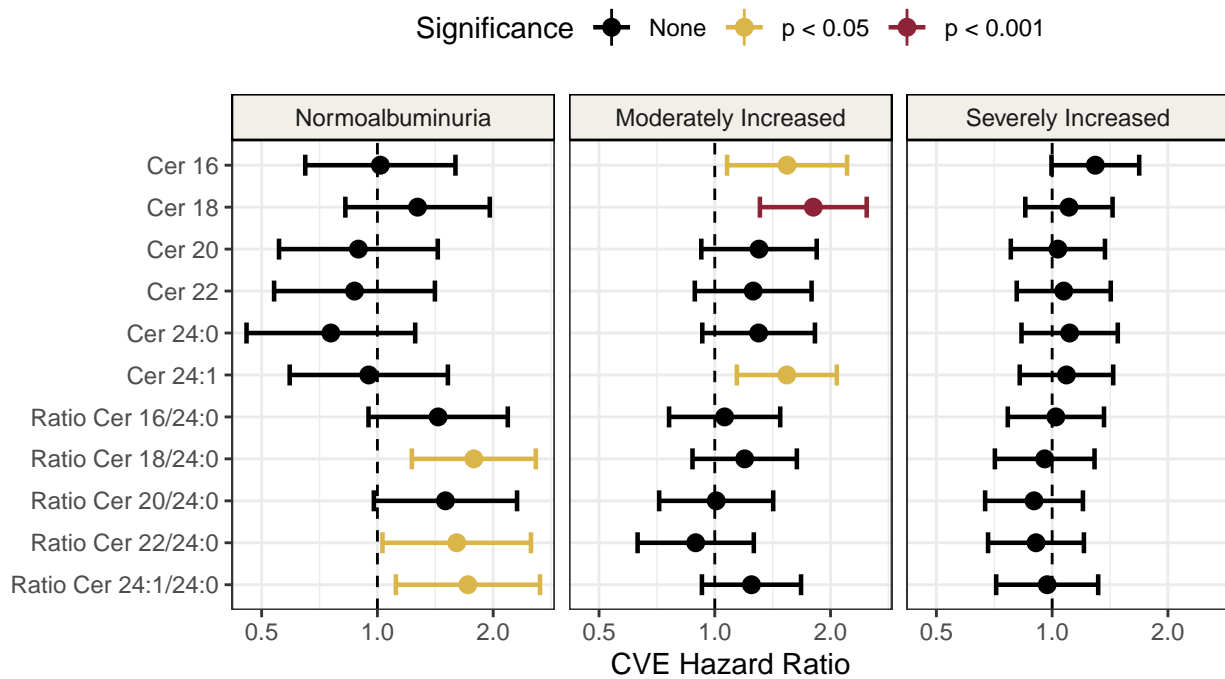
Figure 4 – Forest plot of hazard ratios for ceramide and ratios for cardiovascular events separated by albuminuria status. Normoalbuminuria is defined as <30 mg/g, moderately increased is between 30–299 mg/g and severely increased is >=300 mg/g. These models are unadjusted crude models. Hazard ratios (HRs) are reported per doubling of the log10 ceramide.

```
#export at 4.5X7.1 with legend
#export at 3x6 without

#write supplementary table
#write.csv(Cox_overview_albuminuria, here("data/Cox_overview_albuminuria.csv"))

rm(surv_object, Cox_clinical_overview, Cox_overview, Cox_overview_albuminuria,
   Fig4_forest_plot_albuminuria, Fig4_legend_text)

#Scatterplot UAER and eGFR
data %>%
    select(Target_18, Target_24_0, `Ratio 18/24:0`,
           Albuminuri_3_groups, cv_komb_profil) %>%
    mutate(Albuminuri_3_groups = factor(Albuminuri_3_groups,
                                  labels = c("Normoalbuminuria",
                                             "Moderately Increased",
                                             "Severely Increased"))) %>%
    mutate(cv_komb_profil = factor(cv_komb_profil,
                                  labels = c("No CVE", "CVE"))) %>%
    pivot_longer(cols = starts_with("Target") | starts_with("Ratio"),
                 names_to = "Cer_Name", values_to = "Cer_val") %>%
    mutate(Cer_Name = gsub("Target_", "Cer", Cer_Name)) %>%
    mutate(Cer_Name = gsub("/24", "/Cer24", Cer_Name)) %>%
    mutate(Cer_Name = gsub("_", ":", Cer_Name)) %>%
    mutate(Cer_Name = gsub("Ratio ", "Ratio Cer", Cer_Name)) %>%
    ggplot(aes(x = cv_komb_profil, y = Cer_val , fill = cv_komb_profil)) +
```
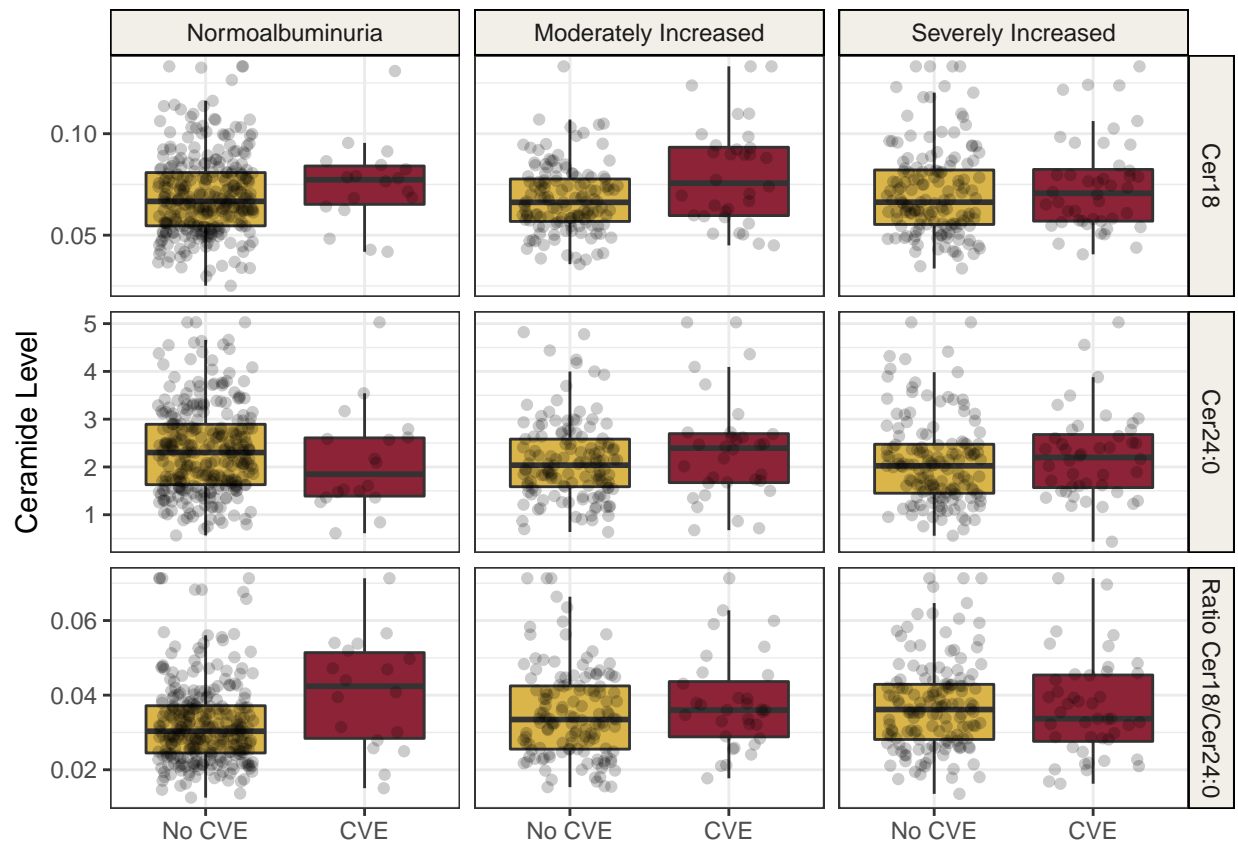
```
geom_boxplot(outlier.shape = NA) +
geom_jitter(position = position_jitter(0.3),
            alpha = 0.2) +
scale_fill_manual(values = c("#D9B54A", "#8C2336"))+
facet_grid(Cer_Name ~ Albuminuri_3_groups, scales = "free")+
ylab("Ceramide Level")+
theme_bw()+
theme(axis.title.x=element_blank(),
      legend.position="none",
      strip.background = element_rect(colour="black",
                                      fill="#F2EFE9"))
```


#export as 7x7