[SDY210] Adding Exhaled Nitric Oxide to Guideline-based Asthma Treatment in Inner-City Adolescents and Young Adults: a Randomized Controlled Trial

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Introduction: The goal of SDY210 clinical trial is to determine whether the measurement of exhaled nitric oxide (NO) could increase the effectiveness of asthma treatment guideline. The report will replicate the statistical analysis of the primary and secondary objectives of the research, *Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomized controlled trial*, including sub-group analysis on gender.

Methods: The original study was conducted under double-blind, parallel group, intention-to-treat (ITT) basis. Centralized block randomization has been applied to the sample. Wilcoxon rank-sum test was used to compare the groups for each patient characteristics. ANCOVA analysis was performed to evaluate if there is any significant difference between the guideline-based group and the guideline-based with exhaled NO measurement group. Patients' asthma-related use of health care was observed and compared by the Chi-square test. Also, additional sub-group analysis on gender was conducted.

Results The required sample size that the study should have had was 764 assuming 35% drop-out rate. The primary outcome demonstrates that no significant difference was shown between the control and treatment group. Of four variables that measured asthma-related use of health care, none of them showed significant results. In sub-group analysis, male adolescents showed a marginally significant result for the primary outcome ($p = 0.047$).

Discussion There was a slight difference in sample size calculation (original study: 780 vs. reproduction: 764). The primary and secondary outcomes were consistent with the result of the original study. However, male adolescents showed a marginally significant result. The only week two and eight data was used to evaluate the effectiveness of adding exhaled NO to the guideline. This is because some essential data was blinded. Thus, a linear mixed model could not have been applied to reproduce the result.

Background

Asthma is a chronic lung disease that inflames and narrows the airways which makes it harder for the person to breathe, affecting more than 25.2 million people in the U.S. In particular, about 10% of children have been diagnosed with asthma with severe symptoms, and are poorly cared despite improved asthma treatment and management among young children and adolescents.

Due to the environment, medical research has been active looking for ways to find out better ways to control asthma treatment.

The role of exhaled nitric oxide (NO) was controversial in previous studies. The study aimed to assess whether measurement of exhaled NO, as a biomarker of airway inflammation, could improve the effectiveness of asthma control, when used as an adjunct to clinical care based on asthma guidelines for inner-city adolescents and young adults.

Szefler et al. (2008) suggested that the fraction of exhaled nitric oxide (FeNO) which has been shown to increase during periods of uncontrolled asthma and decrease during treatment with anti-inflammatory agents, can potentially be a biomarker for direct measurement of airway inflammation due to previous trials that have assessed the use of FeNO in addition to the conventional asthma treatment guideline based on symptoms and pulmonary functions. Also, they wanted to determine whether the use of exhaled nitric oxide (NO) measurements to modify asthma treatment regimens improves asthma control when used as an adjunct to management based on national asthma care guidelines.

Reproduction

The team's purpose of this study is to replicate the statistical analysis of the primary and secondary outcomes of *Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomized controlled trial*, using SDY210 clinical trial data. To be more specific, the team reproduced the sample size calculation, mimicked the centralized block randomization. For the primary outcome, the original study used a linear mixed model. However, different method was used in the reproduction process because some data were not accessible resulted from intentional blinding on regional information due to privacy issue. For secondary outcomes, the team compared the proportions of asthma-related use of health care between control and NO treatment group. Also, extensive sub-group analysis on gender has been

made on primary outcome to evaluate if there is any significant difference between control and NO treatment group.

Methods

Participants

The study has been made among adolescents and young adults from 12 to 20 years from ten inner-cities in the U.S. They recruited 780 inner-city participants, and all of them have been diagnosed with asthma by physicians before. 546 participants were left after the screening process. They applied the restricted eligibility to the participants where their household income should be above the bottom 20% of households which is a recommended federal poverty threshold. All procedures were implemented based on the intention-to-treat (ITT) principle.

Procedures

The study was composed of eight visits, and each visit was made every 6 to 8 weeks, 46 weeks in total. Once a participant is enrolled at first visit, study physicians checked the baseline status of patients. Subsequently, patients got through a run-in period. The purpose of this period was to wash out the effect of each patient's different treatment before the study to make them more equivalent status. In addition to the run-in period, investigators also applied standard treatment to all participants regardless of their study groups.

For the rest of the visits, at each visit, the study physicians assessed the fraction of exhaled NO, days of asthma symptoms, use of rescue drugs, pulmonary function, use of health care, adherence to treatment regimen, and missed days of school because of asthma.

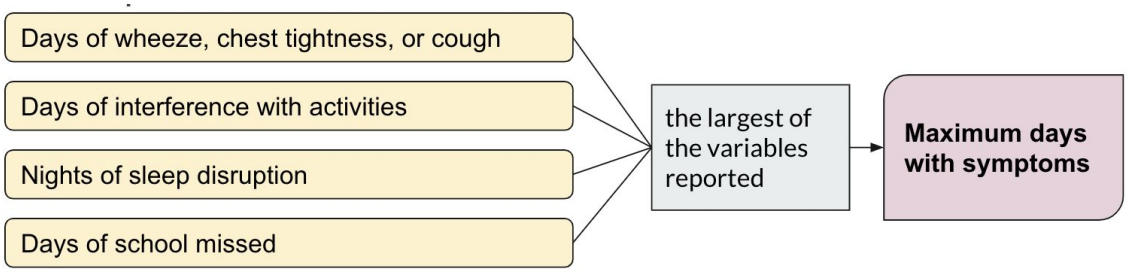
Statistical Methods

Descriptive statistics were implemented using a Wilcoxon rank-sum test to determine if there is any significant difference between control and NO treatment group. Also, assumption checks were done in multiple ways using density plots, normal Q-Q plots, and residual plots including formal tests such as Shapiro-Wilk normality test and Bartlett test for homoscedasticity.

For the primary outcome, in the original study, linear mixed model, with fixed effects for treatment groups and visits and with adjustment for levels at randomization and study site, was used to determine the effect of NO measurement. However, in the reproduction, an ANCOVA model was

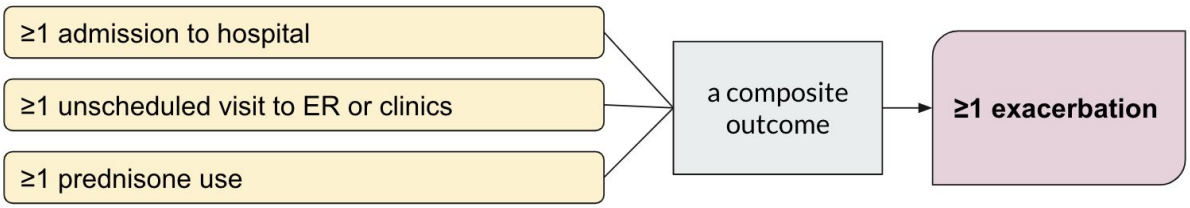
utilized because some necessary information was intentionally blinded due to the privacy issue, for example, regional information. Also, the team decided to use week two measurements as patients' baseline status instead of week 1. This is because week 1 is too early to wash out the previous treatment effect for each treatment. ANCOVA model was chosen since, in a clinical trial, the outcome often is affected by not only the treatments but also other conditions, especially pre-treatment and baseline status of each patient. Patient characteristics such as wheeze, chest tightness, or cough in days, interference with activities in days, nights of sleep disruption in days, and school missed in days were used for the primary outcome. Then, the largest of each variable was reported to the investigator and only maximum days with symptoms were created as one single variable to measure the primary outcome. The relationship between the variables was summarized below:

Relationship between primary outcome variables



For the secondary outcome, investigators also measured the patients' use of health care service during the study. In detail, they recorded the events: hospitalizations, unscheduled visits, prednisone use, and exacerbations. All the events were summed up throughout the study and categorize them into binary format: "any" versus "none." In order to compare the probability of the events happened, all the events were summarized by proportions, and Chi-square test was used to evaluate if there is a significant difference in proportion between control and NO treatment group. The relationship between the variables was summarized below:

Relationship between secondary outcome variables



In addition to the original study's result, the team conducted extensive sub-group analysis on gender. The purpose of this study is to check if each sub-group's result for the primary outcome is consistent with the result of the primary objective as a whole. Likewise, an ANCOVA model was used instead of a linear mixed model to assess the significant difference between the sub-groups.

Results

Sample size calculation

The sample size calculation in the reproduction was inconsistent with the calculation of the original study. The original research reported that they need only 165 patients per group and recruited around 500. However, the team's calculation showed only 496 subjects were required to meet the 0.05 significance level, 90% power, and the given effect size. Applying the 35% as an expected drop-out rate, 764 subjects in total were required to be recruited. Assuming that the given information is correct, the original study might have been either wrongly calculated the sample size or wrongly described in the paper.

Centralized block randomization

Following the original study, by using blockrand R package, the team applied the block randomization to randomize the patients to their assigned treatments. Block size ten was applied since the study was made in ten centers in the U.S. A part of randomization results was displayed in the appendix.

Descriptive statistics

Descriptive statistics showed that demographic characteristics of participants in the two treatment groups did not differ. After the randomization, 276 patients in NO monitoring group had their treatment with eNO measurement, while 270 subjects in control received conventional treatment. Also, assumption checks were made including normality and homoscedasticity. However, none of the

distributions were significantly normal according to the results of normal Q-Q plot and density plots in the appendix. Also, the result of Bartlett's test for homoscedasticity demonstrated that some of the characteristics variables showed insignificant results. Detailed summaries are provided in [Table 2]. Compared to the pre-study levels, asthma-related symptoms were alleviated over the run-in period regardless of the study groups. Detailed summaries are provided in [Table 1]. The result matched with the original study.

Primary outcome

The primary outcome was the number of days with asthma symptoms, which is the maximum days with symptoms for each 2-week recall at each visit during the 46-week treatment period. The mean of maximum days with symptoms for all visits was provided in [Plot 4]. After the run-in period, asthma symptoms remained low in both groups throughout the study. The baseline information is pretty significant, while the treatment is not significant in the univariate model and the multivariate model.

Secondary outcome

The secondary outcome was the use of asthma-related health care due to asthma symptoms. The proportion of patients with these rare events was provided in [Table 1]. Admissions to a hospital, unscheduled use of health care, prednisone use or asthma exacerbations did not differ between groups (Plot 5) but were much lower overall than they had been in the year before the study(Table 0).

Sub-group analysis

To assess whether the intervention could prove effective for patients with certain characteristics, we tested for heterogeneity of treatment effects across several baseline characteristics including gender, race, income, and obesity. We found that the effect of treatment based on primary outcome differs in gender subgroups.

In female, the treatment effect is not significant (p-value = 0.176). While there is significant treatment effect in male group (β = 0.632, p-value = 0.047). The effect is negative, which means the treatment increase the patients' days with asthma symptoms in the male group with marginal significance. The treatment effect difference is not detected when focusing on an entire population

analysis; it may be because of the well-balanced gender distribution in the treatment vs. control group. (53% male in treatment group vs. 53% male in control group).

Discussion

When it comes to the sample size calculation, there was inconsistency between original study and reproduced result. Assuming that the given information is correct, the original study might have been either wrongly calculated the sample size or wrongly described in the paper. This should be clarified by the authors of the original study.

For primary outcome, the result was consistent with the original study, although different statistical method was utilized. There was no significant difference in effectiveness between control and NO treatment group. Due to the limitation of accessing the necessary information, the team could not reproduce the result using linear mixed model. In particular, only two weeks data (baseline status after run-in period and last visit measurement) was used to compare the difference. This could be limitation of this reproduction study.

For secondary outcome, not enough evidence was shown that there is a significant result in participants' asthma-related use of health care. Overall use of health care service was low and rare and did not significantly differ between the groups.

For additional sub-group analysis, the result showed that there is a marginally significant treatment effect in male.

Overall, the team's result was consistent with the original study and could conclude that conventional NAEPP asthma management guideline resulted in good control of symptoms in most participants.

Appendix

Final Dataset

Study dataset name	`Group2_data_2019` or `Group2_data_2019.csv`
Number of rows	546
Number of columns	129

Block randomization result for site1 as an example

stratum	block.id	block.size	treatment
site 1	1	4.000	eNO
site 1	1	4.000	control
site 1	1	4.000	control
site 1	1	4.000	eNO
site 1	2	14.000	eNO
site 1	2	14.000	control
site 1	2	14.000	control
site 1	2	14.000	control
site 1	2	14.000	eNO
site 1	2	14.000	eNO
site 1	2	14.000	eNO
site 1	2	14.000	control
site 1	2	14.000	control
site 1	2	14.000	eNO
site 1	2	14.000	eNO
site 1	2	14.000	control
site 1	2	14.000	control
site 1	2	14.000	eNO
site 1	3	8.000	eNO
site 1	3	8.000	control
site 1	3	8.000	eNO
site 1	3	8.000	eNO
site 1	3	8.000	control
site 1	3	8.000	eNO
site 1	3	8.000	control
site 1	3	8.000	control
site 1	4	2.000	eNO
site 1	4	2.000	control
site 1	5	8.000	eNO
site 1	5	8.000	control
site 1	5	8.000	control
site 1	5	8.000	eNO
site 1	5	8.000	control
site 1	5	8.000	eNO
site 1	5	8.000	control
site 1	5	8.000	eNO
site 1	6	14.000	eNO
site 1	6	14.000	eNO
site 1	6	14.000	control
site 1	6	14.000	control
site 1	6	14.000	eNO
site 1	6	14.000	control
site 1	6	14.000	control
site 1	6	14.000	eNO
site 1	6	14.000	eNO
site 1	6	14.000	control
site 1	6	14.000	eNO
site 1	6	14.000	eNO
site 1	6	14.000	control
site 1	6	14.000	control

Tables

Table 0. Overall reproducted demographic and clinical characteristics at random assignment by study group

	All participants at enrolment (n=546)	Treatment Group (n=276)	Control Group (n=270)
Demographic characteristics			
Age at recruitment (years)	13.89(1.78)	13.88(1.76)	13.9(1.81)
Sex (male)	288/546 (53%)	146/276 (53%)	142/270 (53%)
Ethnic origin			
Black	356/546 (65%)	184/276(67%)	172/270(64%)
Hispanic	148/546 (27%)	69/276(25%)	79/270(29%)
Other or mixed	42/546 (7%)	23/276(8%)	19/270(7%)
Household income <US$15 000	151/502(30%)	70/251(28%)	81/251(32%)
Asthma-related characteristics			
Days with asthma-related symptoms			
Maximum days with symptoms	5.8(4.7)	2.2(2.8)	2.5(3.0)
Days of wheeze, chest tightness, or cough	4.5(4.1)	1.8(2.7)	2.2(3.0)
Days of interference with activities	3.3(4.1)	1.2(1.9)	1.0(1.7)
Nights of sleep disruption	2.7(3.7)	0.6(1.5)	0.6(1.4)
Days of school missed	0.7(1.4)	0.2(0.6)	0.3(1.0)
Lung funtion and exhaled nitric oxide(NO)			
FEV1 (proportion of best FEV1)	92.1%(16.6)	95.9%(15.6)	95.0%(16.5)
FEV1/FVC	77.8(9.4)	79.9(9.1)	80.2(8.4)
Use of asthma-related health care in the year before enrolment			
≥1 admission to hospital	51/546(9%)	26/276(9%)	25/270(9%)
≥1 unscheduled visit	99/546(18%)	47/276(17%)	52/270(19%)
≥1 prednisone course	197/546(36%)	93/276(34%)	104/270(39%)
≥1 exacerbation	249/546(46%)	121/276(44%)	128/270(47%)

Table 1. Primary and secondary outcomes by treatment group with p-values at last visit

	Treatment Group (n=276)	Control Group (n=270)	Difference	p value
Asthma-related symptoms				
Maximum days with symptoms	1.6(2.5)	1.5(2.4)	0.09	0.44
Days of wheeze, chest tightness, or cough	1.4 (2.2)	1.3(2.4)	0.01	0.36
Days of interference with activities*	0.7(1.7)	0.6(1.7)	0.04	0.29
Nights of sleep disruption*	0.4(1.0)	0.4(1.3)	-0.05	0.39
Days of school missed	0.2(0.8)	0.2(0.8)	0.02	0.53
Asthma-related use of health care during 46 weeks				
≥1 admission to hospital	2.9%	3.7%	-0.8%	0.60
≥1 unscheduled visit	15.9%	17.8%	-1.8%	0.57
≥1 prednisone course	29.7%	35.6%	-5.8%	0.15
≥1 exacerbation	34.8%	40.0%	-5.2%	0.21

Table 2. Assumption check: Bartlett test of homogeneity of variances

Primary outcome variables	p.bartlett	p.shapiro
Maximum days with symptoms	0.4898	< 0.001
Days of wheeze, chest tightness, or cough	0.3163	< 0.001
Days of interference with activities	0.9491	< 0.001
Nights of sleep disruption	< 0.001	< 0.001
Days of school missed	0.8745	< 0.001

Table 3. Main effect of ANCOVA model

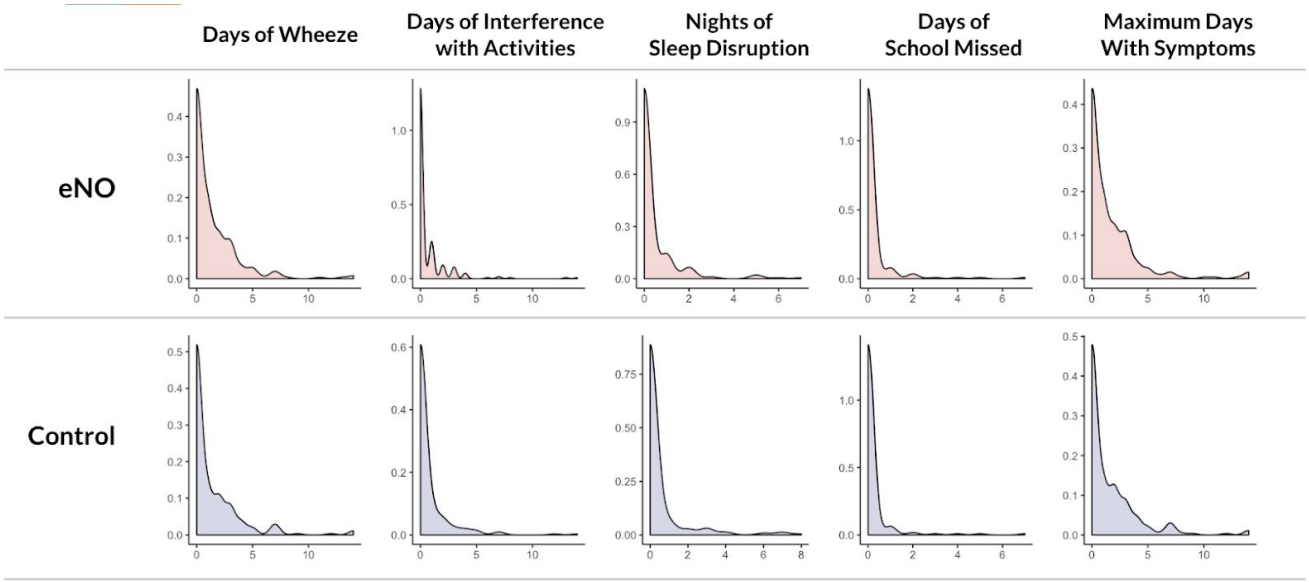
Variable	Crude Effect	Adjusted Effect	95% Wald CI		Marginal p-value	p-value
			Lower	Upper		
Visit 2 measurement Days with asthma symptoms at the second menstrual period	0.137	0.983	0.06	0.22	< 0.001	< 0.001
Treatment Asthma treatment	0.087	0.983	-0.31	0.56	0.698	0.565

Table 4. Assumption check of sub-group analysis on gender: Shapiro test and Bartlett test results

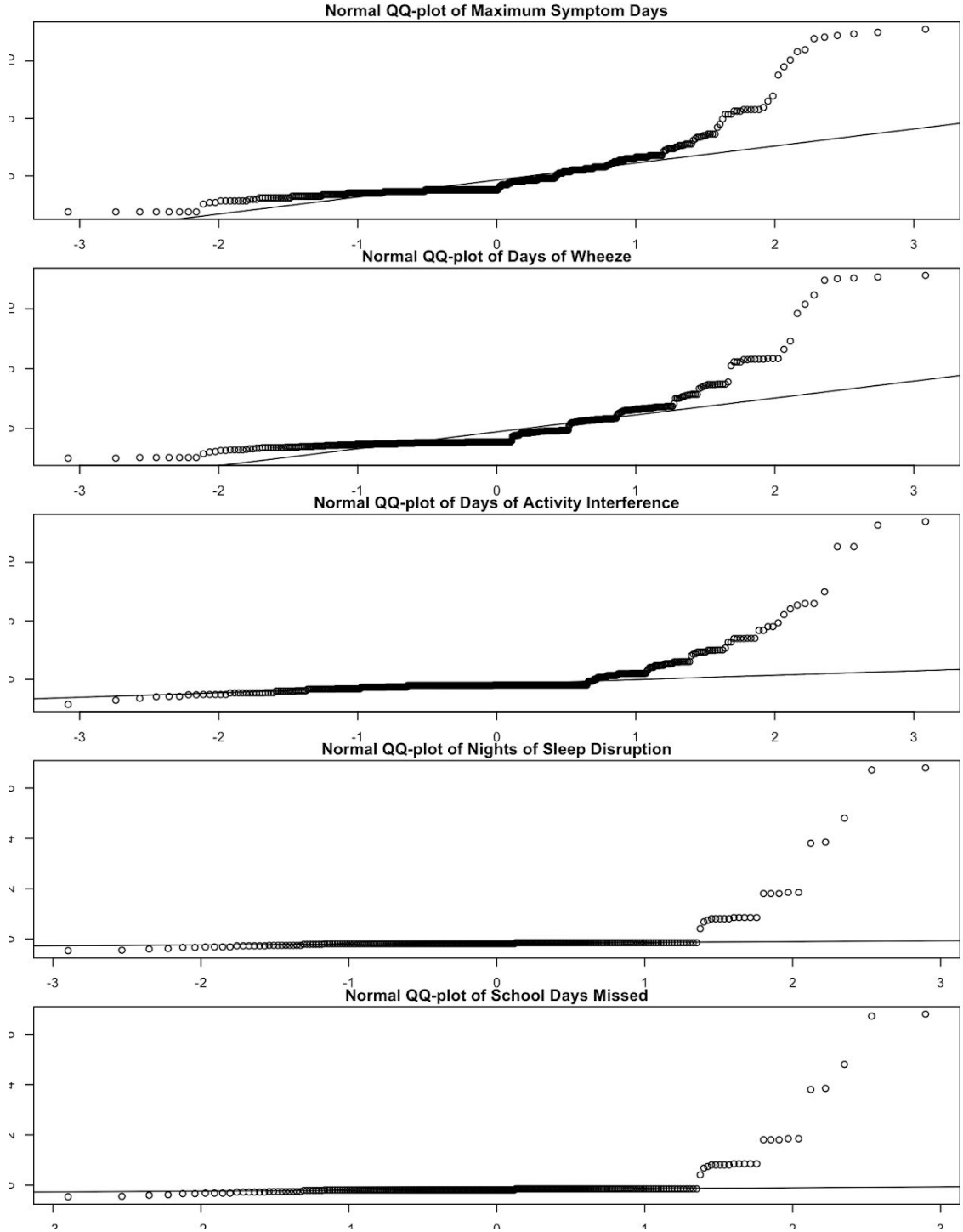
Primary outcome variables	Male p.bartlett	Female p.bartlett	Male p.shapiro	Female p.shapiro
Maximum days with symptoms	0.0129	0.0351	< 0.001	< 0.001
Days of wheeze, chest tightness, or cough	0.1424	< 0.001	< 0.001	< 0.001
Days of interference with activities	0.9884	0.793	< 0.001	< 0.001
Nights of sleep disruption	0.4658	< 0.001	< 0.001	< 0.001
Days of school missed	0.3839	0.5666	< 0.001	< 0.001

Plots

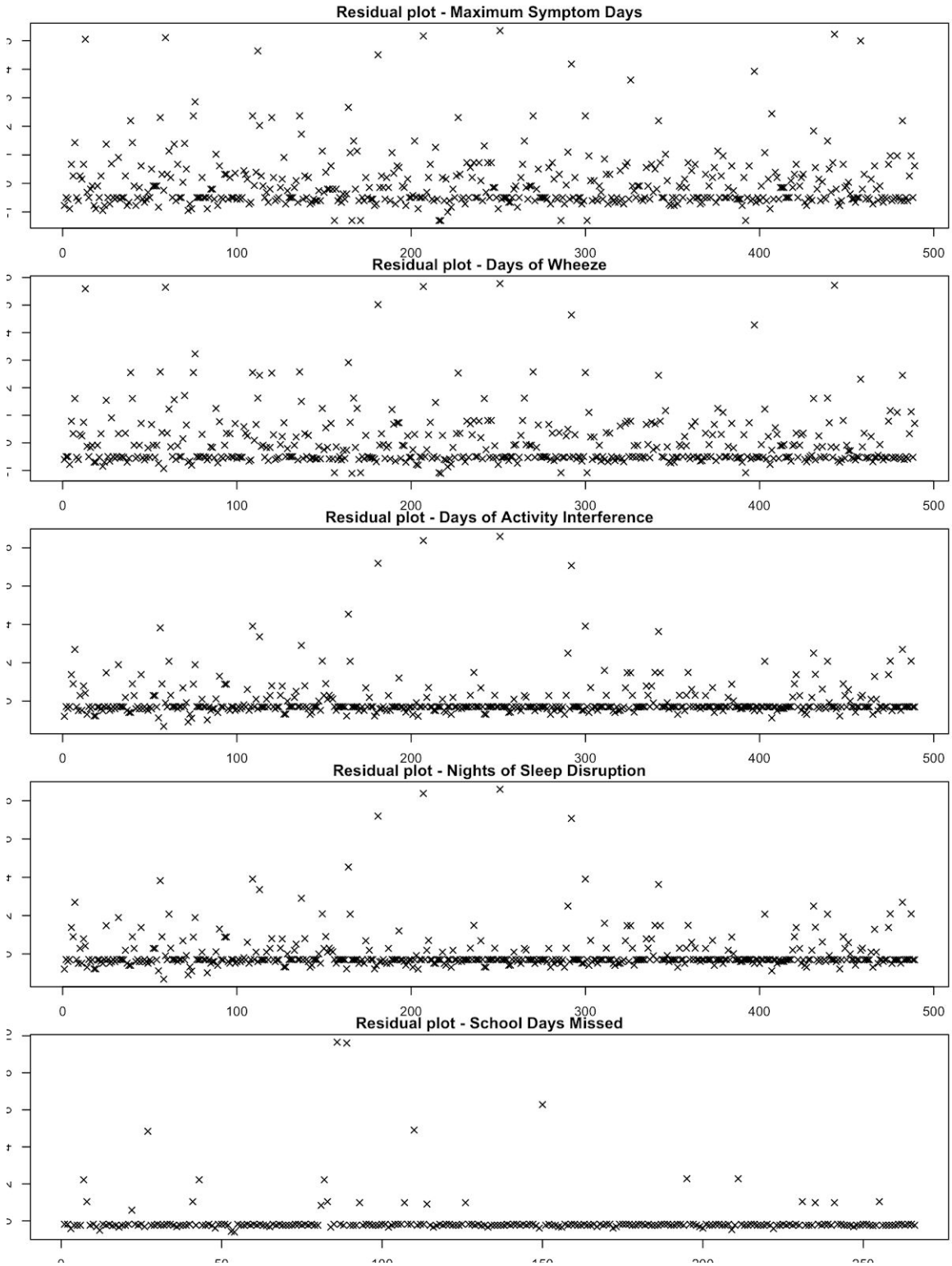
Plot 1. Assumption check: density plots for continuous variables



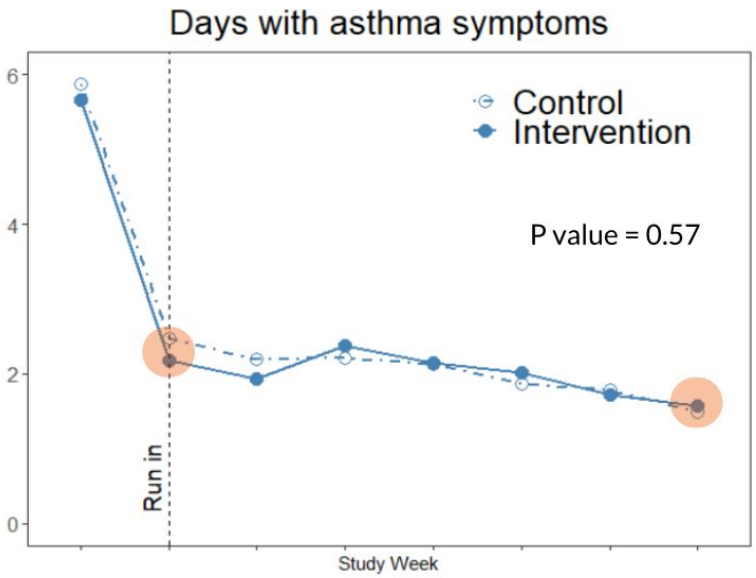
Plot 2. Assumption check: normal Q-Q plots



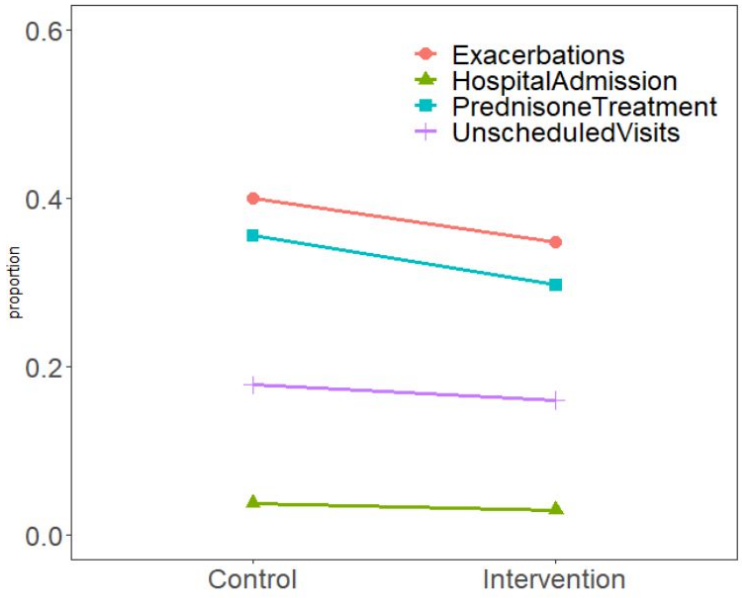
Plot 3. Assumption check: residual plots



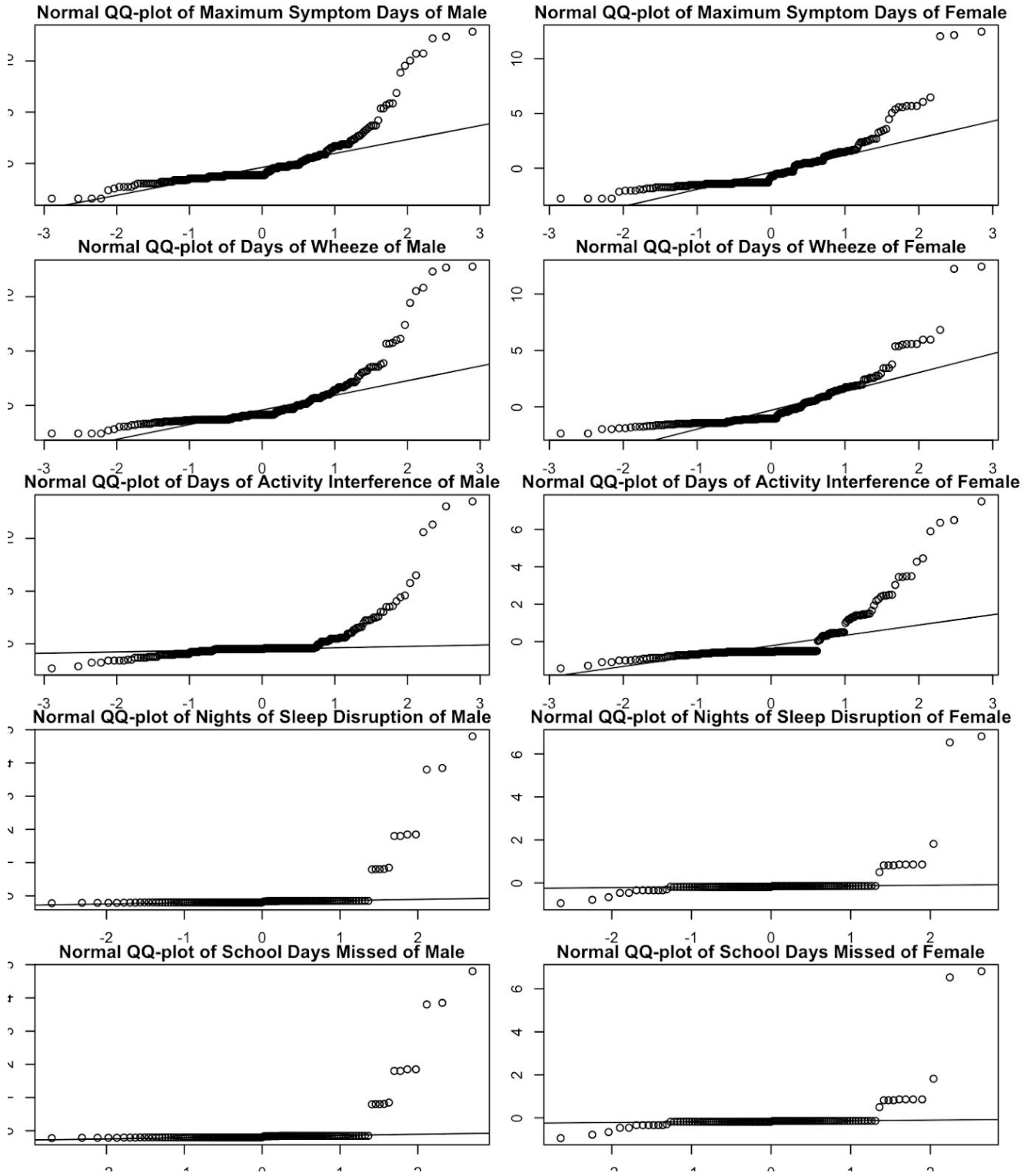
Plot 4. Primary outcome by study group: days with asthma symptoms



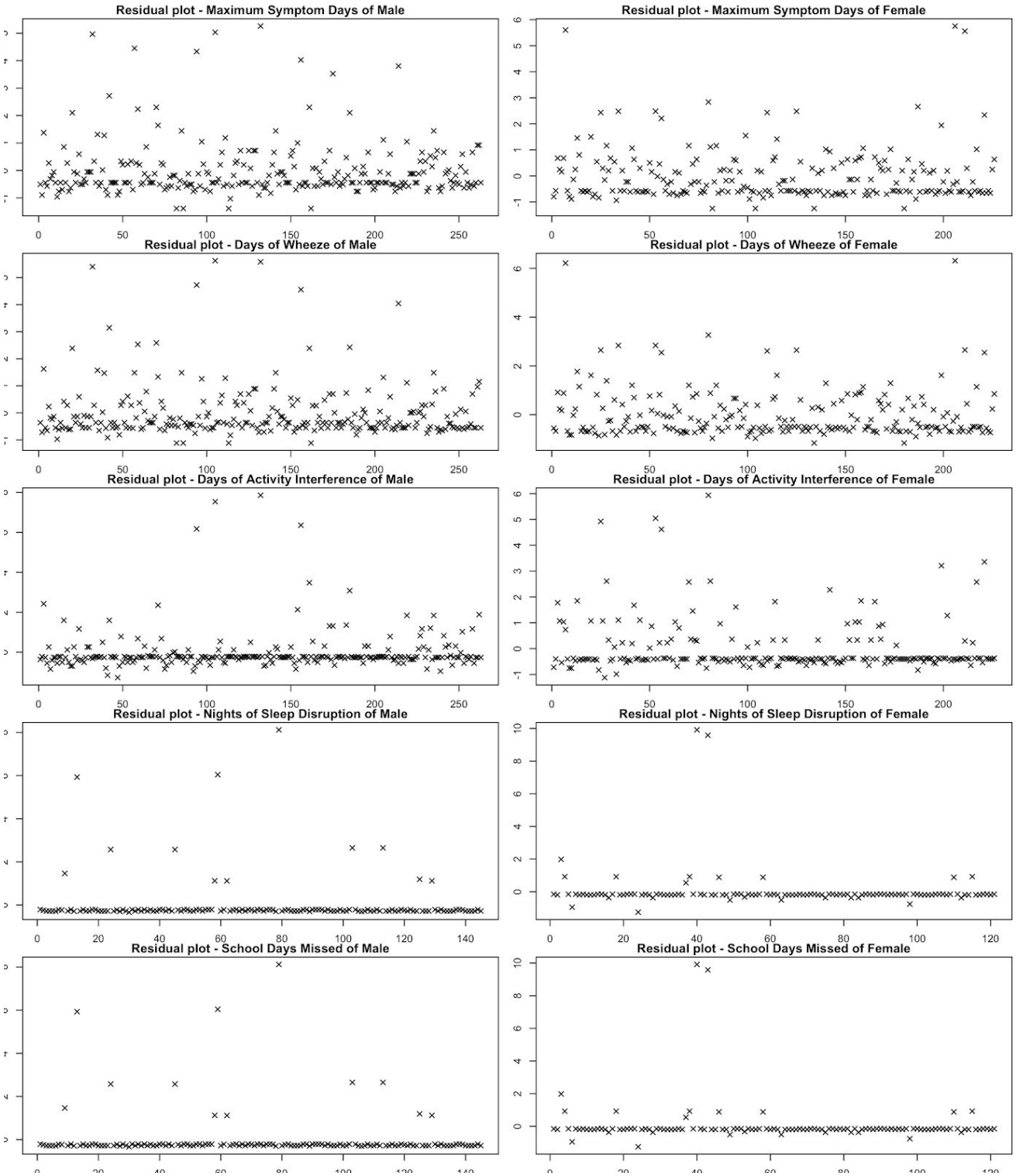
Plot 5. Secondary outcomes by study group: asthma-related use of heatlh care



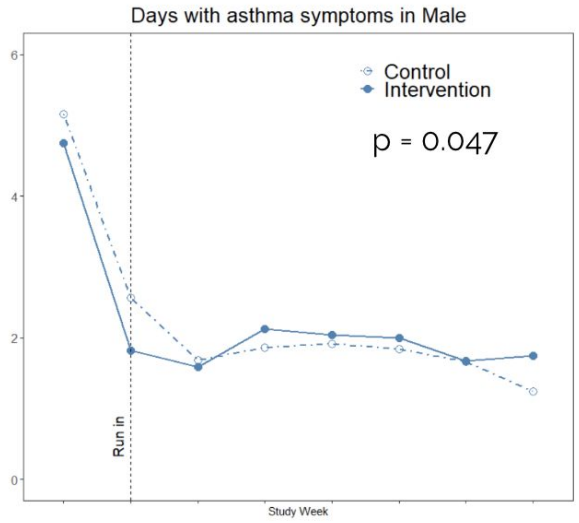
Plot 6. Sub-group analysis on gender: Assumption check - Normal Q-Q plot

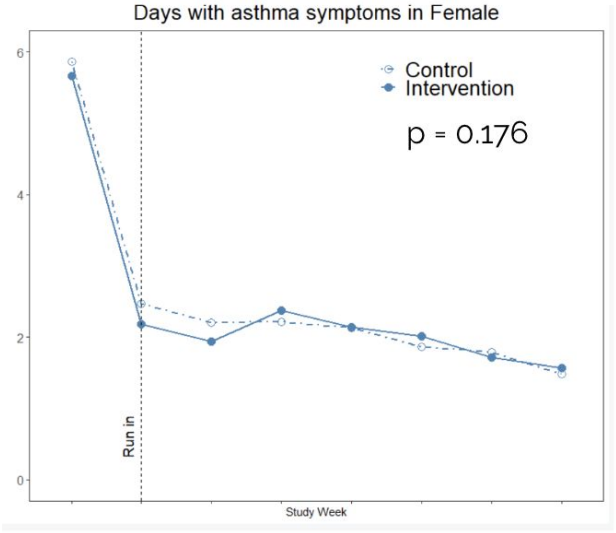


Plot 7. Sub-group analysis on gender: Assumption check - Residual plot



Plot 8. Sub-group analysis on gender: days with asthma symptoms by treatment group by gender





Contributions

- **Taehoon Ha**: Paper review, data preparation and cleaning, data analysis, final presentation, final report, and proofreading
- **Yingtong Lyu**: paper review, data quality check and preparation, literature research, data analysis, final presentation, final report, and proofreading

References

1. Centers for Disease Control and Prevention, Asthma Prevalence in the U.S., 2017
2. Arroyave, Whitney D. et al. Asthma severity, not asthma control, is worse in atopic compared with nonatopic adolescents with asthma (Annals of Allergy, Asthma & Immunology , Volume 116 , Issue 1 , 18 - 25)
3. Szefler SJ, Mitchell H, Sorkness CA, et al. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet*. 2008;372(9643):1065–1072. doi:10.1016/S0140-6736(08)61448-8