Practical Data Processing

BLOOD ALCOHOL CONTENT IMPUTATION

Irritable Bowel Syndrome and a Probiotic Agent

Statistical Analysis using ANCOVA

P. Boily, S. Hagiwara

Contents

Project Description

Project Description
CCNM and IBS

- Irritable Bowel Syndrome (IBS) is a functional colonic disease with high prevalence.
- Typical symptoms include chronic abdominal pain, discomfort, bloating, and alteration of bowel habits, and it has been linked to chronic pain, fatigue, and work absenteeism and is considered to have a severe impact on quality of life.
- Although there is no known cure for IBS, there are treatments that attempt to relieve symptoms, including dietary adjustments, medication, and psychological interventions.
- In 2010, the Canadian College of Naturopathic Medicine (CCNM) was commissioned to conduct a study to investigate the effect of a probiotic agent on IBS. They carried out an analysis using the hierarchical linear models (HLM). Their key findings include:
  - There is a strong placebo/expectation effect, and
  - No strong statistical evidence to suspect that the agent itself has much of an effect on mild to moderate IBS.
- The CCNM was then asked to determine whether these findings still hold, when the trial data is examined using the analysis of covariance (ANCOVA).

Project Description

Data Collection

- The recruitment procedures included advertisements on the radio, in local newsletters and newspapers, on the web and in social media. Local MDs and NDs were also given recruitment posters for their clinic in order to encourage patient referrals.
- The study recruited a total of 129 participants; however, 10 participants did not provide any information past the initial measurement.
- To facilitate a balanced demographical representation in each group, participants were first categorized by their gender group (M/F) and age group (< or ≧50 years). Within each subgroup, participants were then randomly assigned to treatment or placebo groups.
- This randomization process is called an **unbalanced randomized complete block design (RCBD)**.

Group	Male ≥ 50	Male < 50	Female ≥ 50	Female < 50	Total
Placebo	9	5	36	9	59
Treatment	7	4	38	11	60
Total	16	9	74	20	119

Number of participants assigned to each treatment group based on their demographical characteristics.

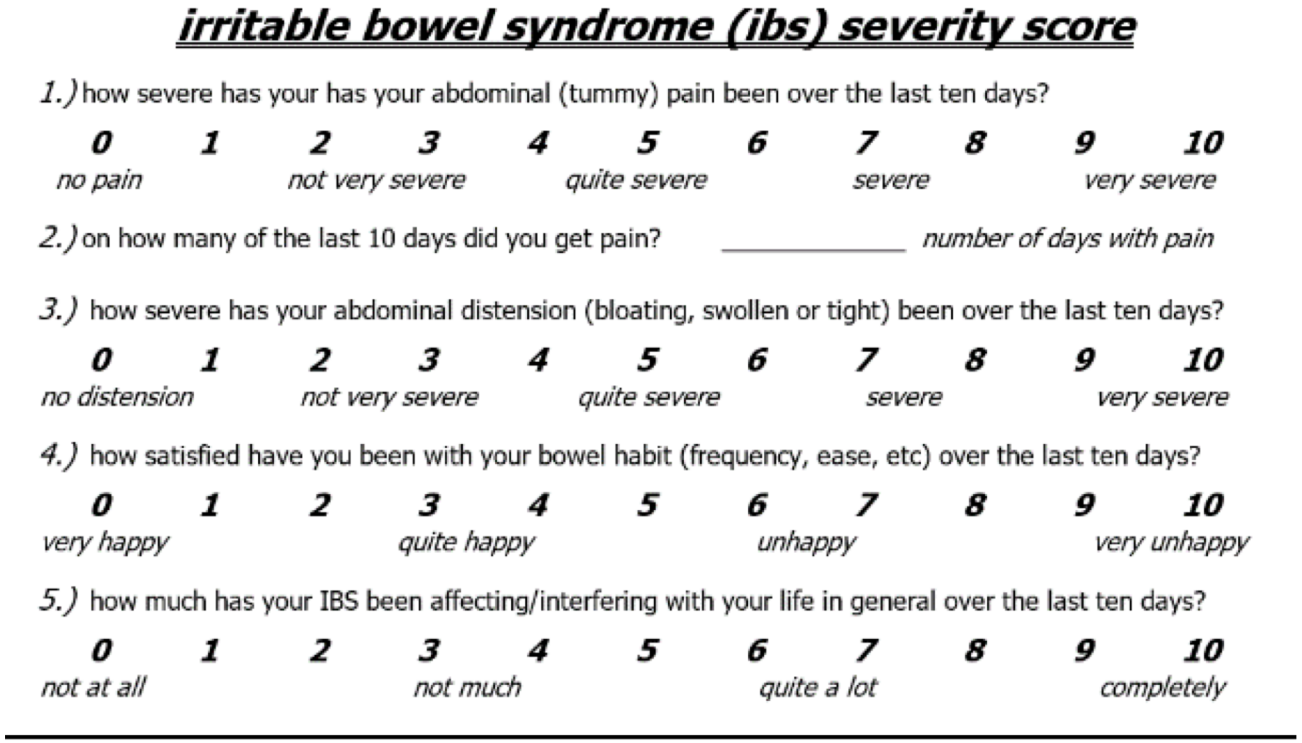
- The study was conducted in a **double-blind fashion**.

Project Description
Outcome Measures

- The study had two response variables of interest:
  - IBS severity score (primary), and
  - IBS quality of life (QoL) measure (secondary)

- IBS severity scores were collected at the beginning of the study (baseline), and at one-month intervals for next three months.
- The participants were also asked to submit the QoL questionnaire at the start of the study, as well as at the second and the third month of their follow-ups.
- Both severity scores and QoL scores are computed using self-reported data.

Project Description
IBS Severity Score Sheet



irritable bowel syndrome (ibs) severity score

1.) how severe has your has your abdominal (tummy) pain been over the last ten days?

0 1 2 3 4 5 6 7 8 9 10
no pain — not very severe — quite severe — severe — very severe

2.) on how many of the last 10 days did you get pain? _____ *number of days with pain*

3.) how severe has your abdominal distension (bloating, swollen or tight) been over the last ten days?

0 1 2 3 4 5 6 7 8 9 10
no distension — not very severe — quite severe — severe — very severe

4.) how satisfied have you been with your bowel habit (frequency, ease, etc) over the last ten days?

0 1 2 3 4 5 6 7 8 9 10
very happy — quite happy — unhappy — very unhappy

5.) how much has your IBS been affecting/interfering with your life in general over the last ten days?

0 1 2 3 4 5 6 7 8 9 10
not at all — not much — quite a lot — completely

Francis C.Y., Morris J. & Whorwell P.J. *The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress.* Aliment Pharmacol Ther. 1997; 11: 395-402

Condition	Score
In remission	<7.5
Mild IBS	7.5 to 17.5
Moderate IBS	17.5 to 30
Severe IBS	30<

Classification of IBS severity (Whorwell et al.)

Project Description
IBS Quality of Life Questionnaire (sample)

The IBS-QOL consists of 34 items, each with a five-point response scale:

Items 1, 2, 4, 8-10, 12, 13, 16, 25-29, 34
1. Not at all 2. Slightly 3. Moderately 4. Quite a bit 5. Extremely

Items 3, 5-7, 11, 14, 15, 17-24, 30-33
1. Not at all 2. Slightly 3. Moderately 4. Quite a bit 5. A great deal

1. I feel helpless because of my bowel problems.
2. I am embarrassed by the smell caused by my bowel problems
3. I am bothered by how much time I spend on the toilet.
4. I feel vulnerable to other illnesses because of my bowel problems.
5. I feel fat because of my bowel problems.
6. I feel like I'm losing control of my life because of my bowel problems.
7. I feel my life is less enjoyable because of my bowel problems.
8. I feel uncomfortable when I talk about my bowel problems.
9. I feel depressed about my bowel problems.
10. I feel isolated from others because of my bowel problems.
11. I have to watch the amount of food I eat because of my bowel problems.
12. Because of my bowel problems, sexual activity is difficult for me.
13. I feel angry that I have bowel problems.
14. I feel like I irritate others because of my bowel problems
15. I worry that my bowel problems will get worse.
16. I feel irritable because of my bowel problems
17. I worry that people think I exaggerate my bowel problems.
18. I feel I get less done because of my bowel problems.
19. I have to avoid stressful situations because of my bowel problems
20. My bowel problems reduce my sexual desire.
21. My bowel problems limit what I can wear.

How is the IBS-QOL scored?

The individual responses to the 34 items are summed and averaged for a total score and then transformed to a 0-100 scale for ease of interpretation with higher scores indicating better IBS specific quality of life. There are also eight subscale scores for the IBS-QOL (Dysphoria, Interference with Activity, Body Image, Health Worry, Food Avoidance, Social Reaction, Sexual, Relationships).

The transformation formula used for the IBS-QOL total and scale scores is:

$$\text{Score} = \frac{\text{The sum of the items - lowest possible score}}{\text{Possible raw score range}} * 100$$

Objective: using IBS severity score and IBS QoL measure, is there a (statistically) significant evidence to believe that the probiotic agent improves the IBS condition?

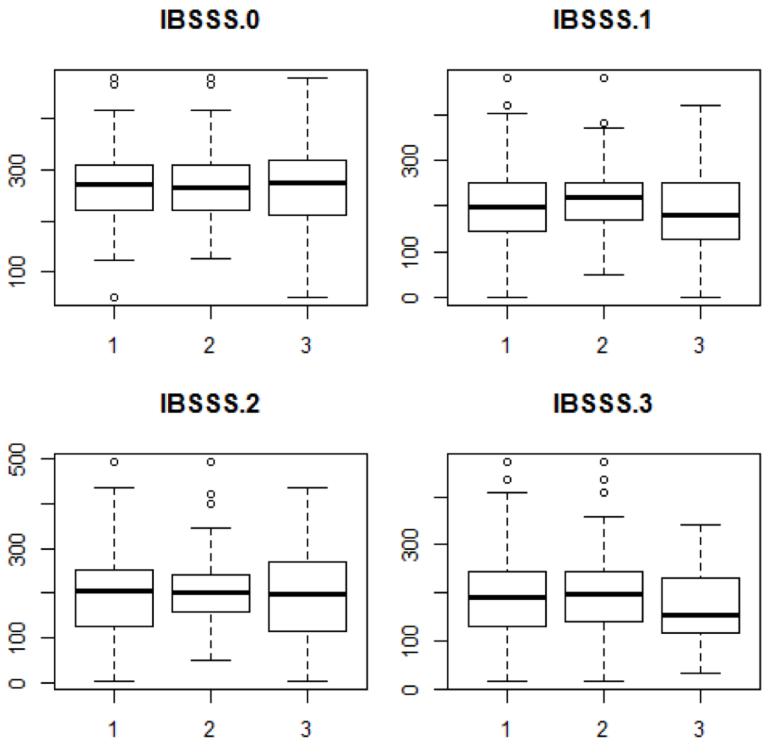
Data Preparation and Methodology

Data Preparation
Drop-outs, Missing Observations, and Imputation

- Out of 129 participants, 10 people did not deliver any information after the baseline measure (i.e., before the administration of agent/placebo).
- In addition, there were six participants who failed to follow-up after the first or the second month of the study.
- While it is difficult to study the exact reasons why some participants terminate the follow-up prematurely, it could be conjectured that participants who complete the study are either more likely to believe in the effect of the active agent or to actually be feeling the effect of the treatment than those who fail to complete the treatment.
- The covariance analysis requires the dataset to be free of missing observations; thus imputations must be performed before proceeding with the analysis.
- The Last Observation Carried Forward (LOCF) imputation was used in this.

Data Preparation
Outlier Detection

- The box-plots (1 = all participants, 2 = placebo group, and 3 = treatment group) and summary tables show that participant #8 is considered anomalous at all observations except for the baseline measure of QoL.



Boxplots of IBS severity scores.

IBSS	Overall	Placebo	Treatment
Baseline	114(480) 74(480) 8(467)* 73(50)	74(480) 8(467)*	114(480) 73(50)
Month 1	8(480)* 45(420)	8(480)* 35(383)	None
Month 2	8(491)*	8(491)* 80(419) 106(400)	None
Month 3	8(472)* 90(453)	8(472)* 90(455)	None

QoL	Overall	Placebo	Treatment
Baseline	None	None	36(91.91)
Month 2	8(86.76)* 39(83.82) 80(83.82) 106(83.09) 123(81.62)	None	39(83.82) 123(81.62) 38(73.53)
Month 3	8(88.24)* 80(83.82)	None	123(72.06)

List of outliers and their values.

Methodology
ANCOVA Models

- On top of the treatment and the block (i.e., demographic) effects, ANCOVA models involve the linear effect of a continuous covariate (i.e., adjustment for initial score): the models that we use are of the following form:

$$y_{ijk} = \mu + \tau_i + \beta_j + \gamma x_{ijk} + \varepsilon_{ijk}$$

where

- y_{ijk} is the i^{th} **response variable** in the i^{th} treatment group and j^{th} block;
- μ is the **overall mean**;
- τ_i is the i^{th} **treatment effect**;
- β_j is the j^{th} **block effect**;
- γ is the **covariate (or regression) effect**;
- $x_{ijk} = X_{ijk} - \bar{X}$ is the k^{th} **covariate (or concomitant variable)** in the i^{th} treatment group and j^{th} block (the baseline IBSS or QoL value adjusted for the mean), and
- ε_{ijk} is the k^{th} **residual** in the i^{th} treatment group and j^{th} block

The indices correspond to $i = 1,2,\ j = 1, \dots, 4,\ k = 1, \dots, n_{ij},\ \sum_i \sum_j n_{ij} = N$, where N is the number of participants.

Methodology
ANCOVA Models (Assumptions)

- In order to use an ANCOVA model, four assumptions must be satisfied:

1. *Independence and Normality of Residuals*: the residuals are thought to be independently and identically distributed random variables following a normal distribution with zero mean (i.e. $\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \sigma_{\boldsymbol{\varepsilon}}^2 \boldsymbol{I})$);
2. *Homogeneity of Residual Variances*: the variance of the residuals must be uniform across treatment groups;
3. *Homogeneity of Regression Slopes*: the regression effect (slope) must be uniform across treatment groups, and
4. *Linearity of Regression*: the regression relationship between the response and the covariate must be linear.

- The third assumption is especially critical to the ANCOVA model. It can be tested with the **equal slope test**: we run an ANCOVA regression with an additional interaction term $x \times \tau$. If the interaction is not significant, the third assumption is satisfied.

- In the event that the interaction term is statistically significant, a different approach (e.g., moderated regression analysis, mediation analyses) is required.

Analyses and Results

Analysis and Results
Actual Sample Size and Effect of Imputation

- A total of 129 participants were recruited for the study, ten of which dropped out after their baseline assessments. A further three drop-outs were removed, leaving a total of $N = 116$ participants for the IBSS analysis.
- To accommodate the three imputations for missing observations, three degrees of freedom are taken from the residual source in the ANCOVA analysis.

Analysis and Results
ANCOVA on IBS Severity Score with Full Dataset

- Using all N=116 sample, the result using IBS severity score is summarized in the following ANOVA table.

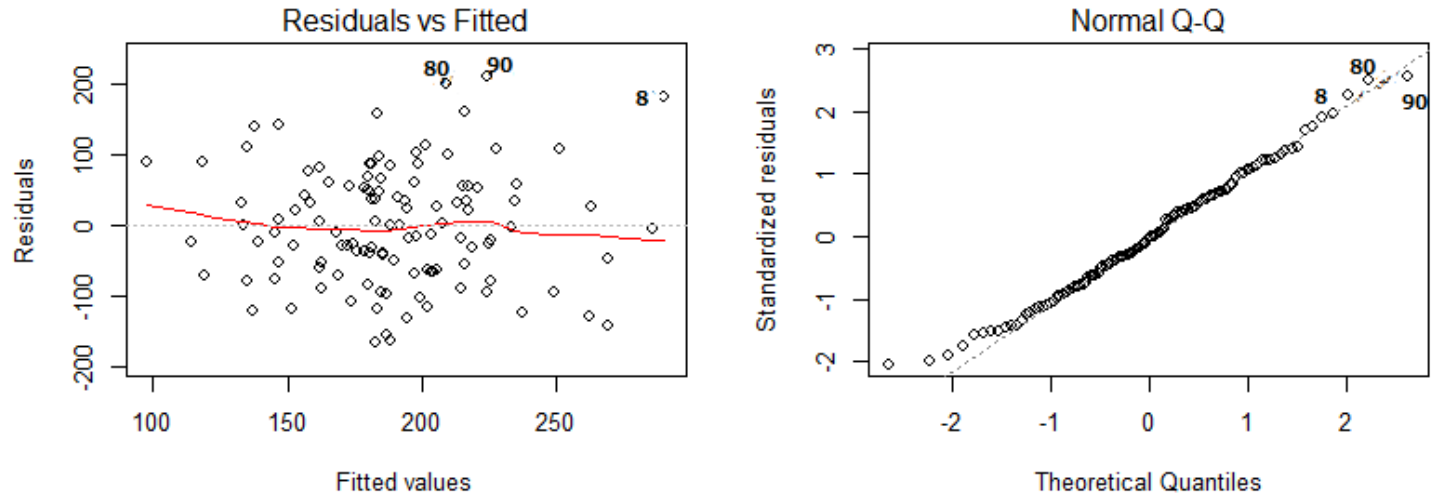
Source	df	Type III SS	MS	*F*	*p*-value
τ (Treatment)	1	20324	20324	2.838	0.09498
β (Block)	3	14090	4696.667	0.65588	0.58108
γ (Covariate)	1	110609	110609	15.4451	0.00015
ε (Residual)	110 – 3 = 107	766275	7161.449		

ANOVA table for the Full IBSS Model with degrees of freedom modified to accommodate imputation.

- As the *p*-value for the treatment effect is about 0.095, we conclude that there is not enough evidence to suggest that the treatment has an effect at the 0.05 significance level (but there appears to be a significant effect of the treatment on IBSS at the 0.10 significance level)
- Also, with a *p*-value of 0.0015 for the covariate effect, it seems reasonable to assume that the relationship between the response and the covariate is indeed linear.

Analysis and Results
ANCOVA on IBS Severity Score (Diagnostic Checks 1)

- The assumption of normality and independence of residuals are satisfied based on the following plots.



Normality and independence of the Full IBSS Model residuals.

- The Bartlett statistic against homogeneous variances of the residuals in the treatment group vs. those in the placebo group is $X^2 = 0.5437$, with a corresponding p-value of 0.4450. Hence it is safe to assume that the assumption of homogeneous variances is met.

Analysis and Results
ANCOVA on IBS Severity Score (Diagnostic Checks 2)

- The test for equal slopes compares the original model $y \sim \tau + \beta + \gamma x$ to the modified interaction model

$$y \sim \tau + \beta + \gamma x + \rho(x \times \tau).$$

- Here, we want to have interaction term to not be statistically significant.
- While the corresponding p-value shows a lack of significance at the 0.05 significance level, it also indicates borderline significance at the 0.10 significance level.

Model	df_ε	RSS	df_{diff}	SS	F	p-value
Original	107	766275				
Interaction	106	748021	1	18254	2.5867	0.1107

Homogeneity of regression slopes across treatment.

- From the diagnostic checks, we re-ran the ANCOVA model without participant ID8 (a potential influential participant).

Analysis and Results

ANCOVA on IBS Severity Score without ID8

- The removal of ID8 has the dramatic effect of changing our conclusions to the point that there is no longer enough evidence to suggest that the treatment has an effect even at the 0.10 significance level.

Source	df	Type III SS	MS	*F*	*p*-value
τ (Treatment)	1	15485	15485	2.2479	0.1368
β (Block)	3	11985	3995	0.5799	0.6295
γ (Covariate)	1	76421	76421	11.0937	0.0010
ε (Residual)	106	730204	6888.717		

ANOVA table for the reduced model with degrees of freedom modified to accommodate imputation.

- The assumption of normality and independence of error terms, homogeneity of variances, and linearity of covariance effect are all met.

- This concludes that participant ID 8 is indeed an influential observation, and based on IBS severity score the probiotic agent does not have statistically significant effect on IBS.

Model	df_ε	RSS	df_{diff}	SS	*F*	*p*-value
Original	106	730204				
Interaction	105	722362	1	7842.5	1.1400	0.2881

Homogeneity of regression slopes across treatment.

Analysis and Results
ANCOVA on IBS QoL Score (Full Dataset)

- As before, a total of 129 participants were recruited for the study, ten of which dropped out after the baseline assessment. This time however, only two drop-outs were removed, leaving a total of $N = 117$ participants for the QoL analysis. In order to accommodate the four imputations, four degrees of freedom are docked from the residual source in the ANCOVA analysis.

Source	df	Type III SS	MS	*F*	*p*-value
τ (Treatment)	1	998	998	3.7453	0.05560
β (Block)	3	398.7	136.7	0.5130	0.6742
γ (Covariate)	1	13949.9	13949.9	52.35163	<0.0001
ε (Residual)	111 – 4=107	28511.8	266.4654		

ANOVA table for the Full QoL Model with degrees of freedom modified to accommodate imputation.

- There is still not enough evidence to suggest that the treatment has an effect at the 0.05 significance level; however, the p-value is very close to the threshold.

Analysis and Results
ANCOVA on IBS QoL Score (Diagnostic checks)

- With the aids of the normal Q-Q plot and the scatter plot of the residuals against the fitted values, there is no strong evidence to suspect the validity of the normality and the independence of the residuals (the two plots are essentially the same as in the severity scores).
- The Levene's test statistic for the Full QoL Model is $W = 1.3327$, with an associated p-value of 0.2508 for equal variances in residuals across two treatment groups
- With the covariate p-value of 0.0010, the linearity of the regression between the response and the covariate seems highly significant.

Model	df_ε	RSS	df_{diff}	SS	F	p-value
Original	107	28512				
Interaction	106	28295	1	216.58	0.8113617	0.3698

Homogeneity of regression slopes across treatment.

- Finally, with a p-value around 0.37, there is no strong evidence to suspect the validity of the most critical ANCOVA assumption: the assumption of the equal slopes.

Analysis and Results
Summary Table on IBS QoL Score (Full Dataset)

- ANCOVA coefficients for the Full QoL Model are given below. The placebo treatment effect $\boldsymbol{\tau_1}$ and the females-over-50 block effect $\boldsymbol{\beta_1}$ are both set to 0.
- Intercept term(the overall mean $\boldsymbol{\mu}$), and the covariate effect $\boldsymbol{\gamma}$ are thought to be significant.
- Blocking does not have statistical significance.
- The treatment effect $\boldsymbol{\tau}_{Treatment}$, has a relatively small p-value; however, it is not significant at $\alpha = 0.05$.

coefficients	estimate	std. error	t-value	p-value
Intercept	30.39	4.01	7.59	<0.0001
$\beta_{Male\geq 50}$	7.59	6.51	1.17	0.25
$\beta_{Feale<50}$	2.80	4.13	0.68	0.50
$\beta_{Male<50}$	0.90	5.48	0.16	0.87
γ	0.57	0.08	7.37	<0.0001
$\tau_{Treatment}$	-5.86	2.97	-1.97	0.051

Consulting Post-Mortem

Consulting Post-Mortem

- **Practical** vs. **Statistical** significance
  - just because we (barely) found statistically significant result, that does not translates to practical significance (i.e., does the agent really provide more than placebo effect?)
- Method of choice (ANCOVA)
  - ANCOVA only allow us to compare before/after treatment scores. Since we have two to three follow-ups, ANCOVA may not be the best choice to test the treatment effect over the course of three months. (We were asked to do ANCOVA analysis by our client).
- Convenient recruitment process
  - as with most medical experiments, participants needed to come forward to participate in this study. This type of recruitment process leads to self-selection bias, and the participants may not be a representative sample of all IBS sufferers.
- Effect of blocking
  - From statistical perspective, blocking should only be used if there are compelling reasons to suspect that treatment effects are different for at least one subgroup as blocking results in a fewer degrees of freedom.
- Never stop digging until we find something
  - After the above results were shown, the client kept asking us to provide further analyses (e.g., considering only severe IBS suffers). Running enough tests, we may find something; however, this does not really mean anything (other than misleading conclusion!)
- Privacy concerns
  - The files we received contained participants' full names.