BIOS 667 Group 5

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week	site	id	treat	age	sex	twstrs
0	1	1	2	65	1	32
2	1	1	2	65	1	30
4	1	1	2	65	1	24
8	1	1	2	65	1	37
12	1	1	2	65	1	39
16	1	1	2	65	1	36

Introduction

The purpose of this project is to examine the effects of botulism toxin type B (BotB) to treat cervical dystonia over time. Cervical dystonia (CD) is a chronic neurological disorder, in which patients have painful involuntary contractions in neck muscles. CD is more prevalent in women (Jankovic et al., 2023). The prevalence of CD is estimated to be 28-183 cases per million. The data comes from a multicenter randomized clinical trial for cervical dystonia patients with 9 U.S. sites. Botulism toxin types A and B are first-line treatments for CD (Wetmore et al., 2025). The treatment groups included in the study were placebo, 5000 U BotB, and 10000 U BotB. The response variable is Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) total score, which ranges from 0 to 85 and is comprised of disability (0-30), pain (0-20), and severity (0-35) subscores. Only total score is included in this data.The TWSTRS score was measured at week 0 (baseline), and 2,4,6,8,12, and 16 weeks after treatment start. Site is included in the dataset but no further details about site were included in the available dataset documentation.

Methods

Study Population

Inclusion and exclusion criteria for the trial were not available. Duration of CD and age at onset were not known. The study included 109 patients (67 (61%)) females.The mean age was 56 (12). Median age was 56 years. The mean TWSTRS score at baseline was 46 (10). It was not known if the patients received prior BotB treatments. Information about the randomization schedule was not provided.

Statistical Analyses

Number of observations, mean, median, standard deviation (SD), minimum (min) and maximum (max) were provided for age. Mean and SD were calculated for TWSTRS score at baseline. Frequencies and percentages were reported for categorical variables. GLM, GLMM, and GEE models were fit using TWSTRS total score as the response variable and blank, blank, blank as covariates.

Results

Table 1: Summary of Demographic and Baseline Characteristics

Characteristic	Overall N = 109	Placebo N = 36	BotB 5000 U N = 36	BotB 10000 U N = 37	p-value
Sex					0.0706
Male	42 (39%)	15 (42%)	18 (50%)	9 (24%)	
Female	67 (61%)	21 (58%)	18 (50%)	28 (76%)	
Age (years)					0.6198
No. obs.	109	36	36	37	
Mean (SD)	56 (12)	54 (12)	57 (12)	56 (12)	
Median	56	56	57	54	
Min, Max	26, 83	26, 79	35, 83	34, 76	
TWSTRS total score at baseline					0.3307
Mean (SD)	46 (10)	44 (9)	46 (10)	47 (10)	

[1] BotB = botulinum toxin type B; TWSTRS = Toronto Western Spasmodic Torticollis Rating Scale.

[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Generalized Linear Model (GLM)

A generalized linear model (GLM model) using a Gaussian link was fit including week, site, treatment, age, and sex as predictors (Table 2).

Table 2: GLM Model Summary (Gaussian Link)

term	estimate	std.error	statistic	p.value
(Intercept)	30.227	2.850	10.605	0.000
week	0.234	0.080	2.923	0.004
treat5000 U	-0.030	1.120	-0.027	0.978
treat10000 U	0.287	1.127	0.254	0.799
age	0.072	0.039	1.854	0.064
sexFemale	1.938	1.006	1.927	0.054
site2	13.216	1.860	7.107	0.000
site3	-2.183	1.933	-1.130	0.259
site4	4.635	2.132	2.174	0.030
site5	7.403	2.413	3.068	0.002
site6	9.617	1.849	5.201	0.000
site7	1.862	1.912	0.974	0.331
site8	-2.493	1.773	-1.406	0.160
site9	10.198	2.011	5.071	0.000

The coefficient for week was statistically significant, indicating an overall linear trend in TWSTRS score over time. There was notable variation across study sites, with several sites showing significantly higher TWSTRS scores compared to the reference site, highlighting site-level differences. However, because the GLM assumes independence of observations, the repeated measures within individuals violate this assumption. As a result, the standard errors and p-values may be underestimated, and inference should be interpreted with caution. This motivates the subsequent use of correlation-aware models such as GEE and GLMM.

GLM Model Diagnostics

Diagnostics were assessed for the GLM model.

Figure 1: Residuals vs Fitted Plot

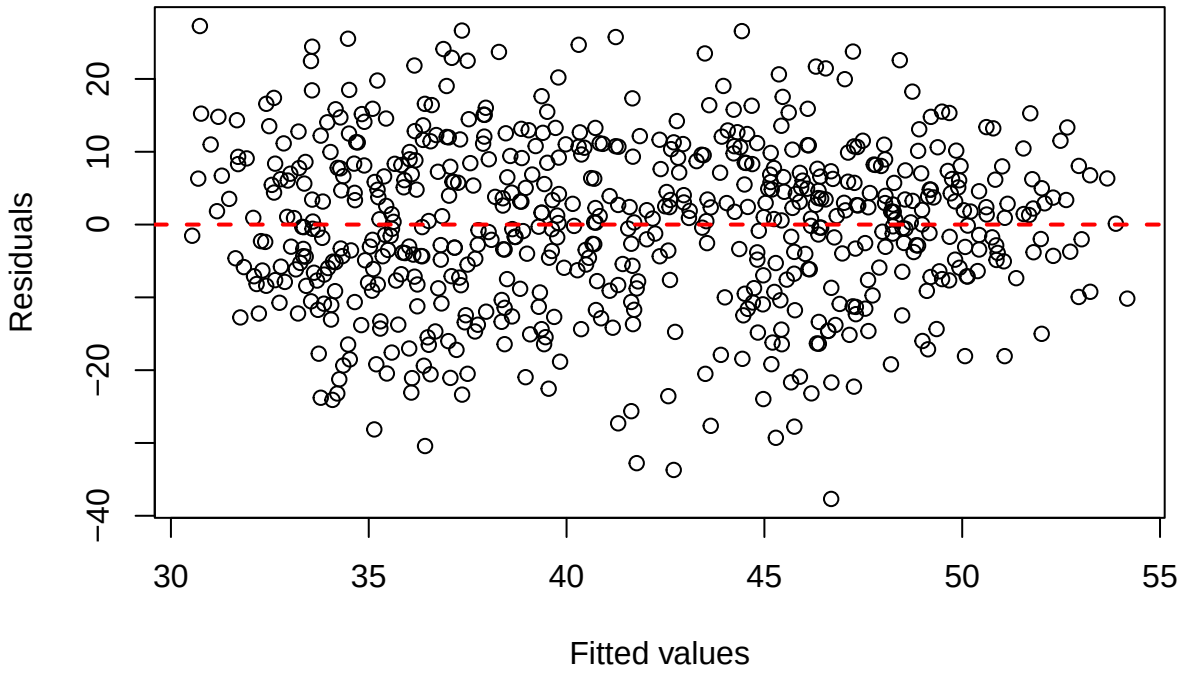


Figure 1 shows the residuals versus the fitted values. The absence of strong patterns or fanning suggests that the linearity and homoscedasticity assumptions are reasonably met.

Figure 2: Q−Q Plot

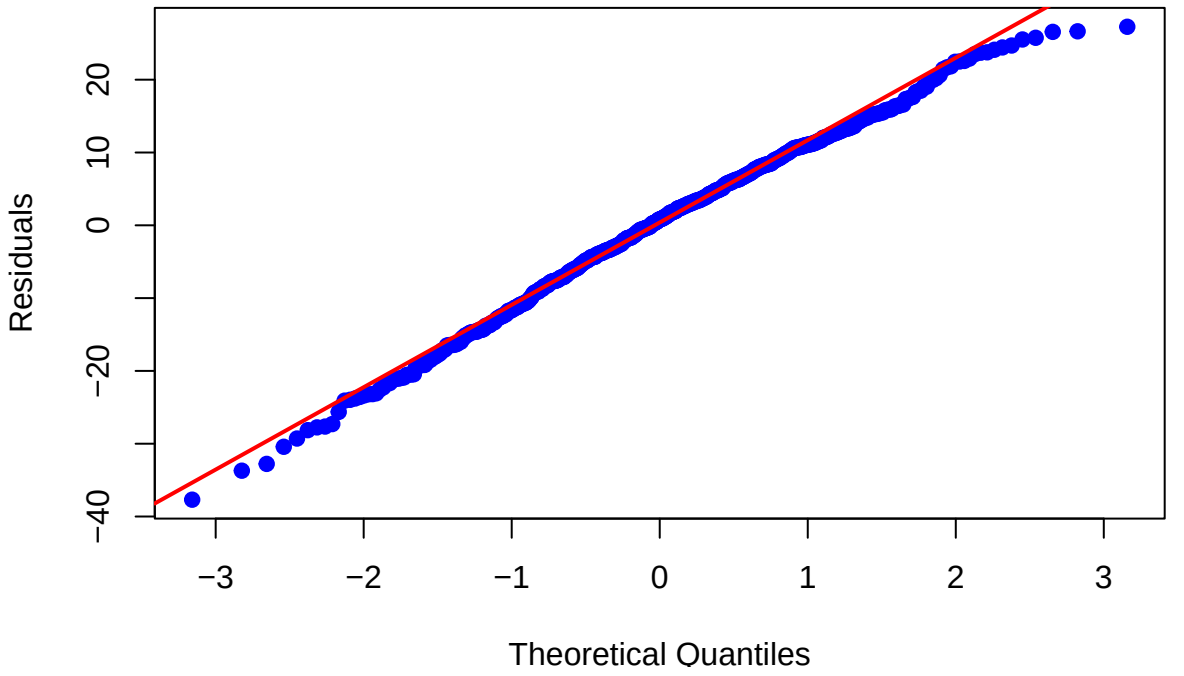
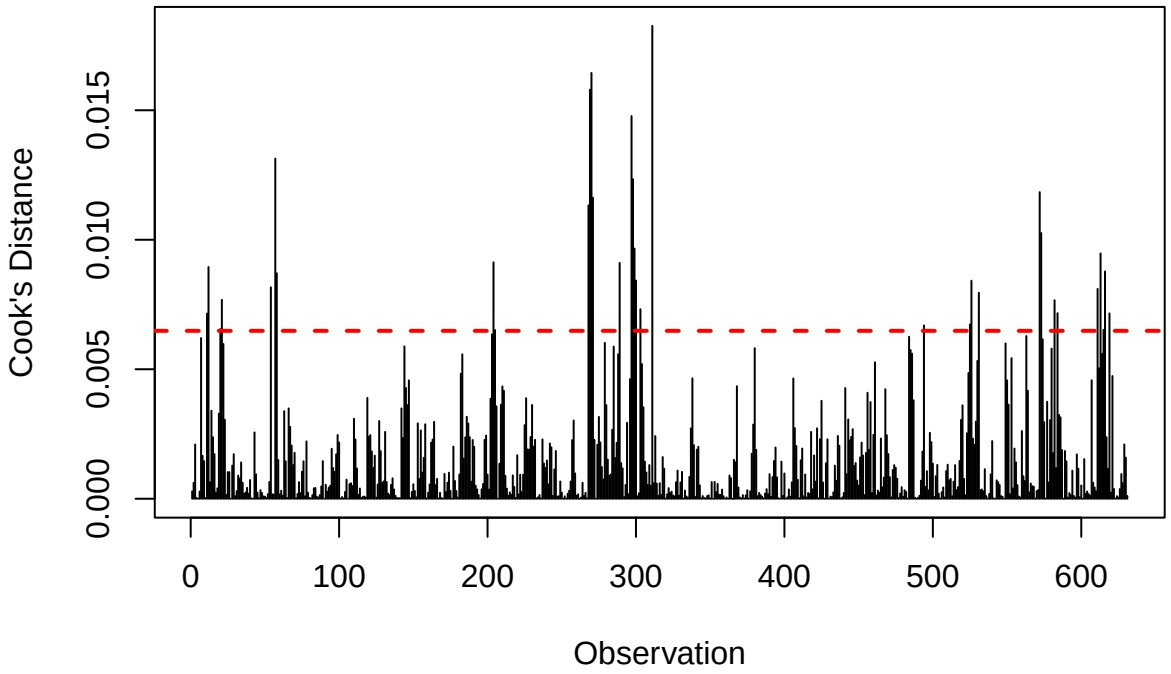


Figure 2 presents a QQ plot of the residuals, which largely follow the 45 degree reference line with mild deviations in the tails. This indicates that the normality assumption is approximately satisfied.

Figure 3: Cook's Distance Plot



Finally, figure 3 displays Cook's distance for all observations. The conventional threshold, 4/(n-k-1), where n is the number of observations and k is the number of predictors, was used to assess the influence of the observations. Several observations exceeded the threshold and were considered potentially influential and warranted further investigation. Therefore, a second GLM was fit, excluding those observations.

Table 3: GLM Model Summary (Excluding Influential Observations)

term	estimate	std.error	statistic	p.value
(Intercept)	31.836	2.571	12.384	0.000
week	0.215	0.072	3.002	0.003
treat5000 U	-0.185	1.004	-0.184	0.854
treat10000 U	0.800	1.001	0.799	0.424
age	0.050	0.035	1.449	0.148
sexFemale	1.046	0.903	1.158	0.247
site2	13.405	1.667	8.043	0.000
site3	-1.417	1.741	-0.814	0.416
site4	7.636	1.947	3.921	0.000
site5	10.761	2.283	4.714	0.000
site6	10.152	1.661	6.111	0.000
site7	2.380	1.707	1.394	0.164
site8	-3.384	1.605	-2.108	0.035
site9	10.049	1.861	5.400	0.000

Excluding influential observations resulted in modest shifts in the parameter estimates and slightly improved precision. Notably, the effect of week remained statistically significant and variation across study sites continued to be pronounced. This underscores that site-level differences remain important even after removing

influential observation.s

Overall, excluding influential points slightly adjusted the estimates and improved precision, but the main patterns of association were consistent with the original GLM. As with the previous model, the GLM assumptions are not appropriate for longitudinal data with correlated repeated measures. The following GEE and GLMM analyses address this limitation by explicitly modeling within-subject correlation and random effects.

Generalized Estimating Equation (GEE)

A GEE model with Gaussian family, identity link, and an exchangeable correlation structure. The mean model included week, treatment, treatment-by-week interaction, and baseline age and sex, with Placebo as the reference treatment group.

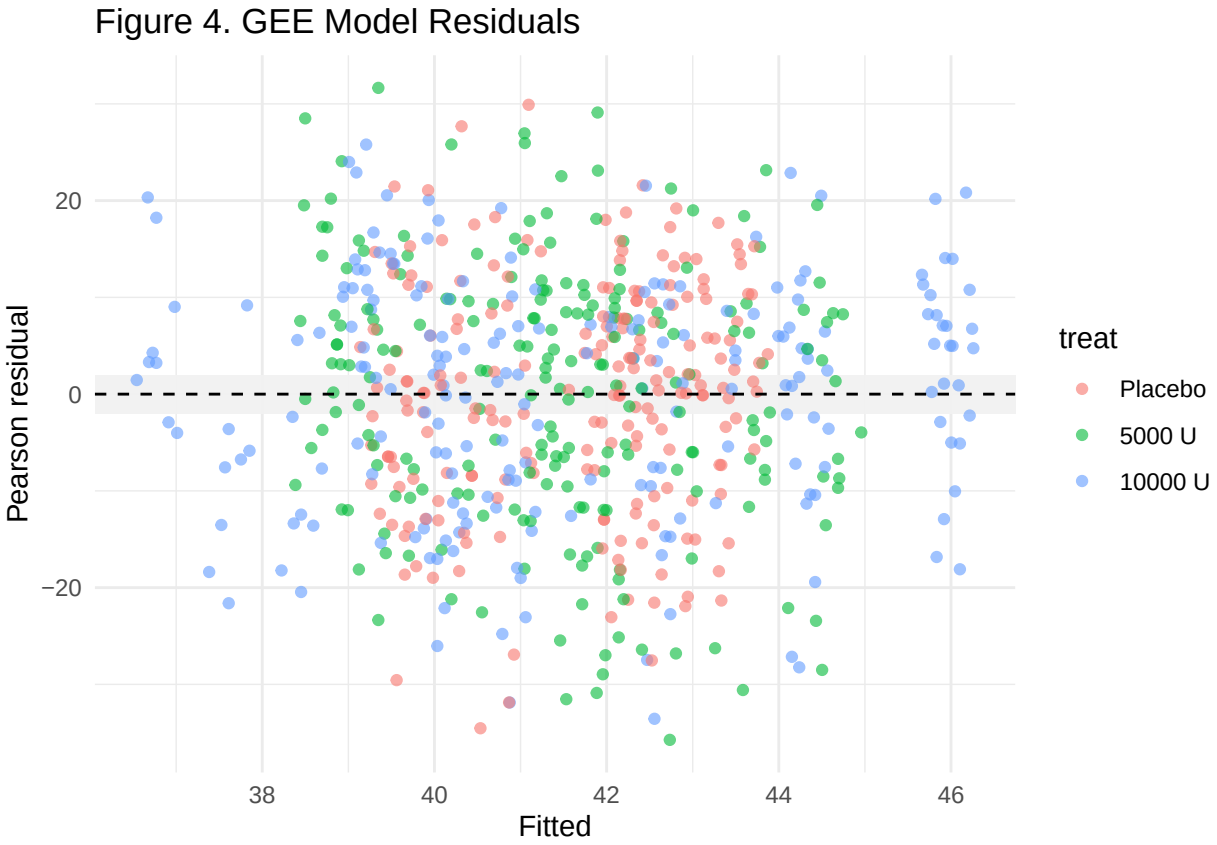


Figure 4 shows Pearson residuals plotted against the fitted TWSTRS values with points colored by treatment group. The residuals are centered around zero and do not show a strong funnel shape, supporting the constant-variance assumption. However, many residuals fall outside ± 2, indicating wide individual variation in TWSTRS trajectories and suggesting that the simple Gaussian working model does not fully capture the variance structure. Even so, because the GEE analysis used robust standard errors, the uncertainty in the regression coefficients is driven by the observed variability of the residuals within subjects, rather than depending on the working variance and correlation.

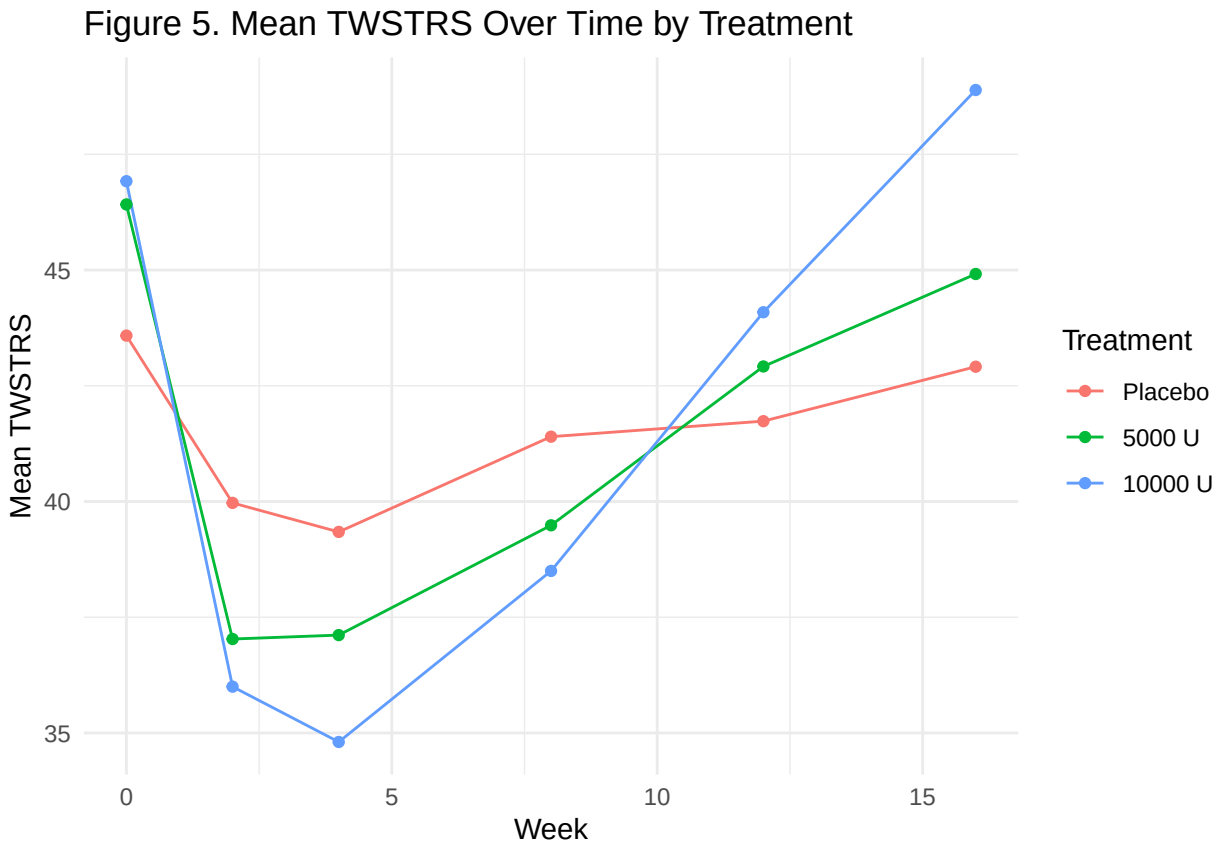


Figure 5 shows mean TWSTRS over time by treatment group. All groups show decreases from baseline through weeks 2–4, suggesting early improvement, followed by increases after week 4. Around week 10, both active treatment groups begin to show higher TWSTRS scores than the placebo group.

Table 4: GEE Model Summary

term	estimate	std.error	statistic	p.value
(Intercept)	38.698	4.760	66.095	0.000
week	0.097	0.114	0.731	0.392
treat5000 U	-0.810	2.758	0.086	0.769
treat10000 U	-2.738	2.372	1.333	0.248
age	0.014	0.078	0.033	0.855
sexFemale	2.494	2.117	1.388	0.239
week:treat5000 U	0.115	0.133	0.745	0.388
week:treat10000 U	0.323	0.146	4.899	0.027

Table 4 summarizes the GEE estimates for TWSTRS over time by treatment, adjusted for age and sex. The main effects for treat5000U (-0.810, $p = 0.769$) and treat10000U (-2.738, $p = 0.248$) compare baseline TWSTRS at week 0 with placebo. Both estimates are negative but not statistically significant, indicating no clear baseline differences in mean TWSTRS between treatment groups and placebo. The treatment-by-week interaction terms describe how each treatment's trajectory differs from placebo over time. For 5000U, the additional slope relative to placebo is small and not significant (0.115, $p = 0.388$), suggesting that its average linear trend over time is similar to placebo. For 10000U, the additional slope is larger and statistically significant (0.323, $p = 0.027$), indicating an average increase in TWSTRS over 16 weeks compared with placebo. Because higher TWSTRS scores reflect worse symptoms, these results imply that low-dose regimen behaves similarly to placebo, while the high-dose regimen is associated with faster worsening over time.

Overall, the GEE model provides a reasonable approach for assessing whether treatment affects population-average TWSTRS trajectories over time, while recognizing that the true time course is somewhat non-linear and that estimated treatment effects are relatively small.

Discussion

Summarization of main points, conclusions based in results Summarization of the various models applied, which ones you prefer and base your interpretations off of and why Discussion of limitations if any

References

Jankovic, J., Tsui, J., & Brin, M. F. (2023). Treatment of cervical dystonia with Botox (Onabotulinumtoxina): Development, insights, and impact. *Medicine*, *102*(S1), e32403. https://doi.org/10.1097/MD.0000000000032403

Wetmore, E., Roberts, H., Livinski, A. A., Camacho, T., Eaton, C., Norato, G., Hallett, M., & Stacy, M. (2025). Clinical response to placebo botulinum toxin injection in cervical dystonia—a systematic review and meta-analysis. *Dystonia*, *4*, 14297. https://doi.org/10.3389/dyst.2025.14297