

Probability and Statistics Final Report

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2025-11-11

Introduction

This report addresses the statistical analysis and regression modelling of 9 randomly selected biomarkers related to inflammation in patients with disc herniation (Moen, 2016). The original data contains levels of 9 biomarkers at inclusion, at 6 weeks and at 12 months along with covariates such as Patient age, sex, smoking status and their reported pain on a scale from 1 to 10 at inclusion and after 12 months (VAS).

Among other covariates, understanding the effect of sex and gender on health is instrumental in delivering appropriate treatment and tailoring dosages of drugs. The first requirement to include women as well as men in medical trials and result analysis was 30 years ago, and progress has been slow and the possibility of sex-specific treatment response is still understudied (Mazure and Jones, 2015). Studying the biomarker levels by sex can help develop new approaches to prevention, diagnosis and treatment specific to patients' sex. If biomarker levels differ between males and females, it suggests a different patophysiology of the condition for men and women. This can help in future applications to advise whether the treatment needs to be tailored based on sex. To narrow down the research question, only biomarker levels at inclusion will be analysed. Hence, the first part of this report will focus on the question: Do the levels of each biomarker vary between males and females at inclusion? The following hypotheses were constructed to answer this question:

- H0: Mean level of biomarker X at inclusion is the same for males and females.
- H1: Mean level of biomarker X at inclusion differs between males and females.

In the second part of the report, a regression model will be constructed to make predictions on the patients' recovery (pain after 12 months) based on the available covariates, including patients' sex, age, smoking, and pain levels.

Methods

Data manipulation, statistical hypothesis testing and regression modelling were performed in R. The full scripts are available in the Appendix.

Data Manipulation

The original datasets biomarkers.xlsx and covariates.xlsx were collated into a single dataframe using left join to only keep patients with complete biomarker values and saved as a csv file “biomarkers_covariates_clean.csv”.

The column names were simplified for easier manipulation, and only values of biomarkers at inclusion were selected for the purposes of the statistical hypothesis testing and regression modelling described below.

2 NAs were found in VAS at 12 months (pain levels after one year), these observations were deleted for the regression modelling. Normality was checked visually with histograms to make assumptions for statistical tests used below.

The R script used for the data manipulation is available in Appendix 1.

Statistical Hypothesis testing

Hypotheses:

- H0: Mean level of biomarker X at inclusion is the same for males and females.
- H1: Mean level of biomarker X at inclusion differs between males and females.

$$H_0 : \mu_{xi} = \mu_{yi}$$

$$H_1 : \mu_{xi} \neq \mu_{yi}$$

where μ_{xi} is mean level of biomarker i for males, and μ_{yi} is mean level of biomarker i for females

Random Variables:

X_i : Level of biomarker i for a randomly selected male patient.

Y_i : Level of biomarker i for a randomly selected female patient.

Distribution:

While most levels of biomarkers for males and females appear to be approximately normally distributed, levels of TGF_beta_1, IL-6, CXCL9 are not normally distributed for either sexes, and level of IL-18 for male patients is not normally distributed. However, we will assume the random variables are independent with normal distributions using the Central Limit Theorem, as sample size of each group is large (>30), and the underlying sampling distribution can be approximately normal.

Test:

A Welch two-sample t-test was used to compare the means of two groups (male, female). Welch's t-test performs better than Student's t-test for unequal sample sizes and variances between groups, and gives the same result when sample sizes and variances are equal (Delacre et al., 2022). Furthermore, variances do not need to be pre-tested. (Rasch et al., 2011). The

Welch t-test was chosen as it is a standard and robust method that can deal with unequal variances and tolerate slight departure from normality.

Multiple Hypothesis Testing:

When doing multiple independent test, the probability of producing Type I error (wrongly accepting the alternative hypothesis) increases (Herzog et al., 2019). In our case, if the null hypotheses are true and the t-tests are all independent, the probability of making at least one Type I error within the 9 t-tests is:

$$1 - (1 - \alpha)^9$$

For $\alpha = 0.05$, the probability of making at least one Type I error is 0.37.

Bonferroni Correction

Bonferroni correction is a classic way to deal with the increasing Type I error with multiple hypothesis testing. Instead of having the probability of Type I error, $\alpha = 0.05$, for all independent tests, this will be the probability of Type I error across all 9 tests, lowering the p value for each individual test to 0.006 to reach significance.

$$p < \left(\frac{0.05}{9}\right)$$

The script for the hypothesis testing is available in Appendix 2.

Regression Modelling

An 80-20 split was performed on the data to obtain a training and testing dataset, and to have the opportunity to adequately estimate true model performance on test data.

Results

Statistical Hypothesis Testing

Welch Two-Sample t-test

Significant difference was found using a Welch t-test without a correction for multiple hypothesis testing, between males and females for four biomarker levels: CSF_1, CXCL1, TGF_beta_1, and VEGF_A. The corresponding difference between means, means of the two groups, p-values and 95% confidence intervals are given in Table 1 belw.

Table 1: Welch t-test results comparing levels of 9 biomarkers between males and females, including mean values, difference between means, p-values, and 95% confidence intervals.

X	biomarker	difference	male	female	p	conf.low	conf.high
1	csf_1	-0.145	8.53	8.67	0.006	-0.25	-0.04
2	cxcl1	-0.619	8.36	8.98	0.006	-1.06	-0.18
3	cxcl9	0.003	6.63	6.62	0.985	-0.33	0.33
4	il_18	0.128	8.43	8.31	0.249	-0.09	0.35
5	il_6	-0.211	3.23	3.44	0.253	-0.58	0.15
6	il_8	-0.161	7.64	7.80	0.329	-0.49	0.16
7	opg	-0.118	10.67	10.79	0.132	-0.27	0.04
8	tgf_beta_1	-0.345	8.07	8.42	0.046	-0.68	-0.01
9	vegf_a	-0.252	11.75	12.00	0.042	-0.49	-0.01

Welch Two-Sample t-test with Bonferroni Correction

There was no significant difference between male and female levels of the 9 biomarkers when the p-values were adjusted with Bonferroni's correction. Results are given in Table 2 below.

Table 2: Welch t-test results with Bonferroni correction comparing levels of 9 biomarkers between males and females, including mean values, difference between means, adjusted p-values, and 95% confidence intervals.

X	biomarker	difference	male	female	p_adj	conf.low	conf.high
1	csf_1	-0.145	8.53	8.67	0.055	-0.25	-0.04
2	cxcl1	-0.619	8.36	8.98	0.056	-1.06	-0.18
3	cxcl9	0.003	6.63	6.62	1.000	-0.33	0.33
4	il_18	0.128	8.43	8.31	1.000	-0.09	0.35
5	il_6	-0.211	3.23	3.44	1.000	-0.58	0.15
6	il_8	-0.161	7.64	7.80	1.000	-0.49	0.16
7	opg	-0.118	10.67	10.79	1.000	-0.27	0.04
8	tgf_beta_1	-0.345	8.07	8.42	0.411	-0.68	-0.01
9	vegf_a	-0.252	11.75	12.00	0.374	-0.49	-0.01

Conclusions

References

herzog moen mazure and jones delacre Datanovia. (2020). How to Perform Multiple T-test in R for Different Variables - Datanovia. <https://www.datanovia.com/en/blog/how-to-perform-multiple-t-test-in-r-for-different-variables/>

perform-multiple-t-test-in-r-for-different-variables/ Rasch, D., Kubinger, K. D., & Moder, K. (2009). The two-sample t test: pre-testing its assumptions does not pay off. Statistical Papers 2009 52:1, 52(1), 219–231. <https://doi.org/10.1007/S00362-009-0224-X>

Appendices

Appendix 1: Initial Data Manipulation Script

Appendix 2: Hypothesis Testing Script

Appendix 3: Regression Modelling Script