PROBABILITY AND STATISTICS

End-of-course summative assessment

Exam Number: B155243
University of Edinburgh

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Introduction

In this assignment, I will be using two datasets of an ongoing research project from two Scandinavian university hospitals.

The two datasets are as follows:

- **Covariates** (covariates.xlsx): This dataset contains basic biodata about each patient and their VAS (scale of pain) at inclusion and 12 months later.
- **Biomarkers** (biomarkers.xlsx): This dataset contains individual measurements of the level of certain proteins (Biomarkers) for each patient and three different timepoints.

To use the two datasets, some basic manipulation had to be done such as renaming columns for better visibility and easier access from the code, validating the datatypes of each column and changing it where necessary and looking for any anomalies in the data.

The table for covariates summarizes as follows:

	PatientID	Age	Sex (1=male, 2=female)	Smoker (1=yes, 2=no)	VAS-at-inclusion	Vas-12months
count	118.00	118.00	118.0	118.00	118.00	116.00
mean	75.45	40.86	1.5	1.67	6.02	3.62
std	43.33	10.14	0.5	0.47	2.66	3.08

We can see that the column names are not very data-friendly and that there is a difference in the number of columns between Vas-12months and the other columns. This difference is due to two missing values in the Vas-12months column (NaN values) as shown below.

PatientID	Age	Sex	Smoker	VAS0	VAS12
42	27	Male	No	6.0	NaN
51	35	Female	Yes	7.5	NaN

The biomarkers dataset has a stray number at the end of the table that is due to a mistake or a human error, so after excluding it, the table summarizes as follows:

	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	CSF-1
count	348.00	347.00	347.00	347.00	347.00	347.00	347.00	347.00	347.00
mean	7.41	11.66	10.67	7.95	3.25	6.47	8.29	8.29	8.54
std	0.96	0.67	0.40	0.95	0.95	0.79	1.25	0.58	0.25

Statistical hypothesis testing

For this part, I have chosen the question: "**Do the levels of biomarkers vary at inclusion between males and females?**"

The answer to this question lies in the comparison between males and females of the mean level of each protein and to test if this difference is significant or not. I find the question interesting because, for each of the eight biomarkers, the observations will be divided into observations for males and observations for females and then compare the difference between the two means. From a biomedical perspective, it's also interesting to explore areas of significant differences between the two sexes and the areas of non-significant or no-differences. The biomarkers dataset contains observations at different timepoints for each individual so for our test we will be using only the observations from the time of inclusion, thus having the value "0weeks" in their Biomarker column.

Our hypothesis would be:

H_0: The level of biomarker {n} at inclusion, does not vary between males and females ⇔ μ-male = μ-female

H_1: The level of biomarker {n} at inclusion, varies between males and females ⇔ μ-male ≠ μ-female

The distributions are thus the samples of male and female patients at inclusion for each biomarker, and the random variable is the sample mean because its value depends on the random sample of patients selected for this research.

We will be using Student's t-test when testing for significant mean differences between the two groups (males and females) as the population's true variance is unknown. (Agrawal, 2019)

Carrying out inference on a mean requires some pre-conditions, on which the accuracy of our methods depends, to be satisfied. The conditions are: **Randomness** of the sample; **Independence** of the individual observations; And **Normality** of the sample. (Khan Academy, n.d.)

The initial assumption that we must make here is that **the sample is random, i.e. the patients were randomly selected.** Though we don't have any information about the data collection mechanism that was employed, it is important to make this assumption to perform the hypothesis test.

The second **condition** for hypothesis testing is **independence**. We also must assume that the individual measurements taken at inclusion for the patients are independent.

Finally, the **third condition of normality** must be checked in two ways: First, by **visually** analyzing the data and its distribution (for each biomarker and sex) and by using a **normality test**. Annexe 1 shows the distribution of biomarkers' levels at inclusion disaggregated by sex. It is clear from these histograms that some of the biomarkers' distributions are either heavily skewed or don't appear to be normally distributed which can be an issue if we were to conduct hypothesis testing using Student's T-test. To numerically verify the non-normality of the distributions, we will use the omnibus K2 statistic of D'Agostino and Pearson's test that combines skew and kurtosis to produce a test of normality. (SciPy.org, n.d.)

		p-value	Test Result (α=0.01)
Biomarker	**Sex**		
CSF-1	**Male**	0.923	Cannot Reject / Is Normal
	Female	0.012	Cannot Reject / Is Normal
CXCL1	**Male**	0.000	Reject / Is not Normal
	Female	0.048	Cannot Reject / Is Normal
CXCL9	**Male**	0.000	Reject / Is not Normal
	Female	0.000	Reject / Is not Normal
IL-18	**Male**	0.675	Cannot Reject / Is Normal
	Female	0.900	Cannot Reject / Is Normal
IL-6	**Male**	0.038	Cannot Reject / Is Normal
	Female	0.000	Reject / Is not Normal
IL-8	**Male**	0.828	Cannot Reject / Is Normal
	Female	0.000	Reject / Is not Normal
OPG	**Male**	0.003	Reject / Is not Normal
	Female	0.237	Cannot Reject / Is Normal
TGF-beta-1	**Male**	0.000	Reject / Is not Normal
	Female	0.051	Cannot Reject / Is Normal
VEGF-A	**Male**	0.092	Cannot Reject / Is Normal
	Female	0.406	Cannot Reject / Is Normal

The above table shows that 7 out of the 18 samples failed the normality test. (The null hypothesis that the distribution is normal was rejected). To deal with the normality issue, a boxplot is generated for each sample. The boxplot in Annexe 2 shows that some samples have outliers that affect both the sample mean and its normality.

To remove the outliers, we use the IQR score method which finds first where the data is majorly situated and then removes any value that is beyond a threshold. (Sharma, 2018)

The IQR score method works as follows:

`Q1 = 25th percentile; Q3 = 75th percentile; IQR = Q3 – Q1; Threshold = 1.5;`

`Outlier < [Q1 - 1.5 * IQR] OR Outlier > [Q3 + 1.5 * IQR]`

Following the removal of the outliers, performing the normality test again gave a better result. Only two samples did not pass the test. Annex 3 shows a table with the test results and Annex 4 and Annex 5 show the histograms and the boxplots of the samples after removing the outliers. Having a sample size large enough (≈ 55 observations/sample) we can now safely conduct the t-test as the three condition are fulfilled.

Now running the t-test gives the following result:

	α	β	power	t-stat	p-value	t-test result
IL-8	0.05	0.902156	0.0978439	-0.646581	0.519261	-
VEGF-A	0.05	0.465889	0.534111	-2.06075	0.0416201	Reject H0
OPG	0.05	0.223169	0.776831	-2.83788	0.00558852	Reject H0
TGF-beta-1	0.05	0.482739	0.517261	-2.02025	0.0456818	Reject H0
IL-6	0.05	0.945143	0.0548574	-0.207751	0.835801	-
CXCL9	0.05	0.909472	0.0905281	0.595527	0.552719	-
CXCL1	0.05	0.209845	0.790155	-2.79027	0.00616716	Reject H0
IL-18	0.05	0.776017	0.223983	1.21358	0.227463	-
CSF-1	0.05	0.267842	0.732158	-2.5896	0.0109716	Reject H0

In **5 out of 9 tests, the null hypothesis is rejected,** which means that with 95% confidence, we infer that **the level of biomarkers at inclusion varies significantly between males and females.**
Since we are running nine consecutive tests, **the probability of making at least one type I error is 37.0%** which is a very high percentage. $P(Type\ I\ error) = 1 - Bin(9, 0.05) = 0.369$
When dealing with multiple t-tests α should be adjusted in a way, so that the probability of observing at least one significant result due to chance remains below our desired significance level. (Goldman, 2008)
One solution to reduce this high probability is to use the Bonferroni correction which divides the significance level α by the number of tests ($\frac{\alpha}{n}$). **By lowering the value of α, it becomes harder to reject the null hypothesis, thus reducing the probability of a type I error.** (Khan Academy, n.d.)
Following this correction on α and as per the following table, we do not reject **any of the hypothesis**, but we notice a large increase in the values of β:

Biomarker	α	β	power	t-stat	p-value	t-test result
IL-8	0.00555556	0.983487	0.0165133	-0.646581	0.519261	-
VEGF-A	0.00555556	0.771724	0.228276	-2.06075	0.0416201	-
OPG	0.00555556	0.530471	0.469529	-2.83788	0.00558852	-
TGF-beta-1	0.00555556	0.784218	0.215782	-2.02025	0.0456818	-
IL-6	0.00555556	0.993445	0.0065553	-0.207751	0.835801	-
CXCL9	0.00555556	0.985322	0.0146781	0.595527	0.552719	-
CXCL1	0.00555556	0.511661	0.488339	-2.79027	0.00616716	-
IL-18	0.00555556	0.943432	0.0565683	1.21358	0.227463	-
CSF-1	0.00555556	0.585287	0.414713	-2.5896	0.0109716	-

β is the probability of a Type II error, i.e. P(Not rejecting a false H_0).

Conclusion

In conclusion, though we did not reject any of the hypothesis when we applied the Bonferroni correction, as a result of decreasing the value α, we have primarily increased the probability of having false-negative tests as a tradeoff. **That is the reason why the Bonferroni correction is criticized for being conservative** (Koehrsen, 2018) **and other corrections are more recommended, such as Holm's Step-Down Procedure which is more powerful.** (Zheng, 2018)

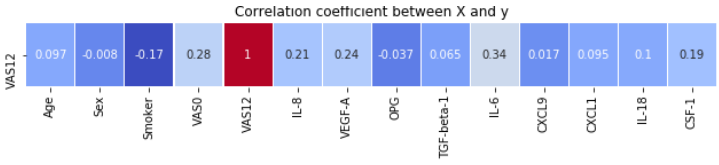
Regression modelling

For the regression modelling, we will use a **linear regression model** with **multiple explanatory variables** and try to fit a line to the data using the **least-squares** method. Our **response variable** is the 12-months VAS.

The explanatory variables used in the model are: Age, Sex, Smoker, VAS0, VAS12, IL-8, VEGF-A, OPG, TGF-beta-1, IL-6, CXCL9, CXCL1, IL-18 & CSF-1

The purpose of the regression model we are fitting to our data is to describe the relationship between the explanatory and the response variables with a linear function and use it to predict values of variables not seen previously by the model then measure how well our model performs (assessing the model fit).

The dataset is divided into two sets, a **testing set** and a **training set**. Observations will be randomly selected and placed in the two sets with a division of 80%/20% for training/test.



The figure on the right shows the correlation between the response variable and the explanatory variables, and it gives a clear indication of the independence of VAS12 from the other variables since the absolute value of the correlation coefficients is relatively small for all variables.

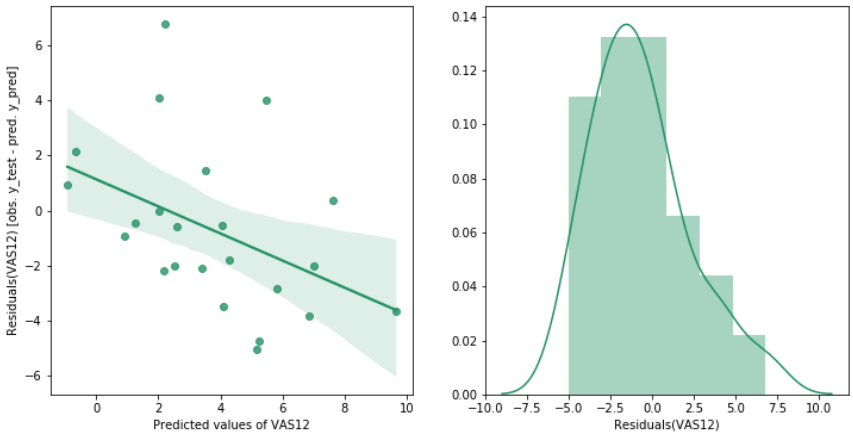
The results of the fitted parameter values are displayed in the table to the right.

The coefficient of determination is R^2=0.365. It implies that 36% of the variability of the response variable has been accounted for, and the remaining 64% of the variability is still unaccounted for. (Wikipedia, n.d.) The coefficient of determination is calculated here based on the result of the training data that is used to fit the model. However, if we recalculate this coefficient on predicted variables from the explanatory variables that are not used while fitting the model (the other 30% of data in the testing set), then the result of R^2 turns out to be much lower, R^2=-0.048. The values of both R^2 are a clear indication that to model is performing very poorly on both known input variables (from the training set) and new input variables (the test set).

Table 1 Parameter Values of the fitted model

	Coef	std err	t	P>|t|
Intercept	-3.2928	10.893	-0.302	0.763
Age	-0.0195	0.034	-0.57	0.571
Sex	-0.3175	0.618	-0.514	0.609
Smoker	-0.1581	0.66	-0.239	0.811
VAS0	0.292	0.114	2.556	**0.013**
IL-8	1.1174	0.62	1.801	0.076
VEGF-A	1.4888	0.761	1.957	0.054
OPG	-2.1262	0.827	-2.572	**0.012**
TGF-beta-1	-1.7945	0.714	-2.515	**0.014**
IL-6	1.2544	0.379	3.306	**0.001**
CXCL9	-0.7432	0.377	-1.972	0.052
CXCL1	0.0864	0.539	0.16	0.873
IL-18	-0.3928	0.543	-0.723	0.472
CSF-1	2.4341	1.462	1.664	0.1

A scatter plot of the residuals and the predicted values of VAS12 shows that the residuals follow a descending pattern as the values of the predicted variable increases which is not a good indicator, and which indicates that there is an existing relationship between them that should not be there. The histogram also indicates that the shape of the distribution of residuals is not normal, and thus, we cannot make an assumption that the errors in the model are normally distributed.



Conclusion

In conclusion, the model is not useful for predicting the 12-month VAS of patients and corrections should be applied to the data to improve the performance of the model.

Possible remedies to improve the model:

If we plot a heatmap of the correlation coefficients between the explanatory variables, we notice that some variables are highly correlated such as CXCL1 with IL-8, VGF-A and TGF-beta-1. These strong correlations can cause the linear regression model's performance to suffer. While we have 9 different predictor variables, some of them appear to be a linear combination of the others, so they don't add any information. If the exact linear relationship holds among more than two variables, we talk about **multicollinearity**. In this case, we can safely remove one of the two variables and try to fit the model again. (Why Collinearity Is a Problem). Another area of improvement could be the standardization of data since it has varying scales, and the regressor can make assumptions about these scales. For instance, age ranges between 18 and 59 while VEGF-A ranges between 10.6 and 13.5 so the regressor can allocate more weight to the age then the biomarker VEGF-A to make a prediction. Finally, the table that describes the parameter values of the fitted model has some rows highlighted in yellow for some variables that have a p-value less than 0.05 (for a 95% confidence). These p-values mean that we can reject the null hypothesis and suggests that changes in the predictor variable are associated with changes in the response variable. This means that the other variables that are not statically significant and can be removed. Finally, I was able to get a much better R^2 score by using only the predictors VAS0, OPG and IL-6.

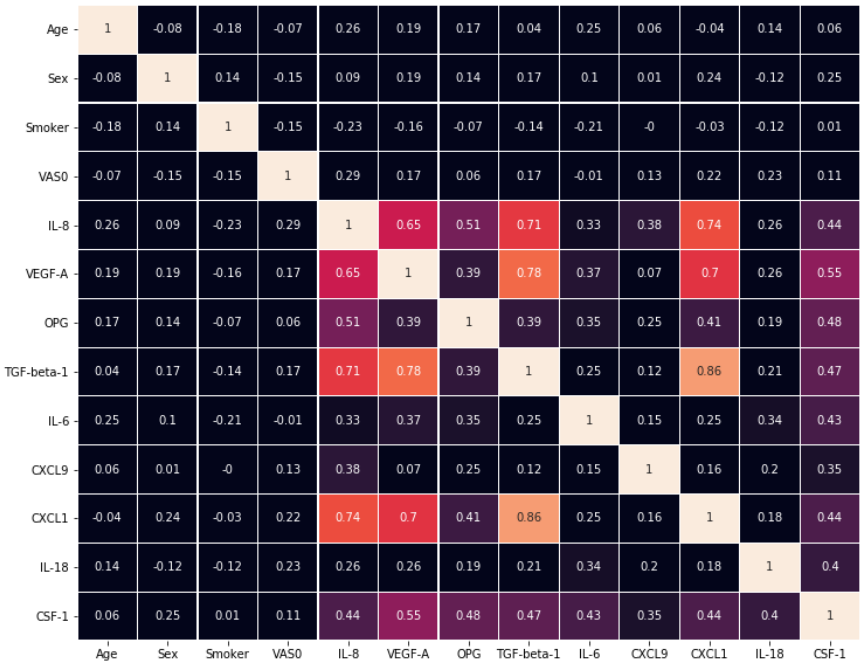
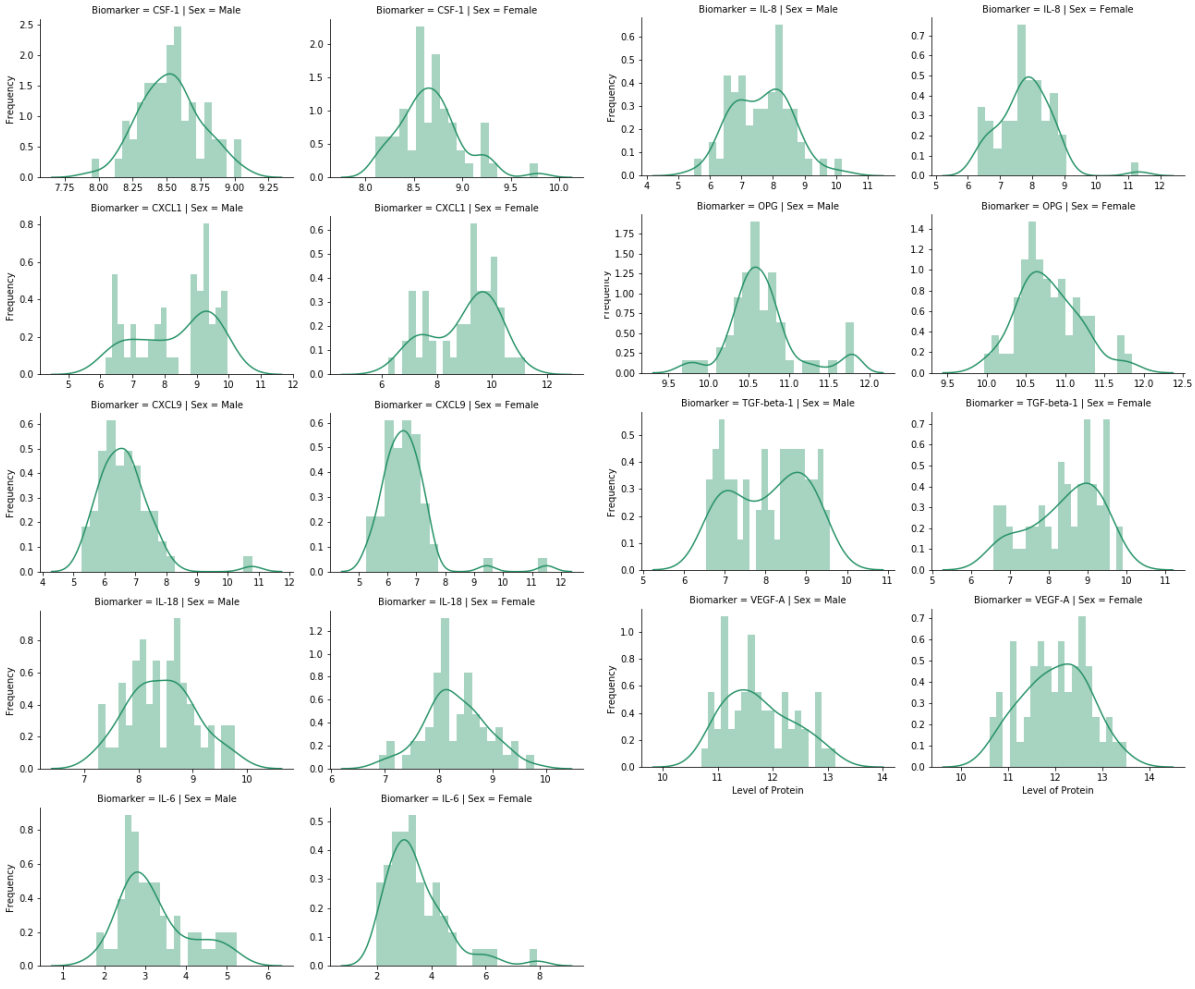


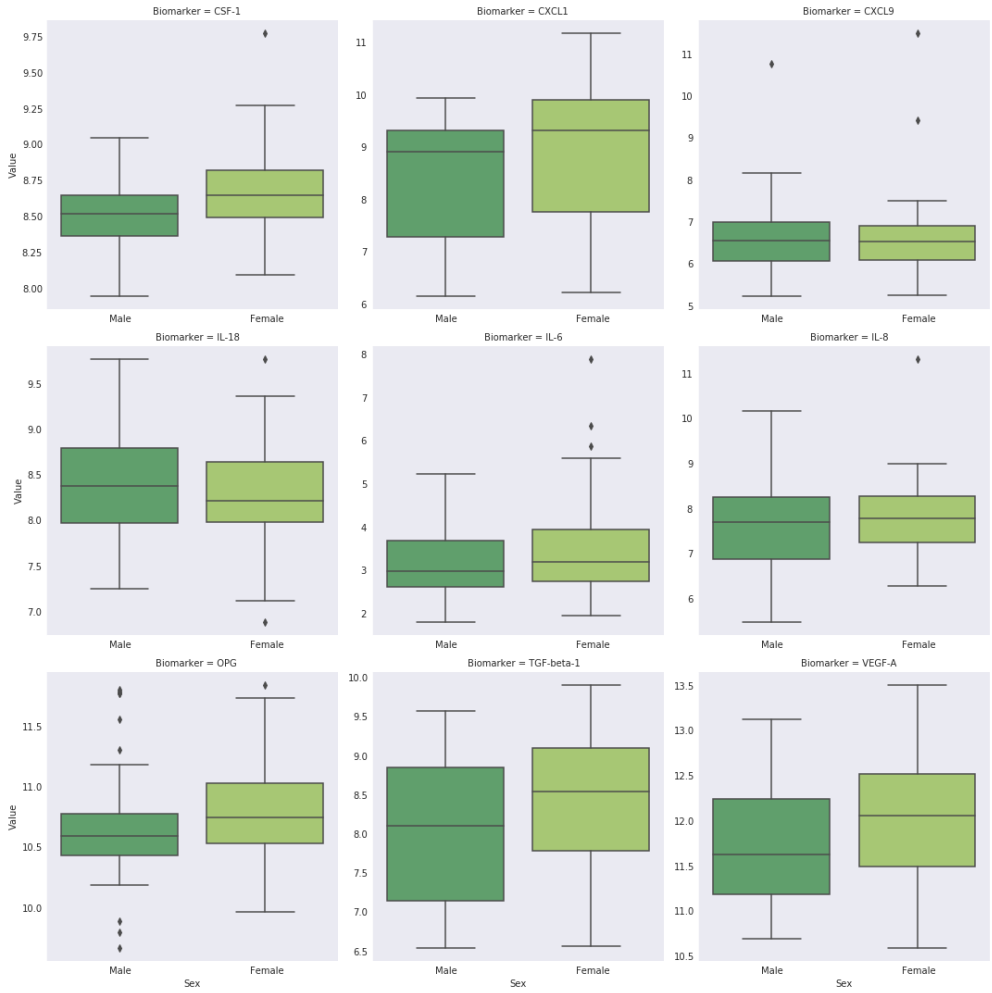
Figure 1 The correlation coefficients between the different explanatory variables

Annexe

1. Distribution of biomarkers' levels at inclusion disaggregated by sex



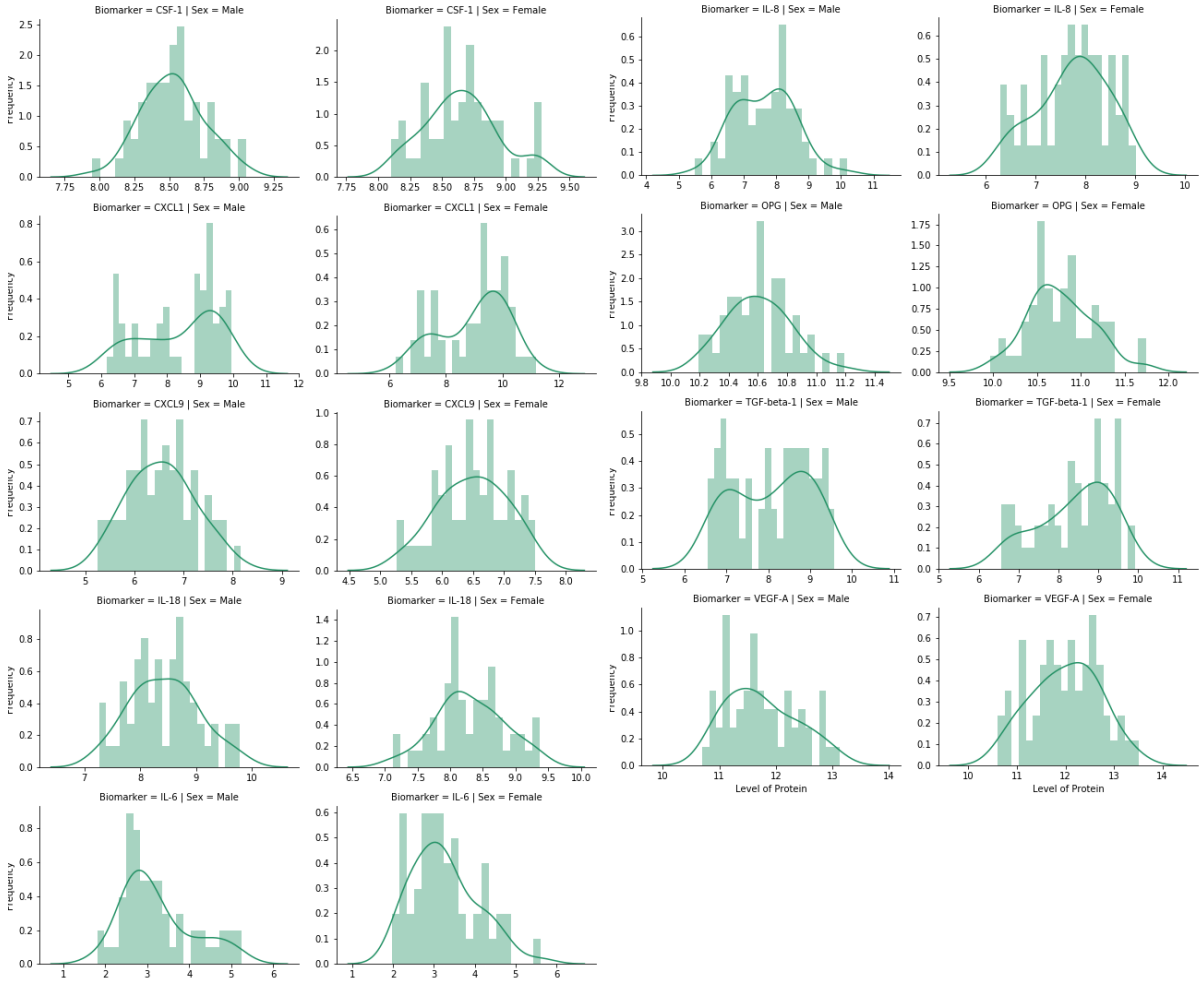
2. Boxplot of biomarkers' levels at inclusion disaggregated by sex



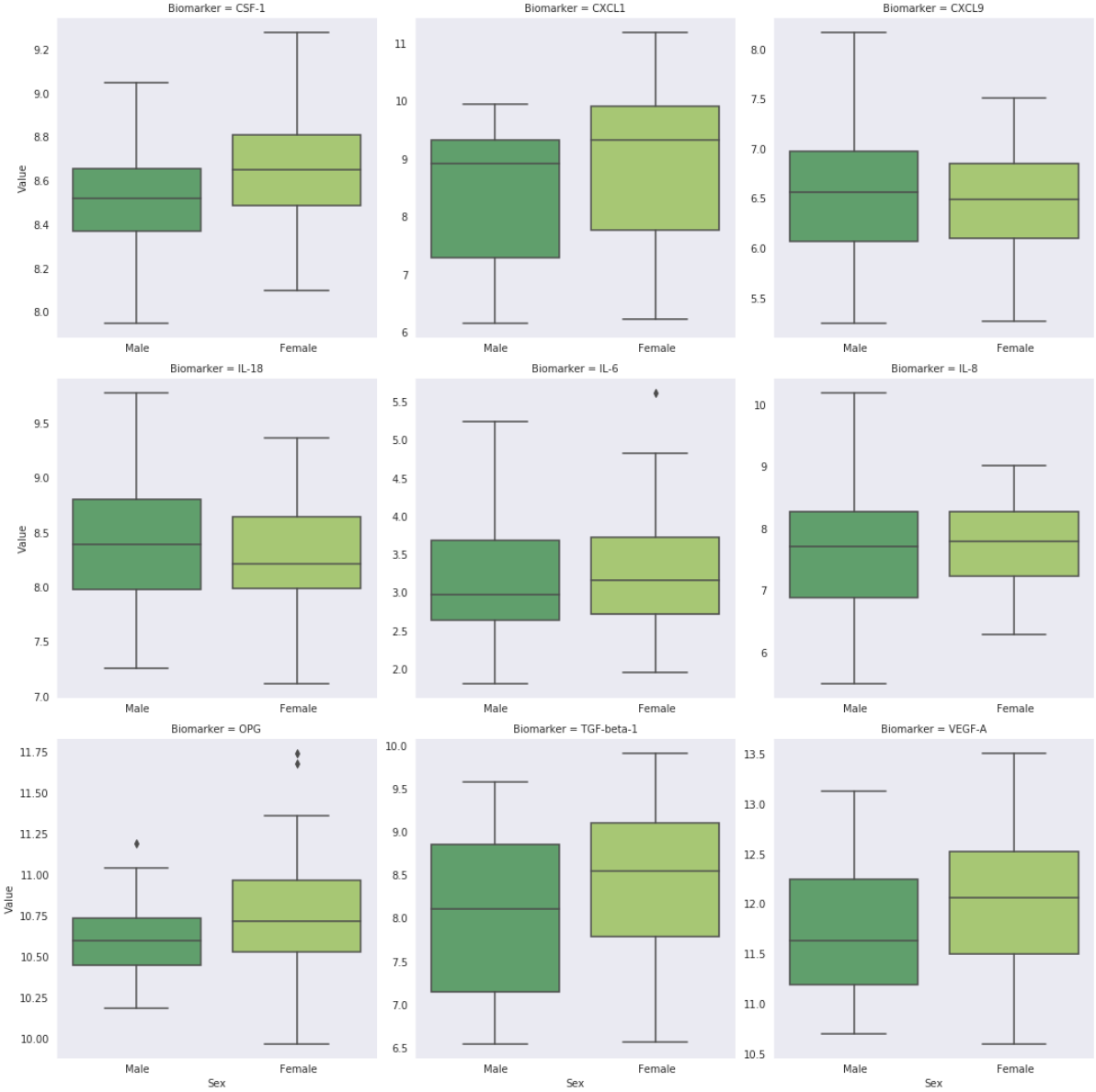
3. The normality test result of biomarkers after removing the outliers

		p-value	Test Result (α=0.01)
Biomarker	**Sex**		
CSF-1	**Female**	0.650	Cannot Reject / Is Normal
	Male	0.923	Cannot Reject / Is Normal
CXCL1	**Female**	0.048	Cannot Reject / Is Normal
	Male	0.000	Reject / Is not Normal
CXCL9	**Female**	0.391	Cannot Reject / Is Normal
	Male	0.594	Cannot Reject / Is Normal
IL-18	**Female**	0.946	Cannot Reject / Is Normal
	Male	0.675	Cannot Reject / Is Normal
IL-6	**Female**	0.110	Cannot Reject / Is Normal
	Male	0.038	Cannot Reject / Is Normal
IL-8	**Female**	0.201	Cannot Reject / Is Normal
	Male	0.828	Cannot Reject / Is Normal
OPG	**Female**	0.535	Cannot Reject / Is Normal
	Male	0.668	Cannot Reject / Is Normal
TGF-beta-1	**Female**	0.051	Cannot Reject / Is Normal
	Male	0.000	Reject / Is not Normal
VEGF-A	**Female**	0.406	Cannot Reject / Is Normal
	Male	0.092	Cannot Reject / Is Normal

4. Distribution of biomarkers' levels at inclusion disaggregated by sex after removing the outliers



5. Boxplot of biomarkers' levels at inclusion disaggregated by sex after removing the outliers



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Wikipedia. (n.d.). *Coefficient of determination*. Retrieved from Wikipedia: https://en.wikipedia.org/wiki/Coefficient_of_determination

Why Collinearity Is a Problem. (n.d.). Retrieved from Carnegie Mellon University: https://www.stat.cmu.edu/~larry/=stat401/lecture-17.pdf

The Source Code:

The language that was used for the coding is in Python 3.7 and it was written inside a Jupyter Notebook for clarity. The libraries used in the code are Pandas, Scipy, Numpy, Statsmodel and Seaborn.

The notebook is exported and attached in the following page and you can use this link here to download it and run it on your computer given that you have the correct python environment and Jupyter Notebooks or Jupyter Labs.

B155243 - Final Report

February 29, 2020

1 PROBABILITY AND STATISTICS

1.1 End-of-course summative assessment

Exam Number: B155243

University of Edinburgh

[63]:
```
import numpy as np
import scipy.stats as st
import scipy.optimize as op
import statsmodels.stats.api as sm
import statsmodels.stats as smt
import statsmodels.api as sma
from statsmodels.api import OLS
import pandas as pd
import matplotlib.pyplot as plt
import seaborn as sn
```

[2]:
```
_ = sn.set_palette("summer")
_ = sn.axes_style("dark")
```

2 Initial Steps - Reading, Summarising and Formatting Datasets

2.1 Read the two datasets: covariates & biomarkers

2.1.1 Step 1 - Reading both datasets

Read the datasets from the csv files

[3]:
```
covariates_df = pd.read_excel("./data/covariates.xlsx")
biomarkers_df = pd.read_excel("./data/biomarkers.xlsx")
```

2.1.2 Step 2 - Fix and summarise the covariates dataset

Display the first 5 records of the covariates dataset

[4]:
```
covariates_df.head()
```

[4]:
	PatientID	Age	Sex (1=male, 2=female)	Smoker (1=yes, 2=no)	VAS-at-inclusion	Vas-12months
0	1	56	1	2	3.0	4.0
1	3	32	1	2	7.2	0.5
2	4	43	2	2	2.7	0.5
3	5	25	2	2	3.0	3.9
4	6	39	1	2	3.5	5.0

Display a summary of the table covariates

[5]:
```
covariates_df.describe().round(2)
```

[5]:
	PatientID	Age	Sex (1=male, 2=female)	Smoker (1=yes, 2=no)	VAS-at-inclusion	Vas-12months
count	118.00	118.00	118.0	118.00	118.00	116.00
mean	75.45	40.86	1.5	1.67	6.02	3.62
std	43.33	10.14	0.5	0.47	2.66	3.08
min	1.00	18.00	1.0	1.00	0.00	0.00
25%	39.25	32.00	1.0	1.00	4.00	0.75
50%	72.50	41.00	1.5	2.00	6.50	3.50
75%	112.75	49.00	2.0	2.00	8.00	6.00
max	152.00	59.00	2.0	2.00	10.00	10.00

Fix the column names for Sex and Smoker and their data types The two columns will be renamed and their data type will become a categorical type instead of integer

[6]:
```
covariates_df.rename(columns={"Sex (1=male, 2=female)": "Sex", "Smoker (1=yes,␣
↪2=no)": "Smoker", "VAS-at-inclusion": "VAS0", "Vas-12months": "VAS12"},␣
↪inplace=True)
covariates_df["Sex"] = covariates_df["Sex"].astype('category')
covariates_df["Sex"] = covariates_df["Sex"].cat.rename_categories({1: 'Male', 2:
↪ 'Female'})
```

```
covariates_df["Smoker"] = covariates_df["Smoker"].astype('category')
covariates_df["Smoker"] = covariates_df["Smoker"].cat.rename_categories({1:␣
↪'Yes', 2: 'No'})
```

Display the first 5 records from the table covariates after the fixes

[7]:
```
covariates_df.head()
```

[7]:

	PatientID	Age	Sex	Smoker	VAS0	VAS12
0	1	56	Male	No	3.0	4.0
1	3	32	Male	No	7.2	0.5
2	4	43	Female	No	2.7	0.5
3	5	25	Female	No	3.0	3.9
4	6	39	Male	No	3.5	5.0

Print the columns datatypes to check if they are correct

[8]:
```
covariates_df.dtypes
```

[8]:
```
PatientID       int64
Age             int64
Sex          category
Smoker       category
VAS0          float64
VAS12         float64
dtype: object
```

Check if there are any NULL values in covariates and show them

[9]:
```
covariates_df.isna().sum()
```

[9]:
```
PatientID    0
Age          0
Sex          0
Smoker       0
VAS0         0
VAS12        2
dtype: int64
```

[10]:
```
covariates_df[covariates_df["VAS12"].isna()]
```

[10]:

	PatientID	Age	Sex	Smoker	VAS0	VAS12
32	42	27	Male	No	6.0	NaN
40	51	35	Female	Yes	7.5	NaN

Display a summary of the values in the dataset Covariates after the fixes

[11]:
```
covariates_df.describe()
```

[11]:

	PatientID	Age	VAS0	VAS12
count	118.000000	118.000000	118.000000	116.000000
mean	75.449153	40.855932	6.019492	3.619828
std	43.330493	10.137748	2.659486	3.079871
min	1.000000	18.000000	0.000000	0.000000
25%	39.250000	32.000000	4.000000	0.750000
50%	72.500000	41.000000	6.500000	3.500000
75%	112.750000	49.000000	8.000000	6.000000
max	152.000000	59.000000	10.000000	10.000000

2.1.3 Step 3 - Fix and summarise the biomarkers dataset

Display the first 5 records

[12]:
```
biomarkers_df.head()
```

[12]:

	Biomarker	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	\
0	126-0weeks	7.63	11.51	10.20	8.83	3.52	6.16	9.45	7.91	
1	126-6weeks	7.12	11.59	10.41	8.87	3.89	6.12	9.06	7.92	
2	127-0weeks	6.93	10.92	10.30	6.59	2.73	6.14	7.31	7.95	
3	127-6weeks	7.16	11.58	10.39	8.61	2.60	6.35	8.61	7.94	
4	127-12months	6.87	11.13	10.25	7.44	3.92	6.15	8.79	7.94	

	CSF-1
0	8.41
1	8.39
2	8.40
3	8.51
4	8.46

Display a summary of the values in the table Biomarkers

[13]:
```
biomarkers_df.describe().round(2)
```

[13]:

	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	\
count	348.00	347.00	347.00	347.00	347.00	347.00	347.00	347.00	
mean	7.41	11.66	10.67	7.95	3.25	6.47	8.29	8.29	
std	0.96	0.67	0.40	0.95	0.95	0.79	1.25	0.58	
min	1.00	10.21	9.67	5.64	1.60	4.61	5.68	6.70	
25%	6.69	11.14	10.41	7.06	2.58	5.99	7.16	7.93	
50%	7.36	11.51	10.62	7.90	3.07	6.35	8.37	8.25	
75%	8.13	12.15	10.84	8.82	3.66	6.85	9.34	8.68	
max	11.33	13.60	11.96	9.91	7.95	11.51	11.19	9.78	

	CSF-1
count	347.00
mean	8.54
std	0.25

```
min      7.95
25%      8.35
50%      8.53
75%      8.70
max      9.78
```

Print the data types of the columns in the biomarkers dataset to check if there are any anomalies

[14]:
```
biomarkers_df.dtypes
```

[14]:
```
Biomarker      object
IL-8          float64
VEGF-A        float64
OPG           float64
TGF-beta-1    float64
IL-6          float64
CXCL9         float64
CXCL1         float64
IL-18         float64
CSF-1         float64
dtype: object
```

Delete the NaN values from the biomarker dataset

[15]:
```
# Drop Biomarker that have a NaN ID
biomarkers_index_to_drop = biomarkers_df[biomarkers_df["Biomarker"].isna()].
 ↪index
biomarkers_df.drop(index=biomarkers_index_to_drop, inplace=True)
```

Create two new columns in the Biomarkers table:

- PatientID
- Timepoint

These columns will be created by splitting the existing column "Biomarker" into two and then setting the correct data type for each of the newly created columns

[16]:
```
biomarkers_new_cols = biomarkers_df["Biomarker"].str.extract(r"(?
 ↪P<PatientID>[0-9]+)\-(?P<Timepoint>.*)")
biomarkers_df = pd.concat([biomarkers_new_cols, biomarkers_df], axis=1)
biomarkers_df["PatientID"] = biomarkers_df["PatientID"].astype("int64")
biomarkers_df["Timepoint"] = biomarkers_df["Timepoint"].astype("category")
biomarkers_df.head()
```

[16]:

	PatientID	Timepoint	Biomarker	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	\
0	126	0weeks	126-0weeks	7.63	11.51	10.20	8.83	3.52	
1	126	6weeks	126-6weeks	7.12	11.59	10.41	8.87	3.89	

```
2        127    0weeks    127-0weeks  6.93   10.92  10.30        6.59  2.73
3        127    6weeks    127-6weeks  7.16   11.58  10.39        8.61  2.60
4        127  12months  127-12months  6.87   11.13  10.25        7.44  3.92

   CXCL9  CXCL1  IL-18  CSF-1
0   6.16   9.45   7.91   8.41
1   6.12   9.06   7.92   8.39
2   6.14   7.31   7.95   8.40
3   6.35   8.61   7.94   8.51
4   6.15   8.79   7.94   8.46
```

Double check the datatypes of the newly created columns

[17]:
```
biomarkers_df.dtypes
```

[17]:
```
PatientID        int64
Timepoint     category
Biomarker       object
IL-8           float64
VEGF-A         float64
OPG            float64
TGF-beta-1     float64
IL-6           float64
CXCL9          float64
CXCL1          float64
IL-18          float64
CSF-1          float64
dtype: object
```

3 Question 1

3.1 Do the levels of biomarkers at inclusion vary between males and females?

H0: Level of Biomaker n at inclusion does not vary between males and females: -male = -female

H1: Level of Biomaker n at inclusion varies between males and females: -male -female

Join the covariates and biomarkers datasets in one dataset to separate each biomarker based on the sex and to filter for "week0" (at inclusion)

[18]:
```
joined_df = covariates_df.merge(biomarkers_df, left_on="PatientID",␣
 ↪right_on="PatientID", how="inner", suffixes=('', '_r'))
filter_condition = joined_df.Timepoint.str.match("0weeks")
at_inclusion_df = joined_df[filter_condition]
at_inclusion_df.head()
```

[18]:
```
   PatientID  Age     Sex Smoker  VAS0  VAS12 Timepoint Biomarker  IL-8  \
0          1   56    Male     No   3.0    4.0    0weeks  1-0weeks  8.13
```

```
3           3   32    Male     No   7.2    0.5    0weeks  3-0weeks  6.55
6           4   43  Female     No   2.7    0.5    0weeks  4-0weeks  6.47
9           5   25  Female     No   3.0    3.9    0weeks  5-0weeks  6.41
12          6   39    Male     No   3.5    5.0    0weeks  6-0weeks  6.54

    VEGF-A    OPG  TGF-beta-1  IL-6  CXCL9  CXCL1  IL-18  CSF-1
0    12.35  10.48        8.66  2.63   6.54   9.54   8.53   8.27
3    11.21  10.49        6.83  2.58   5.31   6.71   7.71   8.30
6    11.13  10.72        6.90  5.62   5.46   7.73   8.02   8.19
9    11.15  10.60        7.26  1.96   5.35   7.15   7.66   8.10
12   11.47  10.20        7.16  3.38   6.51   7.79   8.54   8.35
```

[19]:
```
# Store the name of the biomarkers for later usage
biomarker_columns = at_inclusion_df.loc[:, "IL-8":].columns.values
print(f"The biomarkers columns that will be used: {biomarker_columns}")
```

```
The biomarkers columns that will be used: ['IL-8' 'VEGF-A' 'OPG' 'TGF-beta-1'
'IL-6' 'CXCL9' 'CXCL1' 'IL-18' 'CSF-1']
```

[20]:
```
print(f"The joined dataset's shape is: {at_inclusion_df.shape}")
```

```
The joined dataset's shape is: (117, 17)
```

[21]:
```
at_inclusion_flattened_df = at_inclusion_df.loc[:, ["Sex", *biomarker_columns]]
at_inclusion_flattened_df.head()
```

[21]:
```
      Sex  IL-8  VEGF-A    OPG  TGF-beta-1  IL-6  CXCL9  CXCL1  IL-18  CSF-1
0    Male  8.13   12.35  10.48        8.66  2.63   6.54   9.54   8.53   8.27
3    Male  6.55   11.21  10.49        6.83  2.58   5.31   6.71   7.71   8.30
6  Female  6.47   11.13  10.72        6.90  5.62   5.46   7.73   8.02   8.19
9  Female  6.41   11.15  10.60        7.26  1.96   5.35   7.15   7.66   8.10
12   Male  6.54   11.47  10.20        7.16  3.38   6.51   7.79   8.54   8.35
```

[22]:
```
at_inclusion_flattened_df.shape
```

[22]:
```
(117, 10)
```

3.1.1 Divide the joined dataset into two dataframes, one for males and one for females

[23]:
```
at_inclusion_flattened_df_m = at_inclusion_flattened_df.
 ↪loc[at_inclusion_flattened_df.Sex == "Male", biomarker_columns]
at_inclusion_flattened_df_f = at_inclusion_flattened_df.
 ↪loc[at_inclusion_flattened_df.Sex == "Female", biomarker_columns]

print("Biomarkers for males at inclusion:")
print(at_inclusion_flattened_df_m.head())
print(at_inclusion_flattened_df_m.shape)
```

```
print("\nBiomarkers for females at inclusion:")
print(at_inclusion_flattened_df_f.head())
print(at_inclusion_flattened_df_m.shape)
```

```
Biomarkers for males at inclusion:
    IL-8  VEGF-A    OPG  TGF-beta-1  IL-6  CXCL9  CXCL1  IL-18  CSF-1
0   8.13   12.35  10.48        8.66  2.63   6.54   9.54   8.53   8.27
3   6.55   11.21  10.49        6.83  2.58   5.31   6.71   7.71   8.30
12  6.54   11.47  10.20        7.16  3.38   6.51   7.79   8.54   8.35
18  8.32   12.85  10.41        8.00  3.26   6.04   8.94   8.92   8.39
27  7.78   11.33  10.63        7.42  2.53   6.15   7.29   7.84   8.18
(59, 9)

Biomarkers for females at inclusion:
    IL-8  VEGF-A    OPG  TGF-beta-1  IL-6  CXCL9  CXCL1  IL-18  CSF-1
6   6.47   11.13  10.72        6.90  5.62   5.46   7.73   8.02   8.19
9   6.41   11.15  10.60        7.26  1.96   5.35   7.15   7.66   8.10
15  6.92   11.06  10.28        7.17  2.48   6.41   6.94   8.78   8.17
21  9.01   12.53  11.68        9.26  3.76   6.83  11.19   9.06   8.95
24  8.67   12.23  10.43        8.34  4.00   6.59   9.45   9.13   8.40
(59, 9)
```

3.1.2 Identifying and removing outliers using IQR

https://towardsdatascience.com/ways-to-detect-and-remove-the-outliers-404d16608dba

[24]:
```
Q1_m = at_inclusion_flattened_df_m.quantile(0.25)
Q3_m = at_inclusion_flattened_df_m.quantile(0.75)
IQR_m = Q3_m - Q1_m

Q1_f = at_inclusion_flattened_df_f.quantile(0.25)
Q3_f = at_inclusion_flattened_df_f.quantile(0.75)
IQR_f = Q3_f - Q1_f

pd.DataFrame(data={"Male IQR": IQR_m, "Female IQR": IQR_f})
```

[24]:

	Male IQR	Female IQR
IL-8	1.390	1.0225
VEGF-A	1.065	1.0275
OPG	0.345	0.5025
TGF-beta-1	1.700	1.3225
IL-6	1.055	1.2100
CXCL9	0.920	0.8225
CXCL1	2.025	2.1400
IL-18	0.825	0.6700
CSF-1	0.285	0.3300

Remove the outliers Outliers are first set to NULL then automatically removed in the next operation when a pivot table is created. Cleanup of the outliers should be done for each biomarker and for each sex independtly. So for the same biomarker, outliers for females are not the same for males because they are treated as two different samples. Fot the above reasons, the removal of outliers is executed for each sex independtly then the samples are place together again in one table.

[25]:
```
filter_m = (at_inclusion_flattened_df_m < (Q1_m - 1.5 * IQR_m)) |␣
↪(at_inclusion_flattened_df_m > (Q3_m + 1.5 * IQR_m))
filter_f = (at_inclusion_flattened_df_f < (Q1_f - 1.5 * IQR_f)) |␣
↪(at_inclusion_flattened_df_f > (Q3_f + 1.5 * IQR_f))

at_inclusion_filtered_df_m = at_inclusion_flattened_df_m[~filter_m]
at_inclusion_filtered_df_f = at_inclusion_flattened_df_f[~filter_f]
at_inclusion_filtered_df_m["Sex"] = "Male"
at_inclusion_filtered_df_f["Sex"] = "Female"
at_inclusion_filtered_df = at_inclusion_filtered_df_m.
↪append(at_inclusion_filtered_df_f)

print("Number of values removed:")
at_inclusion_filtered_df.isna().sum() #NAs Will be removed by the pivot␣
↪function below
```

```
Number of values removed:
```

[25]:
```
IL-8           1
VEGF-A         0
OPG           10
TGF-beta-1     0
IL-6           3
CXCL9          3
CXCL1          0
IL-18          2
CSF-1          1
Sex            0
dtype: int64
```

The biomarkers are stacked to have one biomarker per row instead of having them all in one row for each observation

The stacked dataset before filtering outliers:

[26]:
```
# this stacked dataset is created without filtering outliers to test the␣
↪hypothesis results before the filtering
at_inclusion_stacked_df = at_inclusion_df.loc[:, ["Sex", *biomarker_columns]].
↪pivot(columns='Sex', values=biomarker_columns).stack([0,1]).reset_index()
at_inclusion_stacked_df.columns = ["IndexID", "Biomarker", "Sex", "Value"]
at_inclusion_stacked_df.tail()
```

[26]:

	IndexID	Biomarker	Sex	Value
1048	344	IL-6	Male	2.64
1049	344	IL-8	Male	6.93
1050	344	OPG	Male	10.46
1051	344	TGF-beta-1	Male	6.62
1052	344	VEGF-A	Male	10.95

The stacked dataset after filtering outliers:

[27]:
```
# this stacked dataset is created after filtering outliers to test the␣
↪hypothesis results after the filtering
at_inclusion_filtered_stacked_df = at_inclusion_filtered_df.loc[:, ["Sex",␣
↪*biomarker_columns]].pivot(columns='Sex', values=biomarker_columns).
↪stack([0,1]).reset_index()
at_inclusion_filtered_stacked_df.columns = ["IndexID", "Biomarker", "Sex",␣
↪"Value"]
at_inclusion_filtered_stacked_df.head()
```

[27]:

	IndexID	Biomarker	Sex	Value
0	0	CSF-1	Male	8.27
1	0	CXCL1	Male	9.54
2	0	CXCL9	Male	6.54
3	0	IL-18	Male	8.53
4	0	IL-6	Male	2.63

[28]:
```
# Test whether each sample differs from a normal distribution.
# This function tests the null hypothesis that a sample comes from a normal␣
↪distribution.
# It is based on D'Agostino and Pearson's test that combines skew and kurtosis␣
↪to produce an omnibus test of normality.
# Compare the p-value to a significance level

# Ref: https://docs.scipy.org/doc/scipy/reference/generated/scipy.stats.
↪normaltest.html


def _normality_test(a, axis=0, nan_policy='propagate'):
    _, p = st.normaltest(a, axis, nan_policy)
    return p

# The default significance level is set to 0.01
def _normality_test_result(a, alpha=0.01, axis=0, nan_policy='propagate'):
    _, p = st.normaltest(a, axis, nan_policy)
    # Null hypothesis: x comes from a normal distribution
    return  "Reject / Is not Normal" if p < alpha else "Cannot Reject / Is␣
↪Normal"
```

D'Agostino and Pearson's test of normality on the data before removing the outliers:

[29]:
```
# Null hypothesis: The sample comes from a normal distribution
at_inclusion_stacked_df.groupby(by=["Biomarker","Sex"], sort=True,␣
↪as_index=True).agg([_normality_test, _normality_test_result])["Value"].
↪round(6)
```

[29]:

Biomarker	Sex	_normality_test	_normality_test_result
CSF-1	Male	0.923477	Cannot Reject / Is Normal
	Female	0.012154	Cannot Reject / Is Normal
CXCL1	Male	0.000006	Reject / Is not Normal
	Female	0.047508	Cannot Reject / Is Normal
CXCL9	Male	0.000000	Reject / Is not Normal
	Female	0.000000	Reject / Is not Normal
IL-18	Male	0.675231	Cannot Reject / Is Normal
	Female	0.899612	Cannot Reject / Is Normal
IL-6	Male	0.037913	Cannot Reject / Is Normal
	Female	0.000001	Reject / Is not Normal
IL-8	Male	0.828230	Cannot Reject / Is Normal
	Female	0.000119	Reject / Is not Normal
OPG	Male	0.003334	Reject / Is not Normal
	Female	0.236999	Cannot Reject / Is Normal
TGF-beta-1	Male	0.000000	Reject / Is not Normal
	Female	0.050951	Cannot Reject / Is Normal
VEGF-A	Male	0.091760	Cannot Reject / Is Normal
	Female	0.405606	Cannot Reject / Is Normal

D'Agostino and Pearson's test of normality on the data after removing the outliers:

[30]:
```
# Null hypothesis: The sameple comes from a normal distribution
at_inclusion_filtered_stacked_df.groupby(by=["Biomarker","Sex"], sort=True,␣
↪as_index=True).agg([_normality_test, _normality_test_result])["Value"].
↪round(3)
```

[30]:

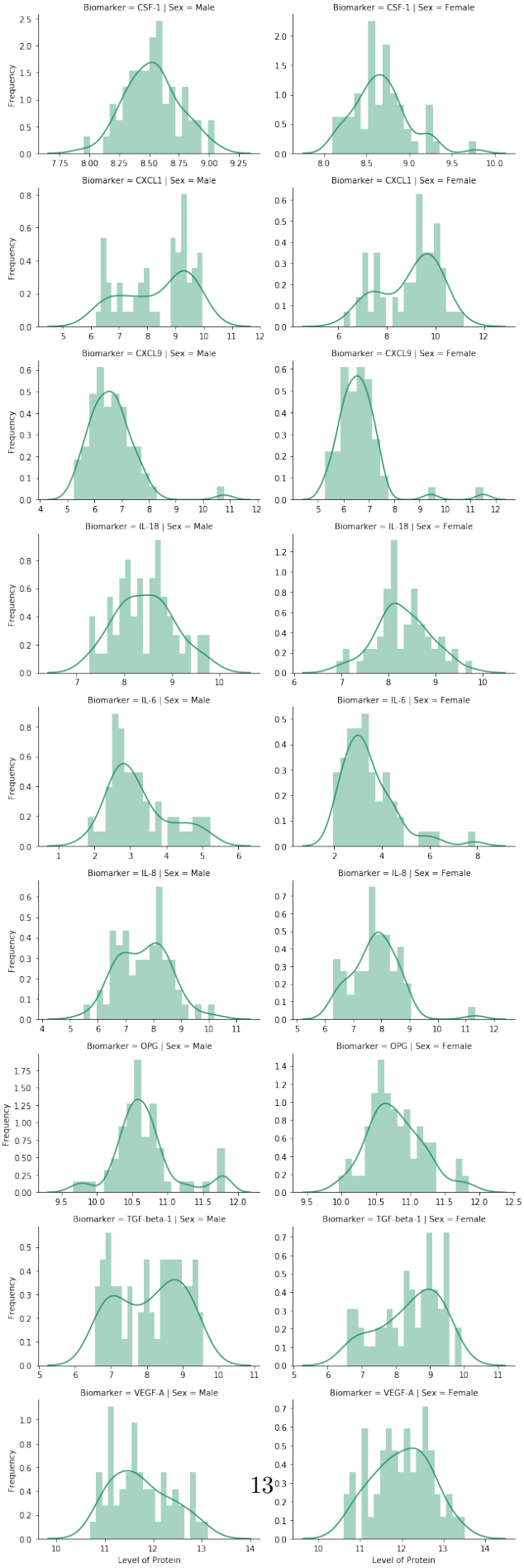
Biomarker	Sex	_normality_test	_normality_test_result
CSF-1	Female	0.650	Cannot Reject / Is Normal
	Male	0.923	Cannot Reject / Is Normal
CXCL1	Female	0.048	Cannot Reject / Is Normal
	Male	0.000	Reject / Is not Normal
CXCL9	Female	0.391	Cannot Reject / Is Normal
	Male	0.594	Cannot Reject / Is Normal
IL-18	Female	0.946	Cannot Reject / Is Normal
	Male	0.675	Cannot Reject / Is Normal
IL-6	Female	0.110	Cannot Reject / Is Normal
	Male	0.038	Cannot Reject / Is Normal
IL-8	Female	0.201	Cannot Reject / Is Normal

```
            Male              0.828  Cannot Reject / Is Normal
OPG         Female            0.535  Cannot Reject / Is Normal
            Male              0.668  Cannot Reject / Is Normal
TGF-beta-1  Female            0.051  Cannot Reject / Is Normal
            Male              0.000     Reject / Is not Normal
VEGF-A      Female            0.406  Cannot Reject / Is Normal
            Male              0.092  Cannot Reject / Is Normal
```

The distribution of biomarkers by sex before filtering outliers:

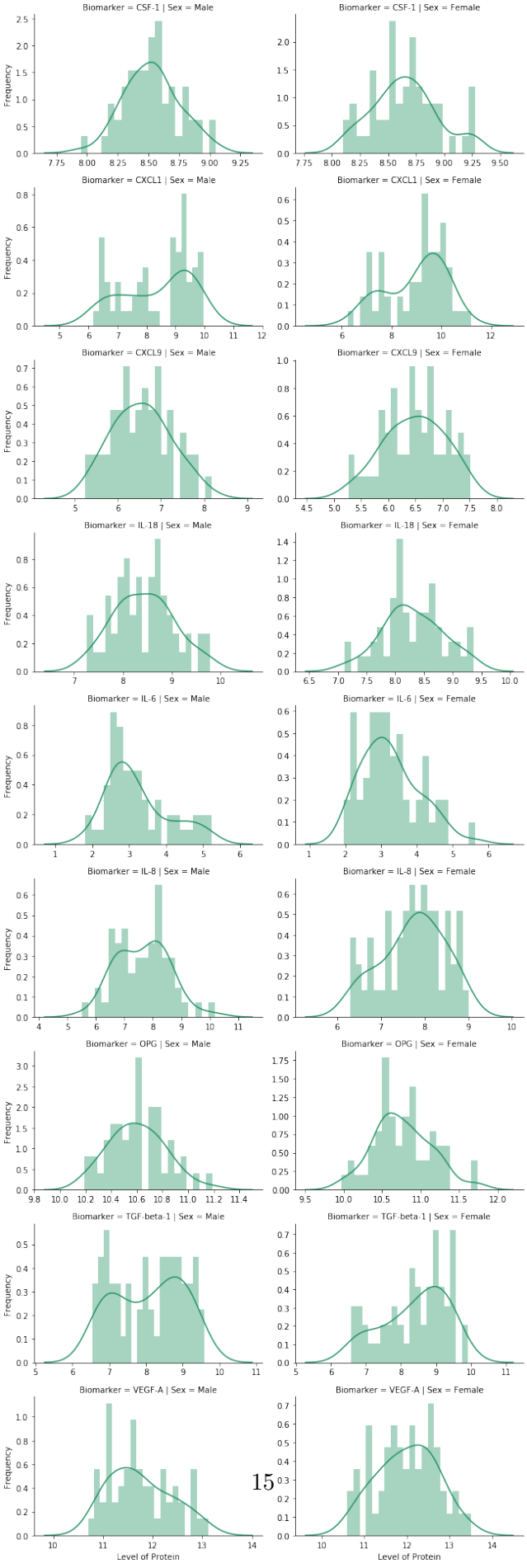
[31]:
```
plt.figure()
g = sn.FacetGrid(at_inclusion_stacked_df, col="Sex", row="Biomarker",␣
 ↪sharex=False, sharey=False, aspect=1.5)
g = g.map(sn.distplot, "Value", bins=20)
g.set_xlabels("Level of Protein")
g.set_ylabels("Frequency")
# g.set_title("The frequency distribution histogram of each protein by")
plt.savefig("./Charts/dists_1.png")
```

```
<Figure size 432x288 with 0 Axes>
```


Biomarker = CSF-1 | Sex = Male
Biomarker = CSF-1 | Sex = Female
Biomarker = CXCL1 | Sex = Male
Biomarker = CXCL1 | Sex = Female
Biomarker = CXCL9 | Sex = Male
Biomarker = CXCL9 | Sex = Female
Biomarker = IL-18 | Sex = Male
Biomarker = IL-18 | Sex = Female
Biomarker = IL-6 | Sex = Male
Biomarker = IL-6 | Sex = Female
Biomarker = IL-8 | Sex = Male
Biomarker = IL-8 | Sex = Female
Biomarker = OPG | Sex = Male
Biomarker = OPG | Sex = Female
Biomarker = TGF-beta-1 | Sex = Male
Biomarker = TGF-beta-1 | Sex = Female
Biomarker = VEGF-A | Sex = Male
Biomarker = VEGF-A | Sex = Female
Frequency
Level of Protein

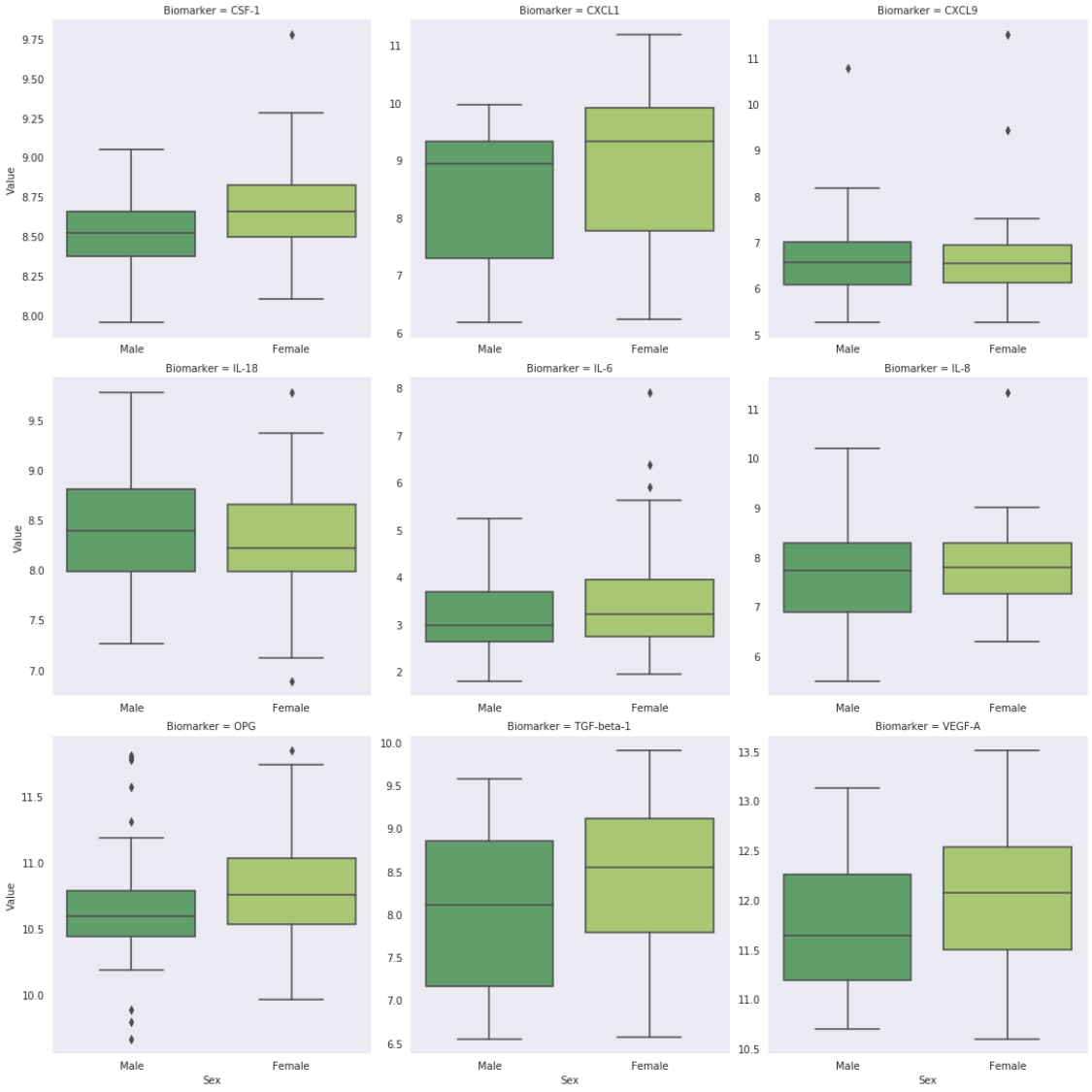
The distribution of biomarkers by sex after filtering outliers:

[32]:
```
g = sn.FacetGrid(at_inclusion_filtered_stacked_df, col="Sex", row="Biomarker",␣
↪sharex=False, sharey=False, aspect=1.5)
g = g.map(sn.distplot, "Value", bins=20)
g.set_xlabels("Level of Protein")
g.set_ylabels("Frequency")
# g.set_title("The frequency distribution histogram of each protein by")
plt.savefig("./Charts/dist_3.png")
```


Biomarker = CSF-1 | Sex = Male
Biomarker = CSF-1 | Sex = Female
Biomarker = CXCL1 | Sex = Male
Biomarker = CXCL1 | Sex = Female
Biomarker = CXCL9 | Sex = Male
Biomarker = CXCL9 | Sex = Female
Biomarker = IL-18 | Sex = Male
Biomarker = IL-18 | Sex = Female
Biomarker = IL-6 | Sex = Male
Biomarker = IL-6 | Sex = Female
Biomarker = IL-8 | Sex = Male
Biomarker = IL-8 | Sex = Female
Biomarker = OPG | Sex = Male
Biomarker = OPG | Sex = Female
Biomarker = TGF-beta-1 | Sex = Male
Biomarker = TGF-beta-1 | Sex = Female
Biomarker = VEGF-A | Sex = Male
Biomarker = VEGF-A | Sex = Female
Frequency
Level of Protein

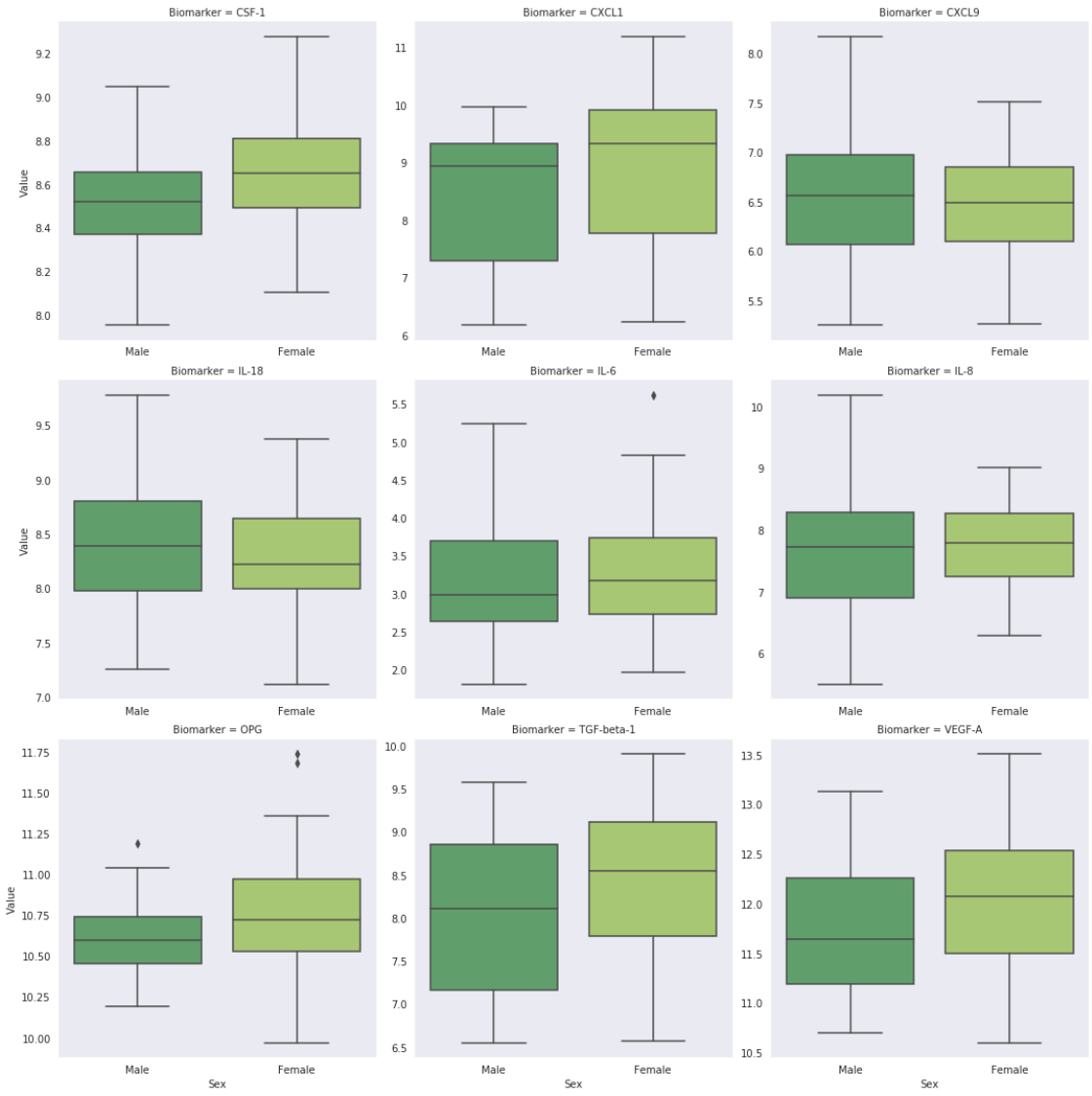
Boxplots of biomarkers by sex before filtering outliers:

[33]:
```
with sn.axes_style("dark"):
    g = sn.catplot(x="Sex", y="Value", col="Biomarker",␣
 ↪data=at_inclusion_stacked_df, kind="box",
                   height=5, aspect=1, sharex=False, sharey=False, col_wrap=3,␣
 ↪palette=sn.color_palette("summer", 2));
plt.savefig("./Charts/dist2.png")
```



Boxplots of biomarkers by sex after filtering outliers:

[34]:
```
with sn.axes_style("dark"):
    g = sn.catplot(x="Sex", y="Value", col="Biomarker",␣
 ↪data=at_inclusion_filtered_stacked_df, kind="box",
                   height=5, aspect=1, sharex=False, sharey=False, col_wrap=3,␣
 ↪palette=sn.color_palette("summer", 2));

plt.savefig("./Charts/dist_4.png")
```



3.1.3 The hypothesis test before removing outliers

A t-test is a type of inferential statistic which is used to determine if there is a significant difference between the means of two groups which may be related in certain features. It is mostly used when the data sets, like the set of data recorded as outcome from flipping a coin a 100 times, would follow

a normal distribution and may have unknown variances. T test is used as a hypothesis testing tool, which allows testing of an assumption applicable to a population.

Student's t test is used if the true variance of the populations from which the samples are extracted is unknown;

The use of Student's t test requires a decision to be taken beforehand on whether variances of the samples are to be considered equal or not. Fisher's F test can be used to test the hypothesis of equality of the variances..

Two-Sampled, two-tailed t-test of the difference of the mean of the samples.

https://towardsdatascience.com/hypothesis-testing-in-machine-learning-using-python-a0dc89e169ce

https://www.xlstat.com/en/solutions/features/two-sample-t-and-z-tests

[35]:
```
filter_condition = at_inclusion_df.Sex.str.match("Male")

# Multiple significance levels were only used for exploratory purposes not for␣
 ↪p-hacking
# significance_levels = [0.1, 0.05, 0.01] # 90%, 95%, 99% Confidence
significance_levels = [0.05] # 95% Confidence

results_df = pd.DataFrame(columns=biomarker_columns, index=significance_levels,␣
 ↪data=None)
results_dict = dict()

for alpha in significance_levels:
    print(f"The significance level alpha = {alpha}")
    for index, biomarker_column in enumerate(biomarker_columns):
        biomarker_1_values_male = at_inclusion_df.loc[filter_condition,␣
 ↪biomarker_column]
        biomarker_1_values_female = at_inclusion_df.loc[~filter_condition,␣
 ↪biomarker_column]


        # Calculate the power of the test
        # The power of the test, is one minus the probability of a
        # type II error. Power is the probability that the test correctly
        # rejects the Null Hypothesis if the Alternative Hypothesis is true.
        # https://towardsdatascience.com/
 ↪introduction-to-power-analysis-in-python-e7b748dfa26
        pow_nobs1 = biomarker_1_values_male.shape[0]
        pow_nobs2 = biomarker_1_values_female.shape[0]
        pow_ratio = pow_nobs2 / pow_nobs1
        pow_mean_1 = np.mean(biomarker_1_values_male)
        pow_mean_2 = np.mean(biomarker_1_values_female)
        pow_var_1 = np.var(biomarker_1_values_male, ddof=1)
        pow_var_2 = np.var(biomarker_1_values_female, ddof=1)
```

```
        pow_effect_size = (pow_mean_1 - pow_mean_2) / np.sqrt(((pow_nobs1 - 1)␣
↪* pow_var_1 + (pow_nobs2 - 1) * pow_var_2) / (pow_nobs1 + pow_nobs2 - 2))
        power = smt.power.tt_ind_solve_power(effect_size=pow_effect_size,␣
↪nobs1=pow_nobs1, alpha=alpha, power=None, ratio=pow_ratio,␣
↪alternative='two-sided')
        beta = 1 - power # Probability of a Type II error - Not rejecting a␣
↪false H0

        # Sample bigger than 30 observation but the variance of the population␣
↪from which the samples are extracted is unknown.
        print(f"Testing hypothesis for biomarker {biomarker_column}:")
        print(f"n_male = {biomarker_1_values_male.shape[0]};␣
↪n_female={biomarker_1_values_female.shape[0]};")
        print(f"H0: At inclusion, levels of biomarker {biomarker_column} do not␣
↪vary between males and females.  -male =  -female")
        print(f"H1: At inclusion, levels of biomarker {biomarker_column} do␣
↪vary between males and females.  -male   -female")

        tstat, pvalue = st.ttest_ind(biomarker_1_values_male,␣
↪biomarker_1_values_female, equal_var=False)
        print(f"t-stat = {tstat}, p-value = {pvalue}, alpha = {alpha}, beta =␣
↪{beta}, power={power}")

        # Store the results of the test for later
        results_dict[f"{biomarker_column}{' (' + str((1-alpha)*100)+'%␣
↪Confidence)' if len(significance_levels) > 1 else ''}"] = {
            "alpha": alpha,
            "beta": beta,
            "power": power,
            "t-stat": tstat,
            "p-value": pvalue,
            "t-test result": "Reject H0" if pvalue < alpha else " - "
        }


        if pvalue <= alpha:
            results_df.loc[alpha, biomarker_column] = "Reject"
            print(f"We reject the null hypothesis <=> we reject the hypothesis␣
↪that inclusion does not vary between males and females for biomarker␣
↪{biomarker_column}.")
        else:
            results_df.loc[alpha, biomarker_column] = "Can't reject"
            print(f"We don't have enough evidence to reject the null hypothesis␣
↪for biomarker {biomarker_column}.")
        print("\n")
    print("==========================================\n")
```

```
The significance level alpha = 0.05
Testing hypothesis for biomarker IL-8:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker IL-8 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-8 do vary between males and females.
 -male   -female
t-stat = -0.9801571664207548, p-value = 0.3290732316677307, alpha = 0.05, beta =
0.8368867242757656, power=0.16311327572423437
We don't have enough evidence to reject the null hypothesis for biomarker IL-8.


Testing hypothesis for biomarker VEGF-A:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker VEGF-A do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker VEGF-A do vary between males and females.
 -male   -female
t-stat = -2.0607498156961435, p-value = 0.04162010149428021, alpha = 0.05, beta
= 0.46588901652063963, power=0.5341109834793604
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker VEGF-A.


Testing hypothesis for biomarker OPG:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker OPG do not vary between males and females.
 -male =  -female
H1: At inclusion, levels of biomarker OPG do vary between males and females.
 -male   -female
t-stat = -1.5160276926030105, p-value = 0.13227339047976225, alpha = 0.05, beta
= 0.6761964089501966, power=0.3238035910498034
We don't have enough evidence to reject the null hypothesis for biomarker OPG.


Testing hypothesis for biomarker TGF-beta-1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker TGF-beta-1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker TGF-beta-1 do vary between males and
females.  -male   -female
t-stat = -2.020246829300327, p-value = 0.04568177954693594, alpha = 0.05, beta =
0.4827388819609081, power=0.5172611180390919
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker TGF-beta-1.


Testing hypothesis for biomarker IL-6:
```

```
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker IL-6 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-6 do vary between males and females.
 -male   -female
t-stat = -1.1499226373266758, p-value = 0.25276023868294506, alpha = 0.05, beta
= 0.7920632149646107, power=0.20793678503538926
We don't have enough evidence to reject the null hypothesis for biomarker IL-6.


Testing hypothesis for biomarker CXCL9:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker CXCL9 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL9 do vary between males and females.
 -male   -female
t-stat = 0.01897905210037341, p-value = 0.9848910140640419, alpha = 0.05, beta =
0.9499593656823275, power=0.050040634317672514
We don't have enough evidence to reject the null hypothesis for biomarker CXCL9.


Testing hypothesis for biomarker CXCL1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker CXCL1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL1 do vary between males and females.
 -male   -female
t-stat = -2.7902717386040914, p-value = 0.006167164236984985, alpha = 0.05, beta
= 0.20984502438772723, power=0.7901549756122728
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker CXCL1.


Testing hypothesis for biomarker IL-18:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker IL-18 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-18 do vary between males and females.
 -male   -female
t-stat = 1.1577143886323786, p-value = 0.24938194900412577, alpha = 0.05, beta =
0.7907740802698509, power=0.2092259197301492
We don't have enough evidence to reject the null hypothesis for biomarker IL-18.


Testing hypothesis for biomarker CSF-1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker CSF-1 do not vary between males and
females.  -male =  -female
```

```
H1: At inclusion, levels of biomarker CSF-1 do vary between males and females.
 -male   -female
t-stat = -2.80239462299329, p-value = 0.006081431050971925, alpha = 0.05, beta =
0.20397819346620316, power=0.7960218065337968
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker CSF-1.


================================================
```

Results of hypothesis testing before removing outliers with 95% confidence:

[36]:
```
results_df
```

[36]:

	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	\
0.05	Can't reject	Reject	Can't reject	Reject	Can't reject	

	CXCL9	CXCL1	IL-18	CSF-1
0.05	Can't reject	Reject	Can't reject	Reject

Details of hypothesis testing before removing outliers with 95% confidence: *The dash (-) means that H0 was not rejected*

[37]:
```
pd.DataFrame(data=results_dict).transpose()
```

[37]:

	alpha	beta	power	t-stat	p-value	t-test result
IL-8	0.05	0.836887	0.163113	-0.980157	0.329073	-
VEGF-A	0.05	0.465889	0.534111	-2.06075	0.0416201	Reject H0
OPG	0.05	0.676196	0.323804	-1.51603	0.132273	-
TGF-beta-1	0.05	0.482739	0.517261	-2.02025	0.0456818	Reject H0
IL-6	0.05	0.792063	0.207937	-1.14992	0.25276	-
CXCL9	0.05	0.949959	0.0500406	0.0189791	0.984891	-
CXCL1	0.05	0.209845	0.790155	-2.79027	0.00616716	Reject H0
IL-18	0.05	0.790774	0.209226	1.15771	0.249382	-
CSF-1	0.05	0.203978	0.796022	-2.80239	0.00608143	Reject H0

3.1.4 The hypothesis test after removing the outliers

[38]:
```
filter_condition = at_inclusion_filtered_stacked_df.Sex.str.match("Male")

# significance_levels = [0.1, 0.05, 0.01] # 90%, 95%, 99% Confidence
significance_levels = [0.05] # 95% Confidence

results_df = pd.DataFrame(columns=biomarker_columns, index=significance_levels,␣
 ↪data=None)
results_dict = dict()
```

```
for alpha in significance_levels:
    print(f"The significance level alpha = {alpha}")
    for index, biomarker_column in enumerate(biomarker_columns):
        biomarker_column_filter = at_inclusion_filtered_stacked_df.Biomarker.
↪str.match(biomarker_column)
        biomarker_1_values_male = at_inclusion_filtered_stacked_df.
↪loc[filter_condition & biomarker_column_filter, "Value"]
        biomarker_1_values_female = at_inclusion_filtered_stacked_df.
↪loc[~filter_condition & biomarker_column_filter, "Value"]

        # Calculate the power of the test
        # The power of the test, is one minus the probability of a
        # type II error. Power is the probability that the test correctly
        # rejects the Null Hypothesis if the Alternative Hypothesis is true.
        # https://towardsdatascience.com/
↪introduction-to-power-analysis-in-python-e7b748dfa26
        pow_nobs1 = biomarker_1_values_male.shape[0]
        pow_nobs2 = biomarker_1_values_female.shape[0]
        pow_ratio = pow_nobs2 / pow_nobs1
        pow_mean_1 = np.mean(biomarker_1_values_male)
        pow_mean_2 = np.mean(biomarker_1_values_female)
        pow_var_1 = np.var(biomarker_1_values_male, ddof=1)
        pow_var_2 = np.var(biomarker_1_values_female, ddof=1)
        pow_effect_size = (pow_mean_1 - pow_mean_2) / np.sqrt(((pow_nobs1 - 1)␣
↪* pow_var_1 + (pow_nobs2 - 1) * pow_var_2) / (pow_nobs1 + pow_nobs2 - 2))
        power = smt.power.tt_ind_solve_power(effect_size=pow_effect_size,␣
↪nobs1=pow_nobs1, alpha=alpha, power=None, ratio=pow_ratio,␣
↪alternative='two-sided')
        beta = 1 - power # Probability of a Type II error - Not rejecting a␣
↪false H0

        # Sample bigger than 30 observation but the variance of the population␣
↪from which the samples are extracted is unknown.
        print(f"Testing hypothesis for biomarker {biomarker_column}:")
        print(f"n_male = {biomarker_1_values_male.shape[0]};␣
↪n_female={biomarker_1_values_female.shape[0]};")
        print(f"H0: At inclusion, levels of biomarker {biomarker_column} do not␣
↪vary between males and females.  -male =  -female")
        print(f"H1: At inclusion, levels of biomarker {biomarker_column} do␣
↪vary between males and females.  -male   -female")

        tstat, pvalue = st.ttest_ind(biomarker_1_values_male,␣
↪biomarker_1_values_female, equal_var=False)
        print(f"t-stat = {tstat}, p-value = {pvalue}, alpha = {alpha}, beta =␣
↪{beta}, power={power}")
```

```
        # Store the results of the test for later
        results_dict[f"{biomarker_column}{' (' + str((1-alpha)*100)+'% Confidence)' if len(significance_levels) > 1 else ''}"] = {
            "alpha": alpha,
            "beta": beta,
            "power": power,
            "t-stat": tstat,
            "p-value": pvalue,
            "t-test result": "Reject H0" if pvalue < alpha else " - "
        }

        if pvalue < alpha:
            results_df.loc[alpha, biomarker_column] = "Reject"
            print(f"We reject the null hypothesis <=> we reject the hypothesis that inclusion does not vary between males and females for biomarker {biomarker_column}.")
        else:
            results_df.loc[alpha, biomarker_column] = "Can't Reject"
            print(f"We don't have enough evidence to reject the null hypothesis for biomarker {biomarker_column}.")
        print("\n")
    print("================================================\n")
```

```
The significance level alpha = 0.05
Testing hypothesis for biomarker IL-8:
n_male = 59; n_female=57;
H0: At inclusion, levels of biomarker IL-8 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-8 do vary between males and females.
 -male   -female
t-stat = -0.646581135107931, p-value = 0.5192605138647244, alpha = 0.05, beta =
0.9021560995483879, power=0.09784390045161204
We don't have enough evidence to reject the null hypothesis for biomarker IL-8.


Testing hypothesis for biomarker VEGF-A:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker VEGF-A do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker VEGF-A do vary between males and females.
 -male   -female
t-stat = -2.0607498156961435, p-value = 0.04162010149428021, alpha = 0.05, beta
= 0.46588901652063963, power=0.5341109834793604
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker VEGF-A.
```

```
Testing hypothesis for biomarker OPG:
n_male = 50; n_female=57;
H0: At inclusion, levels of biomarker OPG do not vary between males and females.
 -male =  -female
H1: At inclusion, levels of biomarker OPG do vary between males and females.
 -male   -female
t-stat = -2.8378755290604567, p-value = 0.005588517883116755, alpha = 0.05, beta
= 0.22316852962824252, power=0.7768314703717575
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker OPG.


Testing hypothesis for biomarker TGF-beta-1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker TGF-beta-1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker TGF-beta-1 do vary between males and
females.  -male   -female
t-stat = -2.020246829300327, p-value = 0.04568177954693594, alpha = 0.05, beta =
0.4827388819609081, power=0.5172611180390919
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker TGF-beta-1.


Testing hypothesis for biomarker IL-6:
n_male = 59; n_female=55;
H0: At inclusion, levels of biomarker IL-6 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-6 do vary between males and females.
 -male   -female
t-stat = -0.20775059411147945, p-value = 0.8358011902025527, alpha = 0.05, beta
= 0.9451426278126318, power=0.054857372187368206
We don't have enough evidence to reject the null hypothesis for biomarker IL-6.


Testing hypothesis for biomarker CXCL9:
n_male = 58; n_female=56;
H0: At inclusion, levels of biomarker CXCL9 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL9 do vary between males and females.
 -male   -female
t-stat = 0.5955269953821581, p-value = 0.5527192103280785, alpha = 0.05, beta =
0.9094718586212036, power=0.09052814137879645
We don't have enough evidence to reject the null hypothesis for biomarker CXCL9.


Testing hypothesis for biomarker CXCL1:
```

```
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker CXCL1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL1 do vary between males and females.
 -male   -female
t-stat = -2.7902717386040914, p-value = 0.006167164236984985, alpha = 0.05, beta
= 0.20984502438772723, power=0.7901549756122728
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker CXCL1.


Testing hypothesis for biomarker IL-18:
n_male = 59; n_female=56;
H0: At inclusion, levels of biomarker IL-18 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-18 do vary between males and females.
 -male   -female
t-stat = 1.2135809065078154, p-value = 0.2274631494235096, alpha = 0.05, beta =
0.776016948326044, power=0.2239830516739561
We don't have enough evidence to reject the null hypothesis for biomarker IL-18.


Testing hypothesis for biomarker CSF-1:
n_male = 59; n_female=57;
H0: At inclusion, levels of biomarker CSF-1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CSF-1 do vary between males and females.
 -male   -female
t-stat = -2.5895995332020694, p-value = 0.010971581561811593, alpha = 0.05, beta
= 0.2678415089412437, power=0.7321584910587563
We reject the null hypothesis <=> we reject the hypothesis that inclusion does
not vary between males and females for biomarker CSF-1.


================================================
```

Results of hypothesis testing before removing outliers with 95% confidence:

[39]:
```
results_df
```

[39]:

	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	CSF-1
0.05	Can't Reject	Reject	Reject	Reject	Can't Reject	Can't Reject	Reject	Can't Reject	Reject

Details of hypothesis testing before removing outliers with 95% confidence: *The dash (-) means that H0 was not rejected*

[40]:
```
results_df1 = pd.DataFrame(data=results_dict).transpose()
results_df1
```

[40]:

	alpha	beta	power	t-stat	p-value	t-test result
IL-8	0.05	0.902156	0.0978439	-0.646581	0.519261	-
VEGF-A	0.05	0.465889	0.534111	-2.06075	0.0416201	Reject H0
OPG	0.05	0.223169	0.776831	-2.83788	0.00558852	Reject H0
TGF-beta-1	0.05	0.482739	0.517261	-2.02025	0.0456818	Reject H0
IL-6	0.05	0.945143	0.0548574	-0.207751	0.835801	-
CXCL9	0.05	0.909472	0.0905281	0.595527	0.552719	-
CXCL1	0.05	0.209845	0.790155	-2.79027	0.00616716	Reject H0
IL-18	0.05	0.776017	0.223983	1.21358	0.227463	-
CSF-1	0.05	0.267842	0.732158	-2.5896	0.0109716	Reject H0

3.1.5 The hypothesis test with Bonferroni correction after removing the outliers

[41]:
```
# We are running one test for each Biomarker so the number of tests equals the␣
 ↪number of Biomarkers
n_biomarkers = len(biomarker_columns) # The number of Biomarkers
alpha = 0.05 # The significance level chosen for the test
n_tests = n_biomarkers
# The probability of making at least one type I error P(X>=1) = 1 - P(X=0) = 1␣
 ↪- Bin(n_tests, alpha)
p_type1_err = 1 - st.binom.cdf(k=0, n=n_tests, p=alpha) # Equivalent of st.
 ↪binom.sf(k=0, n=n_tests, p=alpha)
print(f"Before the Bonferroni correction, the probability of making at least␣
 ↪one type I error by running the test {n_tests} times is {p_type1_err} ie.␣
 ↪{np.round(p_type1_err * 100, 0)}%")
```

```
Before the Bonferroni correction, the probability of making at least one type I
error by running the test 9 times is 0.3697505902753909 ie. 37.0%
```

The Bonferroni correction sets the significance cut-off at /n_tests so we would only reject the null hypothesis if the p-value is less than 0.0056. The downside of this correction is that when is decreased, the power decrease and the probability of making a Type II Error increases. This will be demonstrated in the table below.

https://www.stat.berkeley.edu/~mgoldman/Section0402.pdf

http://grants.hhp.coe.uh.edu/doconnor/PEP6305/Multiple%20t%20tests.htm

There are criticisms that the Bonferroni Correction is too conservative and it may lead us to reject some results which actually are meaningful. However, the important concept behind the method is the significance value needs to be adjusted when we make many comparisons.

https://towardsdatascience.com/the-multiple-comparisons-problem-e5573e8b9578

A Type I error is when we reject a true null hypothesis. Lower values of make it harder to reject the null hypothesis, so choosing lower values for can reduce the probability of a Type I error. The consequence here is that if the null hypothesis is false, it may be more difficult to reject using a low value for . So using lower values of can increase the probability of a Type II error.

A Type II error is when we fail to reject a false null hypothesis. Higher values of make it easier to reject the null hypothesis, so choosing higher values for can reduce the probability of a Type II error. The consequence here is that if the null hypothesis is true, increasing makes it more likely that we commit a Type I error (rejecting a true null hypothesis).

https://www.khanacademy.org/math/statistics-probability/significance-tests-one-sample/error-probabilities-and-power/a/consequences-errors-significance

As an alternative, Holm-Bonferroni formula for the first-ranked (smallest) p-value can be used instead.

Holm's Step-Down Procedure: An update of the Bonferroni correction, this procedure is more powerful. Rather than controlling the FMER, Holm's procedure controls for the false discovery rate (FDR) and performed after conducting all hypothesis tests and finding associated p-values at within a set.

https://towardsdatascience.com/an-overview-of-methods-to-address-the-multiple-comparison-problem-310427b3ba92

[42]:
```
alpha_bonf = alpha / n_tests
p_type1_err = 1 - st.binom.cdf(k=0, n=n_tests, p=alpha_bonf) # Equivalent of st.
↪binom.sf(k=0, n=n_tests, p=alpha_bonf)
print(f"After the Bonferroni correction, the probability of making at least one␣
↪type I error by running the test {n_tests} times becomes {np.
↪round(p_type1_err * 100, 0)}%")
```

```
After the Bonferroni correction, the probability of making at least one type I
error by running the test 9 times becomes 5.0%
```

[43]:
```
filter_condition = at_inclusion_filtered_stacked_df.Sex.str.match("Male")

# significance_levels = np.array([0.1, 0.05, 0.01]) # 90%, 95%, 99% Confidence
significance_levels = np.array([0.05]) # 95% Confidence

significance_levels = significance_levels / n_tests

results_df = pd.DataFrame(columns=biomarker_columns, index=significance_levels,␣
↪data=None)
results_dict = dict()

for alpha in significance_levels:
    print(f"The significance level alpha = {alpha}")
    for index, biomarker_column in enumerate(biomarker_columns):
        biomarker_column_filter = at_inclusion_filtered_stacked_df.Biomarker.
↪str.match(biomarker_column)
```

```
        biomarker_1_values_male = at_inclusion_filtered_stacked_df.
↪loc[filter_condition & biomarker_column_filter, "Value"]
        biomarker_1_values_female = at_inclusion_filtered_stacked_df.
↪loc[~filter_condition & biomarker_column_filter, "Value"]

        # Calculate the power of the test
        # The power of the test, is one minus the probability of a
        # type II error. Power is the probability that the test correctly
        # rejects the Null Hypothesis if the Alternative Hypothesis is true.
        pow_nobs1 = biomarker_1_values_male.shape[0]
        pow_nobs2 = biomarker_1_values_female.shape[0]
        pow_ratio = pow_nobs2 / pow_nobs1
        pow_mean_1 = np.mean(biomarker_1_values_male)
        pow_mean_2 = np.mean(biomarker_1_values_female)
        pow_var_1 = np.var(biomarker_1_values_male, ddof=1)
        pow_var_2 = np.var(biomarker_1_values_female, ddof=1)
        pow_effect_size = (pow_mean_1 - pow_mean_2) / np.sqrt(((pow_nobs1 - 1)␣
↪* pow_var_1 + (pow_nobs2 - 1) * pow_var_2) / (pow_nobs1 + pow_nobs2 - 2))
        power = smt.power.tt_ind_solve_power(effect_size=pow_effect_size,␣
↪nobs1=pow_nobs1, alpha=alpha, power=None, ratio=pow_ratio,␣
↪alternative='two-sided')
        beta = 1 - power # Probability of a Type II error - Not rejecting a␣
↪false H0

        # Sample bigger than 30 observation but the variance of the population␣
↪from which the samples are extracted is unknown.
        print(f"Testing hypothesis for biomarker {biomarker_column}:")
        print(f"n_male = {biomarker_1_values_male.shape[0]};␣
↪n_female={biomarker_1_values_female.shape[0]};")
        print(f"H0: At inclusion, levels of biomarker {biomarker_column} do not␣
↪vary between males and females.  -male =  -female")
        print(f"H1: At inclusion, levels of biomarker {biomarker_column} do␣
↪vary between males and females.  -male   -female")

        tstat, pvalue = st.ttest_ind(biomarker_1_values_male,␣
↪biomarker_1_values_female, equal_var=False)
        print(f"t-stat = {tstat}, p-value = {pvalue}, alpha = {alpha}, beta =␣
↪{beta}, power={power}")

        # Store the results of the test for later
        results_dict[f"{biomarker_column}{' ( =' + str(np.round(alpha, 4))+')'␣
↪if len(significance_levels) > 1 else ''}"] = {
            "alpha": alpha,
            "beta": beta,
            "power": power,
            "t-stat": tstat,
```

```
            "p-value": pvalue,
            "t-test result": "Reject H0" if pvalue < alpha else " - "
        }

        if pvalue < alpha:
            results_df.loc[alpha, biomarker_column] = "Reject"
            print(f"We reject the null hypothesis <=> we reject the hypothesis␣
↪that inclusion does not vary between males and females for biomarker␣
↪{biomarker_column}.")
        else:
            results_df.loc[alpha, biomarker_column] = "Can't Reject"
            print(f"We don't have enough evidence to reject the null hypothesis␣
↪for biomarker {biomarker_column}.")
        print("\n")
    print("================================================\n")
```

```
The significance level alpha = 0.005555555555555556
Testing hypothesis for biomarker IL-8:
n_male = 59; n_female=57;
H0: At inclusion, levels of biomarker IL-8 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-8 do vary between males and females.
 -male   -female
t-stat = -0.646581135107931, p-value = 0.5192605138647244, alpha =
0.005555555555555556, beta = 0.9834867301884093, power=0.016513269811590688
We don't have enough evidence to reject the null hypothesis for biomarker IL-8.


Testing hypothesis for biomarker VEGF-A:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker VEGF-A do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker VEGF-A do vary between males and females.
 -male   -female
t-stat = -2.0607498156961435, p-value = 0.04162010149428021, alpha =
0.005555555555555556, beta = 0.7717235996349865, power=0.22827640036501345
We don't have enough evidence to reject the null hypothesis for biomarker
VEGF-A.


Testing hypothesis for biomarker OPG:
n_male = 50; n_female=57;
H0: At inclusion, levels of biomarker OPG do not vary between males and females.
 -male =  -female
H1: At inclusion, levels of biomarker OPG do vary between males and females.
 -male   -female
t-stat = -2.8378755290604567, p-value = 0.005588517883116755, alpha =
```

```
0.005555555555555556, beta = 0.5304709901559068, power=0.46952900984409324
We don't have enough evidence to reject the null hypothesis for biomarker OPG.


Testing hypothesis for biomarker TGF-beta-1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker TGF-beta-1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker TGF-beta-1 do vary between males and
females.  -male   -female
t-stat = -2.020246829300327, p-value = 0.04568177954693594, alpha =
0.005555555555555556, beta = 0.7842181990638615, power=0.21578180093613852
We don't have enough evidence to reject the null hypothesis for biomarker TGF-
beta-1.


Testing hypothesis for biomarker IL-6:
n_male = 59; n_female=55;
H0: At inclusion, levels of biomarker IL-6 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-6 do vary between males and females.
 -male   -female
t-stat = -0.20775059411147945, p-value = 0.8358011902025527, alpha =
0.005555555555555556, beta = 0.9934447041293836, power=0.006555295870616483
We don't have enough evidence to reject the null hypothesis for biomarker IL-6.


Testing hypothesis for biomarker CXCL9:
n_male = 58; n_female=56;
H0: At inclusion, levels of biomarker CXCL9 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL9 do vary between males and females.
 -male   -female
t-stat = 0.5955269953821581, p-value = 0.5527192103280785, alpha =
0.005555555555555556, beta = 0.9853219008958818, power=0.014678099104118207
We don't have enough evidence to reject the null hypothesis for biomarker CXCL9.


Testing hypothesis for biomarker CXCL1:
n_male = 59; n_female=58;
H0: At inclusion, levels of biomarker CXCL1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CXCL1 do vary between males and females.
 -male   -female
t-stat = -2.7902717386040914, p-value = 0.006167164236984985, alpha =
0.005555555555555556, beta = 0.5116606652882942, power=0.4883393347117057
We don't have enough evidence to reject the null hypothesis for biomarker CXCL1.
```

```
Testing hypothesis for biomarker IL-18:
n_male = 59; n_female=56;
H0: At inclusion, levels of biomarker IL-18 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker IL-18 do vary between males and females.
 -male   -female
t-stat = 1.2135809065078154, p-value = 0.2274631494235096, alpha =
0.005555555555555556, beta = 0.9434316668319106, power=0.05656833316808936
We don't have enough evidence to reject the null hypothesis for biomarker IL-18.


Testing hypothesis for biomarker CSF-1:
n_male = 59; n_female=57;
H0: At inclusion, levels of biomarker CSF-1 do not vary between males and
females.  -male =  -female
H1: At inclusion, levels of biomarker CSF-1 do vary between males and females.
 -male   -female
t-stat = -2.5895995332020694, p-value = 0.010971581561811593, alpha =
0.005555555555555556, beta = 0.585286631111086, power=0.4147133688889139
We don't have enough evidence to reject the null hypothesis for biomarker CSF-1.


==============================================
```

Details of hypothesis testing with Bonferroni correction and 95% confidence: *The dash (-) means that H0 was not rejected*

[44]:
```
results_df2 = pd.DataFrame(data=results_dict).transpose()
results_df2
```

[44]:

	alpha	beta	power	t-stat	p-value	t-test result
IL-8	0.00555556	0.983487	0.0165133	-0.646581	0.519261	-
VEGF-A	0.00555556	0.771724	0.228276	-2.06075	0.0416201	-
OPG	0.00555556	0.530471	0.469529	-2.83788	0.00558852	-
TGF-beta-1	0.00555556	0.784218	0.215782	-2.02025	0.0456818	
IL-6	0.00555556	0.993445	0.0065553	-0.207751	0.835801	
CXCL9	0.00555556	0.985322	0.0146781	0.595527	0.552719	
CXCL1	0.00555556	0.511661	0.488339	-2.79027	0.00616716	-
IL-18	0.00555556	0.943432	0.0565683	1.21358	0.227463	
CSF-1	0.00555556	0.585287	0.414713	-2.5896	0.0109716	-

```
TGF-beta-1              -
IL-6                    -
CXCL9                   -
CXCL1                   -
IL-18                   -
CSF-1                   -
```

Plot of the values of Beta with and without Bonferroni correction

[45]:
```
plt.figure(figsize=(10,6))
ax = plt.subplot()
_beta_results_df = pd.DataFrame([],
            columns=["Beta without Bonferroni correction", "Beta with␣
 ↪Bonferroni correction"])
_beta_results_df["Beta without Bonferroni correction"] = results_df2["beta"].
 ↪astype(np.float32)
_beta_results_df["Beta with Bonferroni correction"] = results_df1["beta"].
 ↪astype(np.float32).T.values
_ = sn.lineplot(data=_beta_results_df, markers=True, ax=ax, palette="Reds")
```

4 Question 2

4.1 Regression modelling

The merged table of covariates and biomarkers

[46]:
```
regression_df = joined_df.copy()
regression_df.head()
```

[46]:

	PatientID	Age	Sex	Smoker	VAS0	VAS12	Timepoint	Biomarker	IL-8	\
0	1	56	Male	No	3.0	4.0	0weeks	1-0weeks	8.13	
1	1	56	Male	No	3.0	4.0	6weeks	1-6weeks	7.09	
2	1	56	Male	No	3.0	4.0	12months	1-12months	8.64	
3	3	32	Male	No	7.2	0.5	0weeks	3-0weeks	6.55	
4	3	32	Male	No	7.2	0.5	6weeks	3-6weeks	6.40	

	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	CSF-1
0	12.35	10.48	8.66	2.63	6.54	9.54	8.53	8.27
1	11.61	10.35	7.59	2.25	7.84	9.44	8.42	8.19
2	12.48	10.68	8.46	2.56	6.64	9.59	8.79	8.41
3	11.21	10.49	6.83	2.58	5.31	6.71	7.71	8.30
4	11.20	10.61	6.65	2.25	5.51	6.62	7.79	8.35

Identification and removal of NULL values

Identify NA's and remove them regression_df.isna().sum()

[47]:
```
# Drop the NAs
regression_df.drop(index=regression_df[regression_df["VAS12"].isna()].index,␣
↪inplace=True)
```

Removing values of timepoints other than Week 0 (at inclusion)

[48]:
```
# Keep only biomarker levels at inclusion => Timepoint is 0weeks
regression_df = regression_df[regression_df.Timepoint.str.match("0weeks")]
regression_df.head()
```

[48]:

	PatientID	Age	Sex	Smoker	VAS0	VAS12	Timepoint	Biomarker	IL-8	\
0	1	56	Male	No	3.0	4.0	0weeks	1-0weeks	8.13	
3	3	32	Male	No	7.2	0.5	0weeks	3-0weeks	6.55	
6	4	43	Female	No	2.7	0.5	0weeks	4-0weeks	6.47	
9	5	25	Female	No	3.0	3.9	0weeks	5-0weeks	6.41	
12	6	39	Male	No	3.5	5.0	0weeks	6-0weeks	6.54	

	VEGF-A	OPG	TGF-beta-1	IL-6	CXCL9	CXCL1	IL-18	CSF-1
0	12.35	10.48	8.66	2.63	6.54	9.54	8.53	8.27
3	11.21	10.49	6.83	2.58	5.31	6.71	7.71	8.30
6	11.13	10.72	6.90	5.62	5.46	7.73	8.02	8.19
9	11.15	10.60	7.26	1.96	5.35	7.15	7.66	8.10
12	11.47	10.20	7.16	3.38	6.51	7.79	8.54	8.35

Removing unecessary columns

[49]:
```
# Remove columns PatientID, Timepoint and Biomarker as they are not needed for␣
 ↪our regression model
regression_df.drop(columns=["PatientID", "Timepoint", "Biomarker"],␣
 ↪inplace=True)
regression_df.head()
```

[49]:

	Age	Sex	Smoker	VAS0	VAS12	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	\
0	56	Male	No	3.0	4.0	8.13	12.35	10.48	8.66	2.63	
3	32	Male	No	7.2	0.5	6.55	11.21	10.49	6.83	2.58	
6	43	Female	No	2.7	0.5	6.47	11.13	10.72	6.90	5.62	
9	25	Female	No	3.0	3.9	6.41	11.15	10.60	7.26	1.96	
12	39	Male	No	3.5	5.0	6.54	11.47	10.20	7.16	3.38	

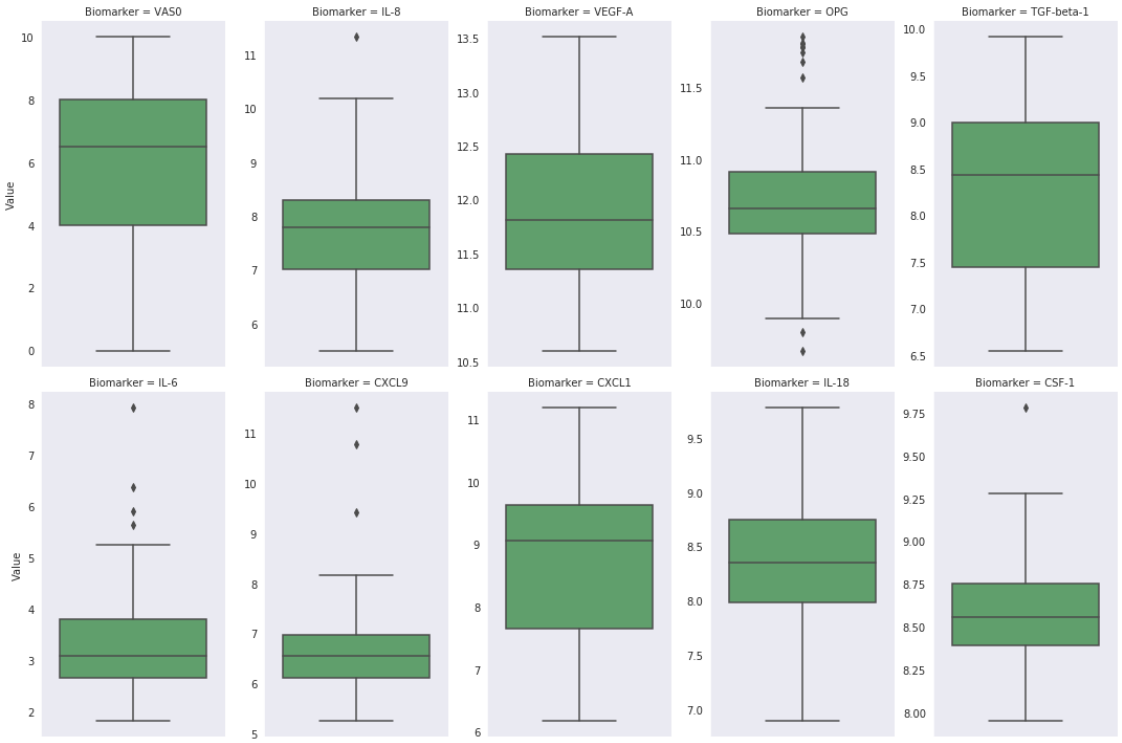
	CXCL9	CXCL1	IL-18	CSF-1
0	6.54	9.54	8.53	8.27
3	5.31	6.71	7.71	8.30
6	5.46	7.73	8.02	8.19
9	5.35	7.15	7.66	8.10
12	6.51	7.79	8.54	8.35

[50]:
```
print(f"Explanatory variables: {regression_df.columns}")
```

```
Explanatory variables: Index(['Age', 'Sex', 'Smoker', 'VAS0', 'VAS12', 'IL-8',
'VEGF-A', 'OPG',
       'TGF-beta-1', 'IL-6', 'CXCL9', 'CXCL1', 'IL-18', 'CSF-1'],
      dtype='object')
```

4.1.1 Boxplots of the different variables for visual inspection

[51]:
```
regression_stacked_df = regression_df.loc[:, ["VAS0", *biomarker_columns]].
 ↪stack(0).reset_index()
regression_stacked_df = regression_stacked_df.iloc[:, 1:]
regression_stacked_df.columns = ["Biomarker", "Value"]

with sn.axes_style("dark"):
    g = sn.catplot(y="Value", col="Biomarker", data=regression_stacked_df,␣
 ↪kind="box",
                   height=5, aspect=.6, sharex=False, sharey=False, col_wrap=5,␣
 ↪palette=sn.color_palette("summer", 2));
    g.set_axis_labels("")
plt.savefig("./Charts/reg_1.png")
```



Converting categorical values to numeric

[52]:
```
# Convert categorical variable into dummy/indicator variables. The process is␣
↪calle Binarization.
# regression_df = pd.get_dummies(regression_df, columns=["Smoker", "Sex"],␣
↪prefix=["Smoker", "Sex"])
regression_df["Smoker"] = regression_df["Smoker"].cat.codes
regression_df["Sex"] = regression_df["Sex"].cat.codes
regression_df.head()
```

[52]:

	Age	Sex	Smoker	VAS0	VAS12	IL-8	VEGF-A	OPG	TGF-beta-1	IL-6	\
0	56	0	1	3.0	4.0	8.13	12.35	10.48	8.66	2.63	
3	32	0	1	7.2	0.5	6.55	11.21	10.49	6.83	2.58	
6	43	1	1	2.7	0.5	6.47	11.13	10.72	6.90	5.62	
9	25	1	1	3.0	3.9	6.41	11.15	10.60	7.26	1.96	
12	39	0	1	3.5	5.0	6.54	11.47	10.20	7.16	3.38	

	CXCL9	CXCL1	IL-18	CSF-1
0	6.54	9.54	8.53	8.27
3	5.31	6.71	7.71	8.30
6	5.46	7.73	8.02	8.19
9	5.35	7.15	7.66	8.10
12	6.51	7.79	8.54	8.35

Summary of the variables in use for the modelling

[53]:
```
regression_df.describe().round(3)
```

[53]:
```
            Age      Sex   Smoker     VAS0    VAS12     IL-8   VEGF-A      OPG  \
count   115.000  115.000  115.000  115.000  115.000  115.000  115.000  115.000
mean     41.009    0.496    0.670    5.998    3.634    7.726   11.879   10.732
std      10.170    0.502    0.472    2.689    3.090    0.894    0.674    0.425
min      18.000    0.000    0.000    0.000    0.000    5.500   10.600    9.670
25%      32.000    0.000    0.000    4.000    0.700    7.010   11.355   10.480
50%      41.000    0.000    1.000    6.500    3.500    7.790   11.810   10.660
75%      49.000    1.000    1.000    8.000    6.000    8.290   12.425   10.910
max      59.000    1.000    1.000   10.000   10.000   11.330   13.510   11.850

        TGF-beta-1     IL-6    CXCL9    CXCL1    IL-18    CSF-1
count      115.000  115.000  115.000  115.000  115.000  115.000
mean         8.236    3.345    6.625    8.672    8.376    8.599
std          0.929    0.998    0.902    1.238    0.601    0.289
min          6.550    1.810    5.250    6.170    6.890    7.950
25%          7.445    2.655    6.110    7.655    7.985    8.390
50%          8.430    3.080    6.560    9.070    8.350    8.560
75%          8.995    3.790    6.970    9.635    8.750    8.755
max          9.910    7.910   11.510   11.190    9.780    9.780
```

[54]:
```
# regression_df.to_csv("regression_df.csv", index=False)
```

Separating the dependant from the independant variables

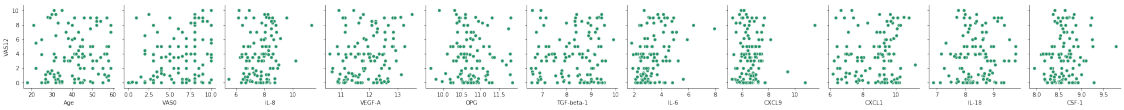
[55]:
```
# 12-month VAS as the response variable => y=VAS12
# biomarker levels at inclusion and covariates as explanatory variables
y = regression_df["VAS12"]
X = regression_df.drop(columns="VAS12")
X.head()
```

[55]:
```
    Age  Sex  Smoker  VAS0  IL-8  VEGF-A    OPG  TGF-beta-1  IL-6  CXCL9  \
0    56    0       1   3.0  8.13   12.35  10.48        8.66  2.63   6.54
3    32    0       1   7.2  6.55   11.21  10.49        6.83  2.58   5.31
6    43    1       1   2.7  6.47   11.13  10.72        6.90  5.62   5.46
9    25    1       1   3.0  6.41   11.15  10.60        7.26  1.96   5.35
12   39    0       1   3.5  6.54   11.47  10.20        7.16  3.38   6.51

    CXCL1  IL-18  CSF-1
0    9.54   8.53   8.27
3    6.71   7.71   8.30
6    7.73   8.02   8.19
9    7.15   7.66   8.10
12   7.79   8.54   8.35
```

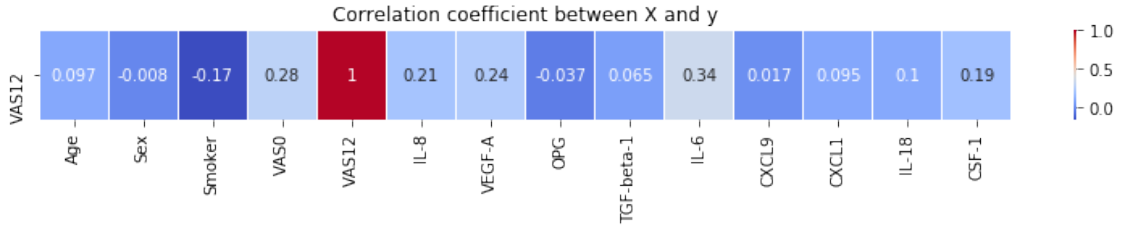
4.1.2 Plotting the correlation between the independent and the dependent variables

[56]:
```
X_non_categorical_columns = list(X.columns)
del(X_non_categorical_columns[1])
del(X_non_categorical_columns[1])
plt.figure(figsize=(30,10))
sn.pairplot(regression_df, y_vars=["VAS12"], x_vars=X_non_categorical_columns)
plt.savefig("./Charts/reg_2.png")
```

```
<Figure size 2160x720 with 0 Axes>
```



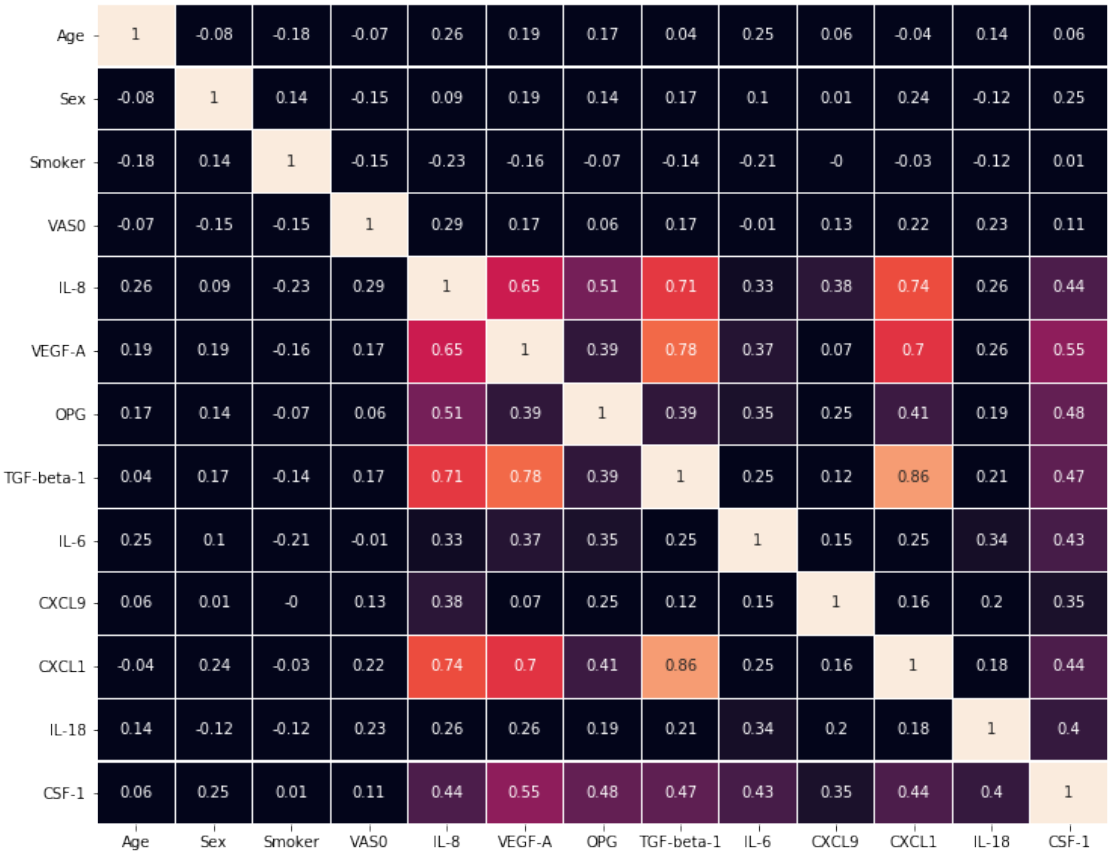
[57]:
```
plt.figure(figsize=(11,2))
variables_corr = pd.DataFrame(regression_df.corr()["VAS12"]).T.round(3)
sn.heatmap(variables_corr, annot=True, linewidth=.1, cmap="coolwarm")
plt.tight_layout()
plt.title("Correlation coefficient between X and y")
plt.savefig("./Charts/reg_3.png")
```



Search for outliers, the outliers identified earlier were withing the same Biomarker for two different samples so we cannot use the previous method and instead we will have to search for outliers in the entire sample of each Biomarker without disaggregation by sex.

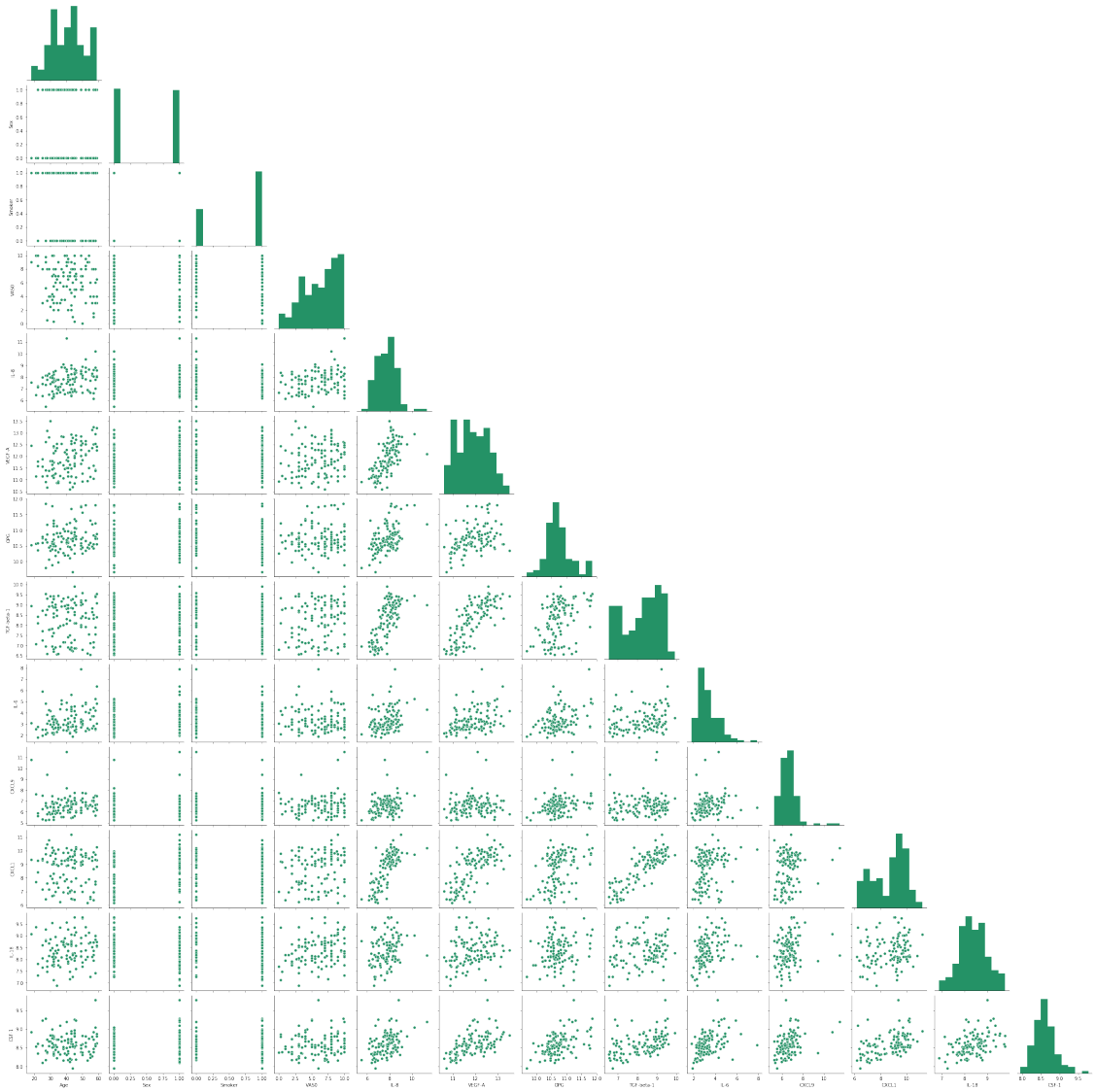
4.1.3 Plotting the correlation between the independent variables

[58]:
```
plt.figure(figsize=(12,10))
variables_corr = X.corr().round(2)
sn.heatmap(variables_corr, annot=True, linewidths=.1, vmin=0.3, cbar=False)
plt.savefig("./Charts/reg_6.png")
```



[59]:
```
sn.pairplot(X.loc[:, "Age":"CSF-1"], corner=True)
```

[59]: <seaborn.axisgrid.PairGrid at 0x1a24840890>

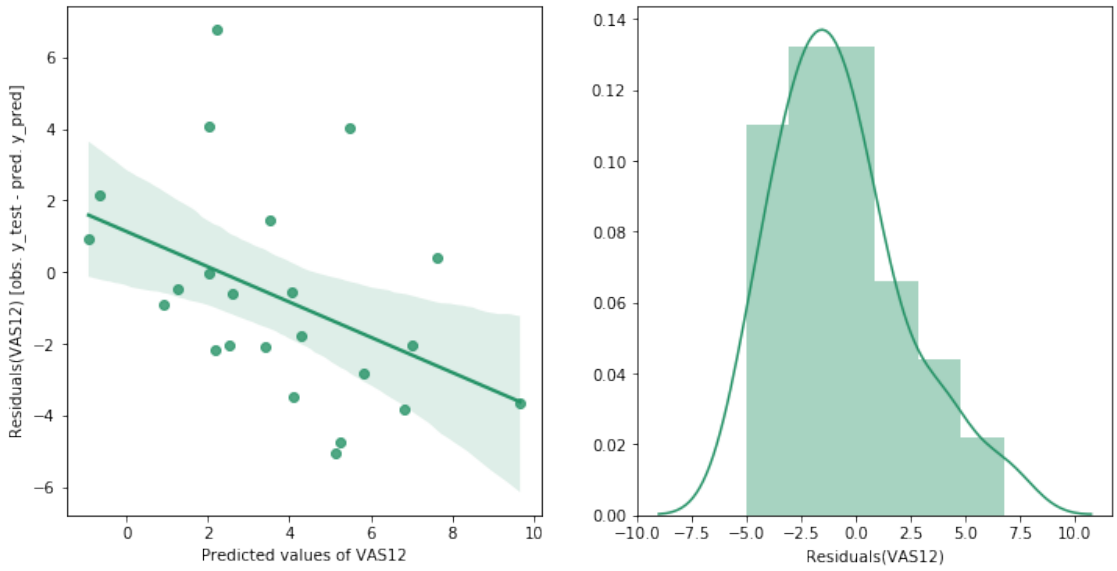


4.1.4 Creating and fitting the Regression model

[60]:
```
from sklearn.model_selection import train_test_split
from sklearn.linear_model import LinearRegression
from sklearn.metrics import mean_squared_error, r2_score

X_train, X_test, y_train, y_test = train_test_split(X, y, train_size=0.80,␣
 ↪random_state=14)
regressor = LinearRegression().fit(X=X_train, y=y_train)
y_pred = regressor.predict(X_test)

print(f"Coeficients: {regressor.coef_}")
print(f"Intercept: {regressor.intercept_}")
```

```
print(f"Rank: {regressor.rank_}")
print(f"Singular: {regressor.singular_}")

# The best possible score is 1.0 and it can be negative (because the model can␣
 ↪be arbitrarily worse).
# A constant model that always predicts the expected value of y, disregarding␣
 ↪the input features, would get a R^2 score of 0.0.
print(f"R^2 for training data: {regressor.score(X_train,y_train)}") # the␣
 ↪coefficient of determination R^2 of the prediction.
print(f"R^2 for test data: {regressor.score(X_test,y_test)}")

# The mean squared error
print('Mean squared error: %.2f'
      % mean_squared_error(y_test, y_pred))
# The coefficient of determination: 1 is perfect prediction
print('Coefficient of determination for test data: %.2f'
      % r2_score(y_test, y_pred))

residuals = y_test - y_pred

plt.figure(figsize=(12,6))
ax = plt.subplot(1,2,1)
sn.regplot(x=y_pred, y=residuals, ax=ax)
ax.set_ylabel("Residuals(VAS12) [obs. y_test - pred. y_pred]")
ax.set_xlabel("Predicted values of VAS12")

ax = plt.subplot(1,2,2)
sn.distplot(residuals)
ax.set_xlabel("Residuals(VAS12)")
plt.savefig("./Charts/reg_5.png")
```

```
Coeficients: [-0.01950044 -0.31753944 -0.15808482  0.29200592  1.11739608
1.48879414
 -2.12617271 -1.79453479  1.25436735 -0.74323047  0.08640174 -0.39275316
  2.43405859]
Intercept: -3.2927544498401584
Rank: 13
Singular: [94.03703773 25.84102834 16.56856551  9.11316398  7.72526429
5.23909697
  4.73472988  4.32085826  4.11422903  3.77958661  3.24704422  2.93553549
  1.75023359]
R^2 for training data: 0.364759585430106
R^2 for test data: -0.04805081169991877
Mean squared error: 8.93
Coefficient of determination for test data: -0.05
```



Verify the results of the previous model using an alternative library

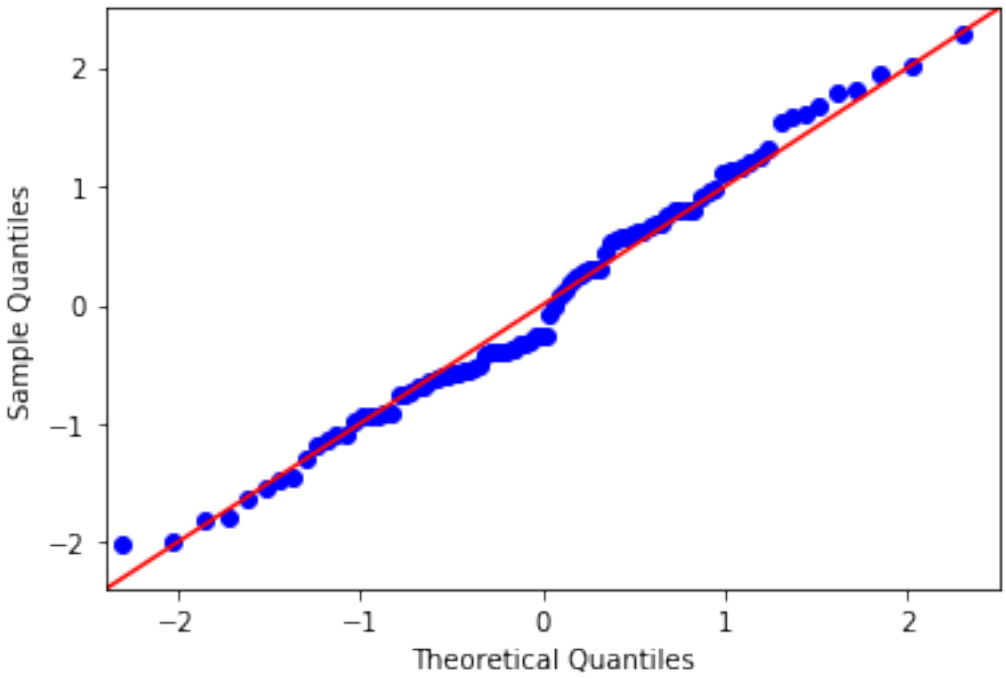
[61]:
```
_n = X_train.shape[0]
_X_train = np.c_[np.ones(_n), X_train]
_X_train = pd.DataFrame(_X_train, columns=["Intercept", *(X_train.columns)])
_ols = OLS(y_train,_X_train.values)
_olsf = _ols.fit()
_olsf.summary(xname=list(_X_train.columns))
```

[61]:
```
<class 'statsmodels.iolib.summary.Summary'>
"""
                            OLS Regression Results
==============================================================================
Dep. Variable:                  VAS12   R-squared:                       0.365
Model:                            OLS   Adj. R-squared:                  0.259
Method:                 Least Squares   F-statistic:                     3.445
Date:                Sat, 29 Feb 2020   Prob (F-statistic):           0.000315
Time:                        16:35:10   Log-Likelihood:                -213.65
No. Observations:                  92   AIC:                             455.3
Df Residuals:                      78   BIC:                             490.6
Df Model:                          13
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept     -3.2928     10.893     -0.302      0.763     -24.980      18.394
Age           -0.0195      0.034     -0.570      0.571      -0.088       0.049
Sex           -0.3175      0.618     -0.514      0.609      -1.549       0.913
```

```
Smoker        -0.1581      0.660     -0.239      0.811      -1.472       1.156
VAS0           0.2920      0.114      2.556      0.013       0.065       0.519
IL-8           1.1174      0.620      1.801      0.076      -0.118       2.353
VEGF-A         1.4888      0.761      1.957      0.054      -0.026       3.003
OPG           -2.1262      0.827     -2.572      0.012      -3.772      -0.481
TGF-beta-1    -1.7945      0.714     -2.515      0.014      -3.215      -0.374
IL-6           1.2544      0.379      3.306      0.001       0.499       2.010
CXCL9         -0.7432      0.377     -1.972      0.052      -1.494       0.007
CXCL1          0.0864      0.539      0.160      0.873      -0.986       1.159
IL-18         -0.3928      0.543     -0.723      0.472      -1.474       0.688
CSF-1          2.4341      1.462      1.664      0.100      -0.478       5.346
==============================================================================
Omnibus:                        3.025   Durbin-Watson:                   1.649
Prob(Omnibus):                  0.220   Jarque-Bera (JB):                2.033
Skew:                           0.160   Prob(JB):                        0.362
Kurtosis:                       2.345   Cond. No.                     1.93e+03
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
specified.
[2] The condition number is large, 1.93e+03. This might indicate that there are
strong multicollinearity or other numerical problems.
"""
```

Plot a QQ-Plot of the residuals This plot is used to visualise the normality of the residuals

[64]:
```
fig = sma.qqplot(_olsf.resid, st.t, fit=True, line='45')
plt.show()
```



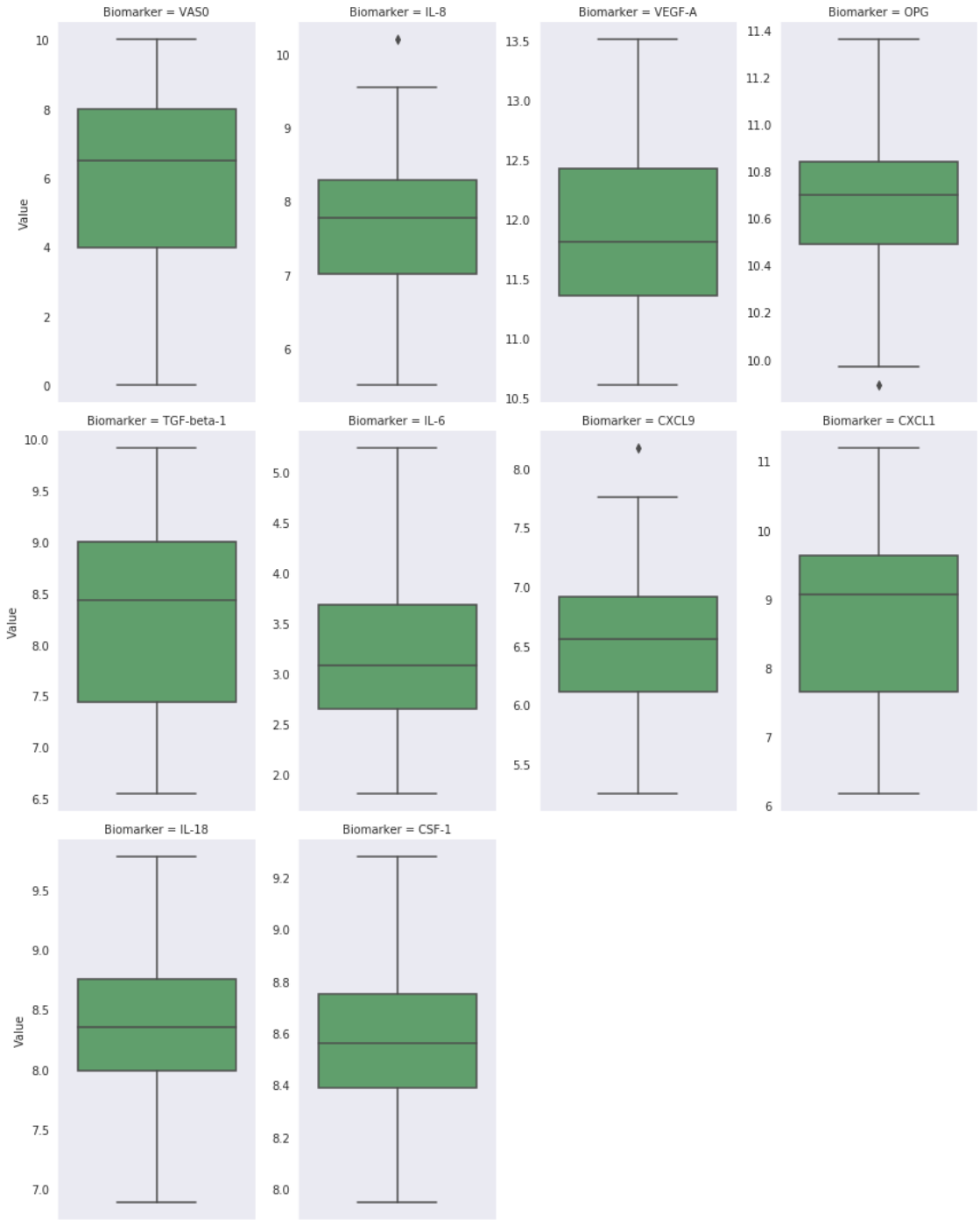
4.1.5 Try to fit the model again but after adjusting the data

Standardization is useful when your data has varying scales and the algorithm you are using does make assumptions about your data having a Gaussian distribution, such as linear regression, logistic regression, and linear discriminant analysis.

Scaling using median and quantiles consists of subtracting the median to all the observations and then dividing by the interquartile difference. It Scales features using statistics that are robust to outliers. *https://medium.com/@swethalakshmanan14/how-when-and-why-should-you-normalize-standardize-rescale-your-data-3f083def38ff https://scikit-learn.org/stable/auto_examples/preprocessing/plot_all_scaling.html#sphx-glr-auto-examples-preprocessing-plot-all-scaling-py https://scikit-learn.org/stable/modules/preprocessing.html#preprocessing-scaler*

[65]:
```
Q1_X = X.quantile(0.25)
Q3_X = X.quantile(0.75)
IQR_X = Q3_X - Q1_X

filter_X = (X < (Q1_X - 1.5 * IQR_X)) | (X > (Q3_X + 1.5 * IQR_X))

filtered_X = X.iloc[:,3:].mask(filter_X.iloc[:,3:]).fillna(X.iloc[:,3:].
 ↪mean(skipna=True))
filtered_X = X.iloc[:,0:3].join(filtered_X)
```

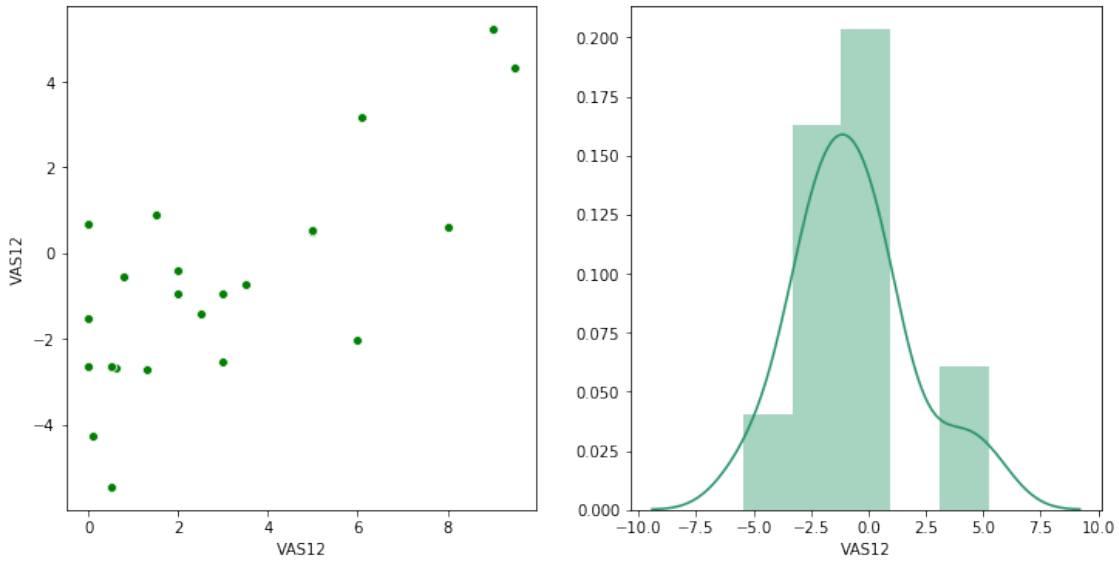
[66]:
```
regression_stacked_df = filtered_X.loc[:, ["VAS0", *biomarker_columns]].
↪stack(0).reset_index()
regression_stacked_df = regression_stacked_df.iloc[:, 1:]
regression_stacked_df.columns = ["Biomarker", "Value"]

with sn.axes_style("dark"):
    g = sn.catplot(y="Value", col="Biomarker", data=regression_stacked_df,␣
↪kind="box",
                   height=5, aspect=.6, sharex=False, sharey=False, col_wrap=4,␣
↪palette=sn.color_palette("summer", 2));
    g.set_axis_labels("")
```



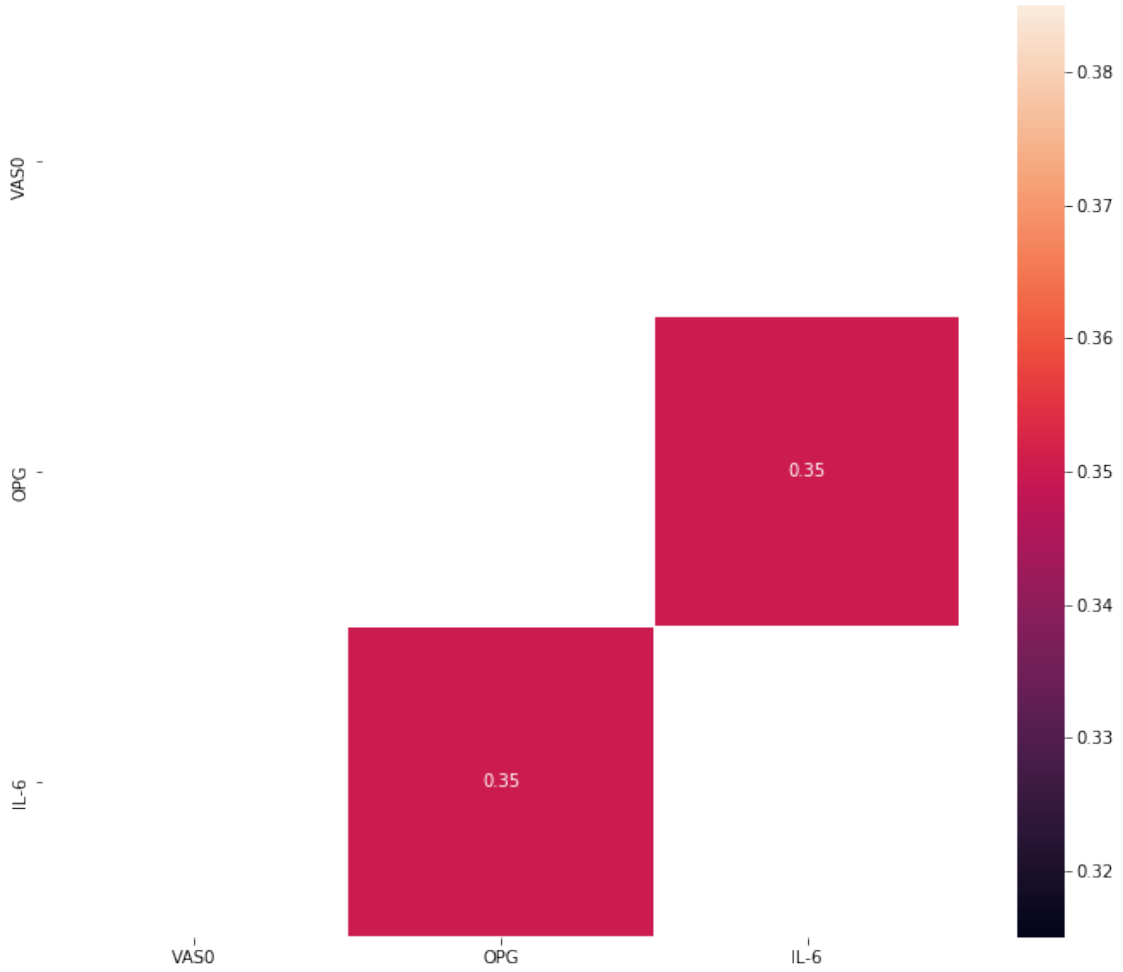
[67]:
```
from sklearn.preprocessing import RobustScaler, StandardScaler
from sklearn.model_selection import train_test_split

# Fit the model only using VASO, OPG and IL-6 variables
X_mod = X[["VASO", "OPG", "IL-6"]]
```

```
# Scale the data using a Standard Scaler to avoid giving more weight to one␣
 ↪variable then the other
X_scaled = StandardScaler().fit_transform(X_mod)
X_train, X_test, y_train, y_test = train_test_split(X_scaled, y, train_size=0.
 ↪8, random_state=14)

regressor = LinearRegression().fit(X=X_train, y=y_train)
y_pred = regressor.predict(X_test)
print(f"Coeficients: {regressor.coef_}")
print(f"Intercept: {regressor.intercept_}")
print(f"Rank: {regressor.rank_}")
print(f"Singular: {regressor.singular_}")

# The best possible score is 1.0 and it can be negative (because the model can␣
 ↪be arbitrarily worse).
# A constant model that always predicts the expected value of y, disregarding␣
 ↪the input features, would get a R^2 score of 0.0.
print(f"R^2 for training data: {regressor.score(X_train,y_train)}") # the␣
 ↪coefficient of determination R^2 of the prediction.
print(f"R^2 for test data: {regressor.score(X_test,y_test)}")

# The mean squared error for the predicted values
print('Mean squared error: %.2f' % mean_squared_error(y_test, y_pred))

# The coefficient of determination of the predicted values: 1 is perfect␣
 ↪prediction
print('Coefficient of determination: %.2f' % r2_score(y_test, y_pred))

residuals = y_test - y_pred

plt.figure(figsize=(12,6))
ax = plt.subplot(1,2,1)
sn.scatterplot(x=y_test, y=residuals, ax=ax, color="green")
ax = plt.subplot(1,2,2)
_ = sn.distplot(residuals)
```

```
Coeficients: [ 0.89502985 -0.64238208  1.42587032]
Intercept: 3.76830567228621
Rank: 3
Singular: [11.07151384  9.57399617  7.04310514]
R^2 for training data: 0.2266573053692228
R^2 for test data: 0.22182503424161068
Mean squared error: 6.63
Coefficient of determination: 0.22
```



[68]:
```
plt.figure(figsize=(12,10))
variables_corr = X_mod.corr().round(2)
_ = sn.heatmap(variables_corr[(variables_corr.abs()>=.3) & (variables_corr.
↪abs()<1)], annot=True, linewidths=.1)
```



Verify the results of the previous model using an alternative library

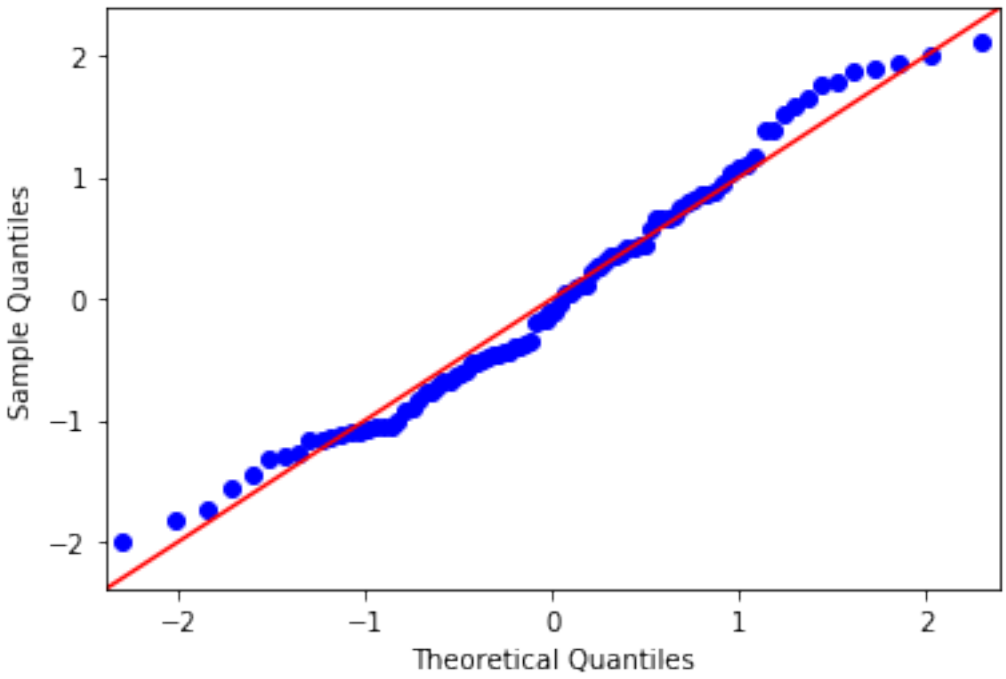
[69]:
```
_n = X_train.shape[0]
_X_train = np.c_[np.ones(_n), X_train]
_X_train = pd.DataFrame(_X_train, columns=["Intercept", "VAS0", "OPG", "IL-6"])
_ols = OLS(y_train,_X_train.values)
_olsf = _ols.fit()
_olsf.summary(xname=list(_X_train.columns))
```

[69]:
```
<class 'statsmodels.iolib.summary.Summary'>
"""
                            OLS Regression Results
==============================================================================
Dep. Variable:                  VAS12   R-squared:                       0.227
Model:                            OLS   Adj. R-squared:                  0.200
Method:                 Least Squares   F-statistic:                     8.597
Date:                Sat, 29 Feb 2020   Prob (F-statistic):           4.57e-05
```

```
Time:                        16:36:16   Log-Likelihood:                -222.70
No. Observations:                  92   AIC:                             453.4
Df Residuals:                      88   BIC:                             463.5
Df Model:                           3
Covariance Type:            nonrobust
==============================================================================
                 coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
Intercept      3.7683      0.291     12.950      0.000       3.190       4.347
VAS0           0.8950      0.292      3.062      0.003       0.314       1.476
OPG           -0.6424      0.322     -1.996      0.049      -1.282      -0.003
IL-6           1.4259      0.339      4.204      0.000       0.752       2.100
==============================================================================
Omnibus:                        5.477   Durbin-Watson:                   1.653
Prob(Omnibus):                  0.065   Jarque-Bera (JB):                3.299
Skew:                           0.270   Prob(JB):                        0.192
Kurtosis:                       2.245   Cond. No.                         1.58
==============================================================================

Warnings:
[1] Standard Errors assume that the covariance matrix of the errors is correctly
specified.
"""
```

Plot a QQ-Plot of the residuals This plot is used to visualise the normality of the residuals

[70]:
```
fig = sma.qqplot(_olsf.resid, st.t, fit=True, line='45')
plt.show()
```


Sample Quantiles
Theoretical Quantiles
2
1
0
−1
−2
−2
−1
0
1
2

[ ]: