EDA Jorick Baron

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```
library(tidyr)
library(ggplot2)
library(gridExtra)
library(stringr)
library(knitr)
library(kableExtra)
library(e1071)
library(foreign)
```

Research question

How accurate can a model be trained to detect the difficult to diagnose pancreatic cancer utilising a patient's urine sample?

Codebook

In this EDA we will explore the data downloaded from $\underline{\text{here}}$. For future reference we will describe the data in the codebook below.

```
codebook <- read.delim("Data/codebook.csv", sep = ",")
kable(codebook, caption = "Table 1: Codebook", align = "lcccr", booktabs = T) %>%
kable_styling(latex_options = c("scale_down"))
```

Table 1: Table 1: Codebook

Name	Fullname	Description	Type	Unit
sample_id	Sample ID	Unique string identifying each subject	string	NA
patient_cohort	Patient's Cohort	Cohort 1, previously used samples; Cohort 2, newly added samples	string	NA
sample_origin	Sample Origin	BPTB: Barts Pancreas Tissue Bank; ESP: Spanish National Cancer Research Centre; LIV: Liverpool University; UCL: University College	string	NA
age	Age	Age in years	int	years
sex	Sex	M = male, F = female	char	NA
diagnosis	Diagnosis (1=Control, 2=Benign, 3=PDAC)	1 = control, 2 = benign hepatobiliary disease; 3 = Pancreatic ductal adenocarcinoma, i.e. pancreatic cancer	int	NA
stage	Stage	For those with pancratic cancer, what stage was it? One of IA, IB, IIA, IIIB, III, IV	string	NA
benign_sample_diagnosis	Benign Samples Diagnosis	For those with a benign, non-cancerous diagnosis, what was the diagnosis?	string	NA
plasma_CA19_9	Plasma CA19-9 U/ml	Blood plasma levels of CA 19–9 monoclonal antibody that is often elevated in patients with pancreatic cancer.	float	plasma units/milliliter
creatinine	Creatinine mg/ml	Urinary biomarker of kidney function	float	mg/ml
LYVE1	LYVE1 ng/ml	Urinary levels of Lymphatic vessel endothelial hyaluronan receptor 1, a protein that may play a role in tumor metastasis	float	ng/ml
REG1B	REG1B ng/ml	Urinary levels of a protein that may be associated with pancreas regeneration.	float	ng/ml
TFF1	TFF1 ng/ml	Urinary levels of Trefoil Factor 1, which may be related to regeneration and repair of the urinary tract	float	ng/ml
REG1A	REG1A ng/ml	Urinary levels of a protein that may be associated with pancreas regeneration.	float	ng/ml

Loading data

First we will load in the data and to check if it has loaded in properly we look at the structure of the loaded data.

```
'data.frame':
                    590 obs. of
                                 14 variables:
                                     "S1" "S10" "S100" "S101" ...
    $ sample_id
                              : chr
    $ patient_cohort
                               chr
                                     "Cohort1" "Cohort1" "Cohort2" "Cohort2" ...
##
                                     "BPTB" "BPTB" "BPTB" ...
    $ sample origin
                                chr
##
    $ age
                                int
                                     33 81 51 61 62 53 70 58 59 56 ...
                                     "F" "F" "M" "M" ...
##
    $ sex
                                chr
##
    $ diagnosis
                                     1 1 1 1 1 1 1 1 1 1 ...
                               int
##
    $ stage
                                chr
                                     NA NA NA NA ...
##
    $ benign_sample_diagnosis: chr
                                     NA NA NA NA ...
##
    $ plasma CA19 9
                               num
                                     11.7 NA 7 8 9 NA NA 11 NA 24 ...
    $ creatinine
                                     1.832 0.973 0.78 0.701 0.215 ...
##
                               num
    $ LYVE1
                                     0.89322 2.03758 0.14559 0.0028 0.00086 ...
##
                                num
##
    $ REG1B
                               num
                                     52.9 94.5 102.4 60.6 65.5 ...
##
    $ TFF1
                                     654.3 209.5 461.1 142.9 41.1 ...
                               num
    $ REG1A
                                     1262 228 NA NA NA ...
##
                               num
```

Thus far it seems to have loaded correctly.

We will also check the first few records to maybe catch some possible errors.

head(data)

```
##
     sample_id patient_cohort sample_origin age sex diagnosis stage
## 1
             S1
                       Cohort1
                                          BPTB
                                                33
                                                      F
                                                                    <NA>
## 2
           S10
                       Cohort1
                                          BPTB
                                                81
                                                      F
                                                                    <NA>
                                                                 1
## 3
          S100
                       Cohort2
                                          BPTB
                                                51
                                                                    <NA>
## 4
          S101
                                          BPTB
                                                                    <NA>
                       Cohort2
                                                61
                                                      М
                                                                 1
## 5
          S102
                       Cohort2
                                          BPTB
                                                62
                                                      M
                                                                    <NA>
## 6
          S103
                       Cohort2
                                          BPTB
                                                53
                                                      М
                                                                 1
                                                                    <NA>
##
     benign_sample_diagnosis plasma_CA19_9 creatinine
                                                               LYVE1
## 1
                          <NA>
                                         11.7
                                                 1.83222 0.89321920
                                                                       52.94884
## 2
                          <NA>
                                                 0.97266 2.03758500
                                                                       94.46703
                                           NA
## 3
                                                 0.78039 0.14558890 102.36600
                          <NA>
                                          7.0
## 4
                          <NA>
                                          8.0
                                                 0.70122 0.00280488
                                                                       60.57900
## 5
                          <NA>
                                          9.0
                                                 0.21489 0.00085956
                                                                       65.54000
## 6
                          <NA>
                                           NA
                                                 0.84825 0.00339300
                                                                       62.12600
##
         TFF1
                  REG1A
## 1 654.2822 1262.000
## 2 209.4882
                228.407
## 3 461.1410
                     NA
## 4 142.9500
                     NA
## 5
      41.0880
                     NA
## 6
      59.7930
```

The data seems to have quite a few NAs, reading further into the description most NAs would be expected i.e. no stage if there is no cancer thus an NA.

NAs

```
Let's check that nothing went wrong with those two anyway.
```

[1] "all these numbers should be the same number 183 183 183"

Those numbers lined up to expectations.

The NAs in columns "plasma_CA19_9" and "REG1A" are supposed to be there because not every patient had been fully tested:

"REG1A ... Only assessed in 306 patients", "plasma_CA19_9 ... Only assessed in 350 patients" see Debernardi et al 2020 documentation.csv in the source files.

However to make sure everything is correct these numbers will be tested.

```
n_plasma_CA19_9 <- nrow(data) - sum(is.na(data$plasma_CA19_9))
n_REG1A <- nrow(data) - sum(is.na(data$REG1A))
paste("REG1A:", n_REG1A, "plasma_CA19_9:", n_plasma_CA19_9)</pre>
```

[1] "REG1A: 306 plasma_CA19_9: 350"

These numbers are correct.

Are there more NAs?

```
sum(is.na(data[, c(1:6, 10:13)]))
```

[1] 0

0 NAs remaining.

Data exploration

Distribution

Class label checking the different diagnoses should be in similar number to each diagnosis.

```
paste("Amount of patients with diagnosis 1:", nrow(subset(data, diagnosis == 1)))
```

```
## [1] "Amount of patients with diagnosis 1: 183"
```

```
paste("Amount of patients with diagnosis 2:", nrow(subset(data, diagnosis == 2)))
```

```
## [1] "Amount of patients with diagnosis 2: 208"
```

```
paste("Amount of patients with diagnosis 3:", nrow(subset(data, diagnosis == 3)))
```

[1] "Amount of patients with diagnosis 3: 199"

These are quite balanced and should not influence statistics.

Let's look at a summary of the data for a quick overview of the distributions.

```
summary(data[,c(4, 9:14)])
```

```
plasma_CA19_9
                                      creatinine
                                                         LYVE1
##
        age
##
                  Min. : 0.0
                                          :0.05655
                                                     Min. : 0.000129
   Min.
         :26.00
                                  Min.
   1st Qu.:50.00
                  1st Qu.:
                              8.0
                                   1st Qu.:0.37323
                                                     1st Qu.: 0.167179
## Median :60.00
                  Median :
                             26.5
                                   Median :0.72384
                                                     Median: 1.649862
##
  Mean :59.08
                  Mean : 654.0
                                  Mean
                                          :0.85538
                                                     Mean : 3.063530
                                                     3rd Qu.: 5.205037
   3rd Qu.:69.00
                                   3rd Qu.:1.13948
##
                  3rd Qu.: 294.0
##
  Max.
          :89.00
                         :31000.0
                                          :4.11684
                                                           :23.890323
                  Max.
                                   Max.
                                                     Max.
##
                   NA's
                         :240
##
       REG1B
                           TFF1
                                             R.F.G1A
##
  Min. : 0.0011
                      Min.
                                 0.005
                                                     0.00
   1st Qu.: 10.7572
                                 43.961
##
                      1st Qu.:
                                          1st Qu.:
                                                    80.69
                                                   208.54
##
   Median: 34.3034
                      Median :
                                259.874
                                         Median :
## Mean
         : 111.7741
                             : 597.869
                                         Mean
                                                : 735.28
                      Mean
   3rd Qu.: 122.7410
                       3rd Qu.: 742.736
                                          3rd Qu.: 649.00
##
  Max. :1403.8976
                      Max.
                             :13344.300
                                          Max.
                                                :13200.00
##
                                          NA's
                                                :284
```

Much of the data seems to be imbalanced with outliers.

Now let's take a closer look at the data itself using box-plots.

```
p1<- ggplot(data=data)+
  geom_boxplot(mapping = aes(x = "",
                            y = age))+
  ylab("age in years") +
  xlab(NULL)
p2<- ggplot(data=data)+
  geom_boxplot(mapping = aes(x = "",
                            y = plasma_CA19_9))+
  ylab("plasma_CA19_9 in U/ml") +
  xlab(NULL)+
  ylim(0,500)
p3<- ggplot(data=data)+
  geom_boxplot(mapping = aes(x = "",
                            y = creatinine))+
  ylab("creatinine in mg/ml") +
  xlab(NULL)
p4<- ggplot(data=data)+
  geom_boxplot(mapping = aes(x = "",
                            y = LYVE1))+
  ylab("LYVE1 in ng/ml") +
  xlab(NULL)+
  ylim(0,17)
p5<- ggplot(data=data)+
  geom_boxplot(mapping = aes(x = "",
                            y = REG1B))+
  ylab("REG1B in ng/ml") +
  xlab(NULL)+
  ylim(0,600)
```

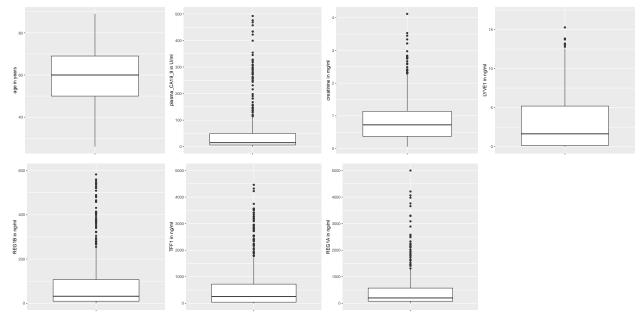


Figure 1: boxplots of different values

There are many outliers to take a good look at the whiskers y-limits are in place. Still it's a lot, maybe adding another dimension can correct this.

To add this extra dimension let's look at the difference in diagnoses. To properly do this we will also assign levels to the diagnosis column in the dataframe.

```
"pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("age in years")
gp2 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
                             y = plasma_CA19_9,
                             group=diagnosis,
                             fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("plasma_CA19_9 in U/ml")+
  ylim(0,500)
gp3 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
                             y = creatinine,
                             group=diagnosis,
                             fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("creatinine in mg/ml")
gp4 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
                             y = LYVE1,
                             group=diagnosis,
                             fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("LIVE1 in ng/ml")+
 ylim(0,17)
gp5 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
                             y = REG1B,
                             group=diagnosis,
                             fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("REG1B in ng/ml")+
 ylim(0,600)
gp6 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
```

```
y = TFF1,
                             group=diagnosis,
                             fill=diagnosis))+
  scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
 xlab("diagnosis")+
  ylab("TFF1 in ng/ml")+
 ylim(0,5000)
gp7 <- ggplot(data = data)+</pre>
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = REG1A,
                            group=diagnosis,
                            fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                             "benign hepatobiliary disease",
                             "pancreatic cancer"))+
 xlab("diagnosis")+
 ylab("REG1A in ng/ml")+
 ylim(0,5000)
grid.arrange(gp1, gp2, gp3, gp4, gp5, gp6, gp7, nrow = 3)
```

Figure 2: boxplots with added dimension (diagnosis)

The data still has many outliers but by many columns a pattern does emerge.

Log transformation

Let's use statistical tests to test the skewness to see how imbalanced the data is.

```
skewness(data$age)
## [1] -0.2157312
skewness(data$plasma_CA19_9, na.rm = T)

## [1] 7.950382
skewness(data$creatinine)
## [1] 1.458965
skewness(data$LYVE1)
## [1] 1.386933
skewness(data$REG1B)
## [1] 3.316992
skewness(data$LYVE1)
## [1] 1.386933
skewness(data$LYVE1)
```

[1] 4.425404

Here we see that everything is skewed greatly except age.

A way of dealing with this skewness is to apply a log transformation on the data due to the high positively skewed data.

```
trans <- as.data.frame(log(data$plasma_CA19_9))
names(trans) <- "plasma_CA19_9"
trans$creatinine <- log(data$creatinine)
trans$LYVE1 <- log(data$LYVE1)
trans$REG1B <- log(data$REG1B)
trans$TFF1 <- log(data$TFF1)
trans$REG1A <- log(data$REG1A + 1)
trans$diagnosis <- data$diagnosis
head(trans)</pre>
```

```
plasma_CA19_9 creatinine
                                       REG1B
##
                               LYVE1
                                                TFF1
                                                       REG1A diagnosis
## 1
        1
## 2
             NA -0.02772069 0.7117653 4.548251 5.344668 5.435498
                                                                   1
## 3
        1.945910 -0.24796148 -1.9269684 4.628555 6.133704
                                                         NA
                                                                   1
## 4
        2.079442 -0.35493360 -5.8763945 4.103948 4.962495
                                                         NA
                                                                   1
        2.197225 -1.53762901 -7.0590899 4.182661 3.715716
## 5
                                                         NA
                                                                   1
              NA -0.16457988 -5.6860408 4.129165 4.090889
                                                                   1
```

Now having transformed the data lets see how this influences the distribution.

```
xlab("diagnosis")+
  ylab("plasma_CA19_9 in U/ml (log transformed)")
tgp2 <- ggplot(data = trans)+
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = creatinine,
                            group=diagnosis,
                            fill=diagnosis))+
   scale_x_discrete(labels=c("control",
                            "benign hepatobiliary disease",
                            "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("creatinine in mg/ml (log transformed)")
tgp3 <- ggplot(data = trans)+
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = LYVE1,
                            group=diagnosis,
                            fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                            "benign hepatobiliary disease",
                            "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("LIVE1 in ng/ml (log transformed)")
tgp4 <- ggplot(data = trans)+
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = REG1B,
                            group=diagnosis,
                            fill=diagnosis))+
    scale_x_discrete(labels=c("control",
                            "benign hepatobiliary disease",
                            "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("REG1B in ng/ml (log transformed)")
tgp5 <- ggplot(data = trans)+
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = TFF1,
                            group=diagnosis,
                            fill=diagnosis))+
  scale_x_discrete(labels=c("control",
                            "benign hepatobiliary disease",
                            "pancreatic cancer"))+
  xlab("diagnosis")+
  ylab("TFF1 in ng/ml (log transformed)")
tgp6 <- ggplot(data = trans)+
  geom_boxplot(mapping = aes(x = diagnosis,
                            y = REG1A,
                            group=diagnosis,
                            fill=diagnosis))+
    scale_x_discrete(labels=c("control",
```

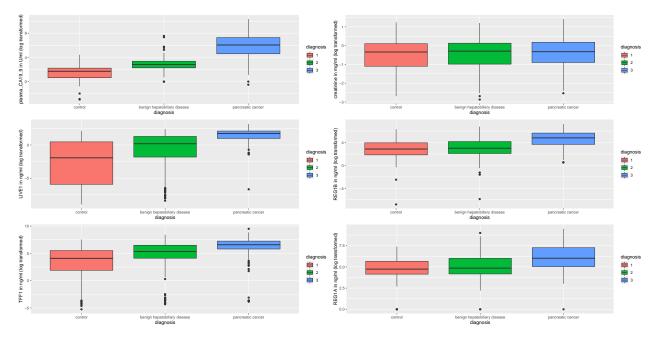


Figure 3: boxplots of transformed values

This data looks more normalized than before.

However it's good practice to test normality after transformations.

shapiro.test(trans\$plasma_CA19_9)

```
##
    Shapiro-Wilk normality test
##
##
## data: trans$plasma_CA19_9
## W = NaN, p-value = NA
qq1 <- ggplot(trans, aes(sample = plasma_CA19_9, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("plasma_CA19_9")
shapiro.test(trans$creatinine)
##
##
    Shapiro-Wilk normality test
##
## data: trans$creatinine
## W = 0.98211, p-value = 1.254e-06
```

```
qq2 <- ggplot(trans, aes(sample = creatinine, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("creatinine")
shapiro.test(trans$LYVE1)
##
##
   Shapiro-Wilk normality test
##
## data: trans$LYVE1
## W = 0.81496, p-value < 2.2e-16
qq3 <- ggplot(trans, aes(sample = LYVE1, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("LYVE1")
shapiro.test(trans$REG1B)
##
   Shapiro-Wilk normality test
##
## data: trans$REG1B
## W = 0.9695, p-value = 9.888e-10
qq4 <- ggplot(trans, aes(sample = REG1B, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("REG1B")
shapiro.test(trans$TFF1)
## Shapiro-Wilk normality test
##
## data: trans$TFF1
## W = 0.82765, p-value < 2.2e-16
qq5 <- ggplot(trans, aes(sample = TFF1, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("TFF1")
shapiro.test(trans$REG1A)
    Shapiro-Wilk normality test
##
## data: trans$REG1A
## W = 0.97007, p-value = 5.564e-06
qq6 <- ggplot(trans, aes(sample = REG1A, colour = diagnosis)) +
  stat_qq() +
  stat_qq_line() +
  ggtitle("REG1A")
```

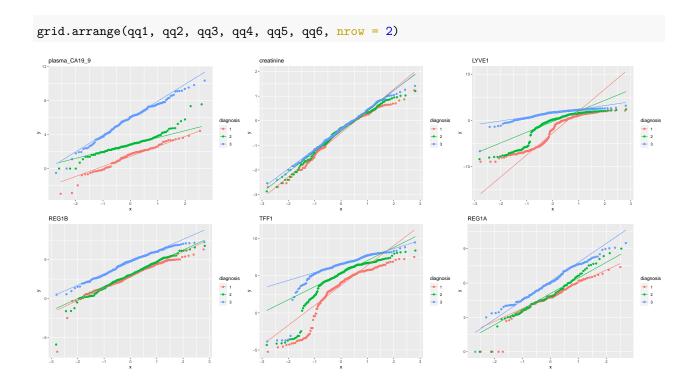


Figure 4: qqplots displaying normalcy

The data is despite the transformation still not fully normalised however we can still continue but this should be kept this in mind in case of future problems.

Correlations

Now using the transformed data let's create a new dataframe.

```
new_data <- cbind(data[3:6], trans[2:6])</pre>
```

Using the new dataframe let's explore if the data is correlated.

```
matrix_data <- drop_na(new_data[,c(2, 5:9)])
cor_matrix <- cor(matrix_data)
heatmap(cor_matrix, scale = "column", col = heat.colors(5, rev = T))
legend(x="right", legend=c("full", "strong", "medium", "minimal", "none"),fill=heat.colors(5))
title(main = "Heatmap depicting correlations")</pre>
```

REG1 A and B seem moderately correlated (0.7641084), otherwise no real strong correlation is observed.

Now we also should check if any variable is seemingly influential for the diagnosis so we can see later if the machine learning picks up on this.

```
new_data$has_cancer <- ifelse(new_data$diagnosis == 3, 1, 0)
new_data$has_cancer <- factor(new_data$has_cancer)

t.test(new_data$age ~ new_data$has_cancer)

##
## Welch Two Sample t-test</pre>
```

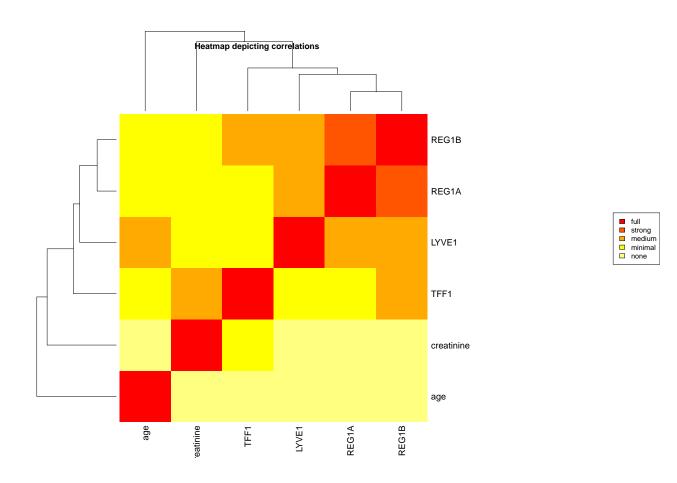


Figure 5: heatmap displaying correlation of values

```
##
## data: new_data$age by new_data$has_cancer
## t = -10.846, df = 473.94, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -12.656785 -8.774077
## sample estimates:
## mean in group 0 mean in group 1
          55.46547
                          66.18090
t.test(new_data$creatinine ~ new_data$has_cancer)
## Welch Two Sample t-test
##
## data: new_data$creatinine by new_data$has_cancer
## t = -1.427, df = 411.11, p-value = 0.1543
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.2372607 0.0376788
## sample estimates:
## mean in group 0 mean in group 1
       -0.4793594
                        -0.3795684
t.test(new_data$LYVE1 ~ new_data$has_cancer)
##
## Welch Two Sample t-test
##
## data: new_data$LYVE1 by new_data$has_cancer
## t = -17.495, df = 520.79, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -3.536142 -2.822156
## sample estimates:
## mean in group 0 mean in group 1
##
         -1.746755
                         1.432394
t.test(new_data$REG1B ~ new_data$has_cancer)
##
## Welch Two Sample t-test
##
## data: new_data$REG1B by new_data$has_cancer
## t = -13.059, df = 456.65, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.982107 -1.463572
## sample estimates:
## mean in group 0 mean in group 1
          2.910900
                          4.633739
t.test(new_data$TFF1 ~ new_data$has_cancer)
##
   Welch Two Sample t-test
##
```

```
## data: new_data$TFF1 by new_data$has_cancer
## t = -10.433, df = 532.25, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -2.679849 -1.830573
## sample estimates:
## mean in group 0 mean in group 1
          3.910662
                          6.165872
t.test(new_data$REG1A ~ new_data$has_cancer)
##
##
   Welch Two Sample t-test
##
## data: new_data$REG1A by new_data$has_cancer
## t = -6.923, df = 300.15, p-value = 2.687e-11
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.5725494 -0.8764183
## sample estimates:
## mean in group 0 mean in group 1
                          6.074772
##
          4.850289
```

No p-value except Creatinine seems to be small enough to not be statistically significant. We will expect to see this in the model.

```
finalData <- new_data[,c(2:3, 5:10)]
write.arff(finalData, "Data/data.arff")</pre>
```