INVESTIGATING THE EFFICACY OF FORMOTEROL COMPARED TO SALBUTAMOL USING ADJUSTED ANALYSIS

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Abstract

Asthma is a non-commutable disease characterised by recurrent breathing difficulties [30]. Presently there is no cure for asthma, however treatments are available that can help reduce or prevent its symptoms [17]. In this study we investigate the efficacy of two such treatments, Formoterol and Salbutamol. An Analysis of Covariance is used to compare response PEF in patients to determine whether efficacy differs between the two treatments, whilst adjusting for baseline imbalance [2] [20]. A non-significant result for treatment group provides no evidence of a difference in the efficacy of the two treatments. This supports previous studies [23] [19] [26]. We do, however, raise issues with the design of the trial, particularly concerning sample size, and suggest that further research should be conducted with certain methodological improvements and a more thorough approach to trial design.

1. Introduction

Asthma is a chronic respitory disease affecting an individuals airways [30] [6]. It is characterised by attacks of breathlessness and wheezing [30] [17], with symptoms varying in both frequency and severity between individuals. Asthma occurs globally [8] [1] [30] and it is estimated to affect 235 [30] to 300 [15] million people. The World Health Organisation estimates that there were 383,000 deaths due to asthma in 2015 [30], with many deaths being preventable [15].

Simple breathing tests, combined with the knowledge of a patients symptoms, can be used to diagnose asthma. There are, however, differences in diagnosing a child and diagnosing an adult [5] [14]. There are many tests to aid in diagnosing asthma [18] [5] [4], however, no one test can definitively conclude that a patient has asthma [5]. Using a combination of tests and patient knowledge, it can be deduced whether treatment can help to reduce patient symptoms. For adults, the peak expiratory flow (PEF) test measures lung function [18] [5]. Patients exhale as hard and as fast as they can into a small device called a peak flow meter, and the PEF value is recorded [18] [5]. PEF is the maximum air exhaled with maximal force starting with maximal lung inflation [5] [18] [21].

Although there is currently no cure for asthma there are many treatments that can help control the symptoms [17] [6] [30]. Inhalers are the main delivery device of the treatments, which are designed to either relieve symptoms for a short time, or to prevent the symptoms occurring [17] [30] [3].

In this study we investigated the efficacy of two types of treatment for asthma, Formoterol and Salbutamol, and aimed to establish if one was better than the other at reducing the symptoms of asthma. They are both long lasting treatments that aim to prevent the symptoms of asthma [23] [19]. Previous research has found no difference in the efficacy of the two treatments [23] [19] [26]. This trial utilised the PEF test, where PEF was measured in litres of air per minute. We used clinical trial data of PEF readings taken before and after the application of treatments to two groups of patients, and used adjusted analysis methods to access whether any difference in efficacy exists between the treatments. We used Analysis of Covariance in order to account for the difference in baseline PEF readings between the two groups [28] [2].

2. Methods

A randomised trial was conducted to compare the efficacy of two treatments for Asthma, "Salbutamol" and "Formoterol". The variables included; "Gender" (Male or Female), "Treatment" (Formoterol or Salbutomol), "Baseline PEF" (Peak expiratory flow rate before treatment), "Response PEF" (Peak expiratory flow rate taken 8 hours after patients received a single dose of a randomly assigned treatment), and "Age" (in years). Peak expiratory flow was measured in litres of air per minute.

The study consisted of 17 patients being treated for Asthma using one of the two treatments. Treatments were assigned to each patient using simple randomisation [13], with a probability of 50% of receiving either Salbutamol or Formoterol. There were 9 patients treated with Salbutamol and 8 treated with Formoterol. Across all groups patients' age ranged from 26 to 48 years.

In baseline/response clinical trials such as this, statistical comparisons can be made in several ways. If we wished to perform an unadjusted test (which should be our main focus in most scenarios [2] [20]), we could either compare the response variable scores between treatment groups, or we could compute a change score and compare change scores between groups [28]. Change scores, in the case of our data, are calculated by subtracting the baseline score from the response score [28]. However, if by chance the first treatment group has baseline scores that are higher than the second treatment group, the effectiveness of the treatment will be underestimated by comparing the response scores, and overestimated by comparing the change scores [28]. This is because baseline scores are negatively correlated with change scores [28]. In the context of our data, this is because patients with low baseline PEF scores (relative to the sample population) will usually improve more than those higher

baseline PEF scores, simply because they have greater potential to improve [28]. This is known as regression to the mean [9] [10].

An alternative approach is to use Analysis of Covariance (ANCOVA). Analysis of Covariance is unaffected by baseline imbalance between groups, thus it gives the same unbiased [25] estimate for treatment affect regardless of if baseline imbalance is present or not [28]. It has been suggested that ANCOVA should be the default significance test for baseline/response data regardless of whether there is an imbalance in baselines [25] [24] [2] [20]. From Figure 2a and 2b respectively, we can see that there is a difference in the baseline scores of the two treatment groups, and that the baseline scores a negatively correlated with change scores, confirming that for this data it is most appropriate to us Analysis of Covariance.

Analysis of Covariance is a regression method [28]. We wish fit to a linear regression model to the data with the following form:

$$\text{Response PEF} = \beta_0 + \beta_1 * \text{Baseline PEF} + \beta_2 * \text{Treatment}, \tag{1}$$

$$\text{where Treatment} = \begin{cases} 0, & \text{if the patient receives Formoterol and,} \\ 1, & \text{if the patient receives Salbutamol.} \end{cases}$$

The intercept is denoted by β_0, and β_1 and β_2 are estimated coefficients. The coefficient β_2 can be seen as representing the estimate of the difference between the two treatment groups [28]. The effect of Analysis of Covariance is to adjust each patients Response PEF score for their Baseline PEF score. An additional benefit of ANCOVA is that it has higher statistical power than the other types of analysis we have mentioned [27]. All analyses were done in `R` [22] version 3.4.2, and some made use of the function `Ancova()` from the `car` [12] package.

Assumptions of ANCOVA. Analysis of Covariance has a set of assumptions [11] that must be satisfied for the results of the analysis to be valid.

(i) Normally distributed data within groups.
(ii) Homogeneity of variance.
(iii) Interval Data.
(iv) Independent observations.
(v) The covariate(s) is independent of the treatment effect.
(vi) Homogeneity of regression slopes.

Outliers. We test for outliers with the equation that is used by `geom_boxplot()` in the `R` package `ggplot2` [29]. An observation is treated as an outlier if its value is greater than 1.5 times the 75th quantile of the data or less than 1.5 times the 25th quantile of the data.

From Figure 1 we can see that there exists one outlier in the Salbutomol treatment group. We have chosen to remove this observation from our analysis due the extremely low Response PEF score.

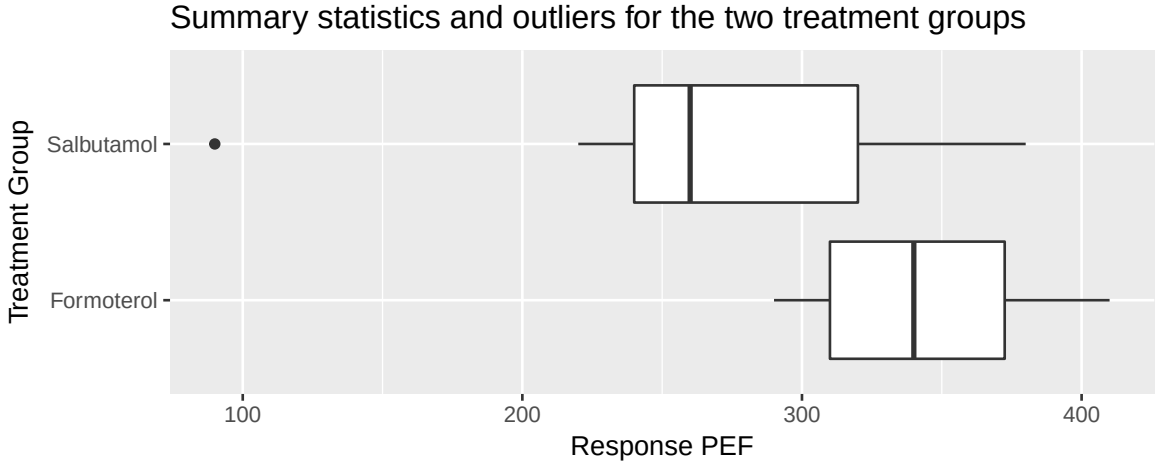

FIGURE 1. Box-plots of the two treatment groups. The centre lines are the medians, the outer edges of the boxes are the 25th and 75th percentiles, the whiskers extend to the largest values within 1.5 times the interquartile range (IQR). Any points beyond 1.5 times the IQR are outliers and are denoted separately using a point.

3. Results

Testing the assumptions of ANCOVA. As previously mentioned, Analysis of Covariance has certain assumptions that must be satisfied in order for the our statistical inferences to be valid. We will now prove that these assumptions are satisfied for the data in this trial.

(i) Normally distributed distributed data within groups. We can check the first assumption of Analysis of Covariance in multiple ways. We first use a Shapiro-Wilks test to test for normality of the data. For a Shapiro-Wilks Normality test;

$$H_0 : \text{The data is Normally distributed,} \quad vs. \quad H_A : \text{The data is not Normally distributed.}$$

The p-value for the Salbutamol group was 0.7195 and the p-value for the Formoterol group was 0.1954. At the 5% significance level we can comfortably reject the alternate hypotheses and state that there is not enough evidence to suggest that the within group distribution of either group was different from a Normal distribution.

It is known, however, that formal normality tests such as the Shapiro-Wilks test have low power for small sample sizes [16], so we combine this test with Figure 2c. From Figure 2c we can see that both groups lie on approximately straight lines. It is difficult to tell due to the small sample size, but based on the evidence we will assume that both groups have a normal distribution.

(ii) Homogeneity of variance. We can check the second assumption of Analysis of Covariance using Levene's test. In the context of our data, under Levene's test;

$$H_0 : \text{The variances in each group are equal,} \quad vs. \quad H_A : \text{The variances in each group are different.}$$

The p-value for the Levene Test was 0.6738. At the 5% significance level we can comfortable reject the alternate hypothesis and state that there is not enough evidence to suggest that the variances of the two treatment groups differ.

(iii) Interval Data. We know that the Response PEF variable is measured in "litres of air per minute" which is continuous, so we know that the third assumption is satisfied.

(iv) Independent observations. We know that one patients response to the either treatment does not affect any other patients response to either treatment. Thus the fourth assumption is satisfied.

(v) The covariate(s) is independent of the treatment effect. The covariate in the context of our data is the baseline PEF. We know that baseline PEF is independent of treatment effect, as the baseline PEF score is measured before the treatment is applied, so there is no way that treatment effect can affect the covariate. Thus we can state that the fifth assumption is satisfied.

(vi) Homogeneity of regression slopes. We can check the final assumption through Figure 2d. We can see that the regression lines fitted for each treatment group are approximately parallel. Thus the sixth assumption for Analysis of Covariance is satisfied.

Analysis of Covariance. We performed an Analysis of Covariance with Type II sum of squares. The linear model that was fitted, including coefficient estimates, was:

$$\text{Response PEF} = 172.7519 + 0.5429 * \text{Baseline PEF} \ - 26.6253 * \text{Treatment}.$$

The p-value for treatment group was 0.25688. Testing at the 5% significance level we cannot accept the alternate hypothesis. In this case, the p-value for treatment was the same to 5 d.p. for ANCOVAs performed with Type I, Type II, and Type III sum of squares.

4. Discussion

Analysis of Covariance. From the results of the Analysis of Covariance we can state there was not enough evidence to support the idea that one of the two treatments, Salbutamol or Formoterol, was any better than the other at increasing the Peak Expiratory Flow rate in adult patients with asthma. This supports other studies comparing the efficacy of Salbutamol and Formoterol [23] [19] [26].

Strengths and limitations of the study. Due to the nature of the data, and the imbalance in the baselines between treatment groups, we strongly believe that Analysis of Covariance was the appropriate methodology to use over unadjusted methods [2] [20] [28]. We recommend that future investigations based upon baseline/response data use this report as an initial framework for their analyses.

This study, however, had many flaws that should be rectified in future trials when exploring the efficacy of Formoterol over Salbutamol.

We have very little information about how the study was conducted, we do not know how the variables (both dependent and independent) were measured and the reliability of that method of measurement, we have no information about where the trial was conducted, and we do not know the patients history with asthma (whether they were diagnosed as a child or an adult for instance). These are all very important considerations when contemplating the analysis, and with such little information on the background of the data and how it was collected, readers should be cautious about any results presented by this report. We have no way of knowing if any serious design flaws or biases were introduced during the trial, nor do we have any intuition about any additional factors that could have affected the results. For instance, we know from Beasley, R. (1998) [8] that prevalence of asthma in centres across the world differ greatly: the covariates that affect prevalence could

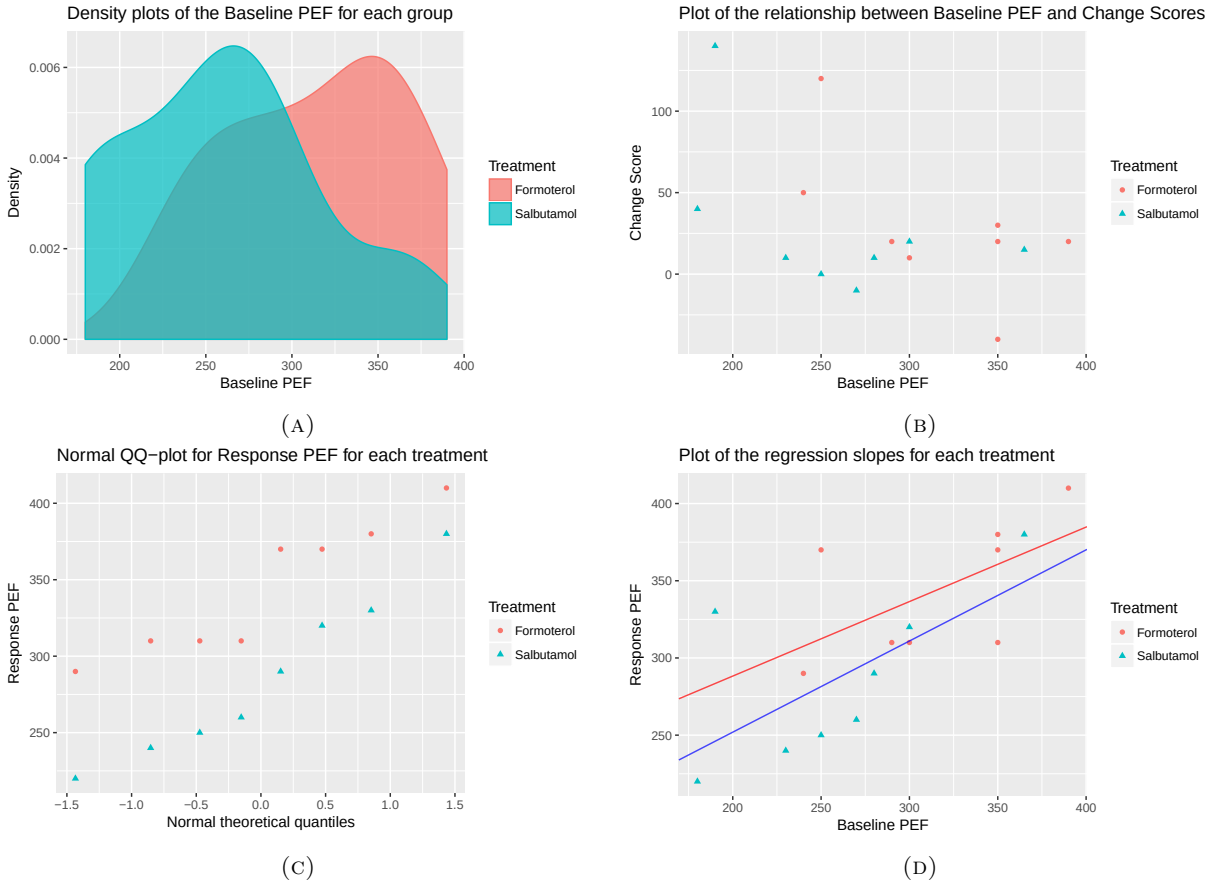

FIGURE 2. Checking the assumptions of the Analysis of Covariance: (A) A density plot demonstrating the difference in the baseline PEF of the two groups. (B) A plot showing the negative correlation of Baseline PEF with Change Score. (C) A QQ-plot for each treatment group, showing that the within group distributions are assumed normal. (D) A plot showing the regression slopes for each treatment group. We can see that the two slopes are approximately parallel and so the assumption of homogeneity of regression slopes is satisfied.

also affect the efficacy of one or both of these treatments. It is also known that various factors affect PEF performance in patients [7], such as age and gender [21]. These covariates were not accounted for in the Analysis of Covariance due to the small sample size.

The number of participants was far too small. Given that there were less than 10 patients per treatment group, we would again recommend being very wary of the results presented in this report. Subgroup analysis based on other factors, such as gender, were not considered because of this reason. If we had divided the groups further, by gender say, we would have had less than 5 patients per group. Hence we would not have returned any reliable results for the efficacy of the two treatments, nor for the effect of gender on the efficacy of the treatments. Whilst we do believe that the sample size was far too small, it is of the same magnitude as other similar studies [23] [19] [26], which range from n = 12 to n = 30.

Avenues for further research. As previously stated we would recommend that the reader be cautious of the results presented in this report for the established reasons. To this end we would recommend that the trial be repeated, with explicit detail of the trial design provided and the number of participants increased dramatically.

For these future studies, Analysis of Covariance should still be the default choice of analysis over unadjusted methods, for the same reasons as stated earlier in this report. Depending on the number of participants in future studies, sub-group analysis and alternative methods of analysis could be investigated. However, this should be stated in the trial design, and if the aim of the trial remains the same (to investigate the efficacy of Formoterol compared to Salbutamol), then the main focus of any reports should still be the results of the ANCOVA for the full treatment groups.

REFERENCES

[1] M Innes Asher, Stephen Montefort, Bengt Bjrkstn, Christopher KW Lai, David P Strachan, Stephan K Weiland, and Hywel Williams. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: Isaac phases one and three repeat multicountry cross-sectional surveys. *The Lancet*, 368(9537):733 – 743, 2006.

[2] Susan F Assmann, Stuart J Pocock, Laura E Enos, and Linda E Kasten. Subgroup analysis and other (mis)uses of baseline data in clinical trials. *The Lancet*, 355(9209):1064 – 1069, 2000.
[3] Asthma UK. Asthma inhalers, medicines and treatments. `https://www.asthma.org.uk/advice/inhalers-medicines-treatments/`, 2016. [Online; accessed 2017-11-05].
[4] Asthma UK. Tests for severe asthma. `https://www.asthma.org.uk/advice/severe-asthma/diagnosing-severe-asthma/tests-for-severe-asthma/`, 2016. [Online; accessed 2017-11-05].
[5] Asthma UK. Tests to diagnose asthma. `https://www.asthma.org.uk/advice/diagnosis/tests/`, 2016. [Online; accessed 2017-11-05].
[6] Asthma UK. What is Asthma? `https://www.asthma.org.uk/advice/understanding-asthma/what-is-asthma/`, 2016. [Online; accessed 2017-11-02].
[7] E. D. Bateman, S. S. Hurd, P. J. Barnes, J. Bousquet, J. M. Drazen, M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S. E. Pedersen, E. Pizzichini, S. D. Sullivan, S. E. Wenzel, and H. J. Zar. Global strategy for asthma management and prevention: Gina executive summary. *European Respiratory Journal*, 31(1):143–178, 2008.
[8] Richard Beasley. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: Isaac. *The Lancet*, 351(9111):1225 – 1232, 1998.
[9] J M Bland and D G Altman. Statistic notes: Regression towards the mean. *BMJ*, 308(6942):1499, 1994.
[10] J M Bland and D G Altman. Statistics notes: Some examples of regression towards the mean. *BMJ*, 309(6957):780, 1994.
[11] Andy P Field. *Discovering statistics using R*. SAGE, London, 2012.
[12] John Fox and Sanford Weisberg. *An R Companion to Applied Regression*. Sage, Thousand Oaks CA, second edition, 2011.
[13] Suresh Kp. An overview of randomization techniques: An unbiased assessment of outcome in clinical research. 4:8–11, 03 2011.
[14] F D Martinez, A L Wright, L M Taussig, C J Holberg, M Halonen, and W J Morgan. Asthma and wheezing in the first six years of life. the group health medical associates. *The New England journal of medicine.*, 332(3):133–138.
[15] Matthew Masoli, Denise Fabian, Shaun Holt, Richard Beasley, and Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the gina dissemination committee report. *Allergy*, 59(5):469–478, 2004.
[16] Nornadiah Mohd Razali and Bee Yap. Power comparisons of shapiro-wilk, kolmogorov-smirnov, lilliefors and anderson-darling tests. 2, 01 2011.
[17] NHS. Asthma. `https://www.nhs.uk/conditions/asthma/`, 2016. [Online; accessed 2017-11-02].
[18] NHS. Asthma: Diagnosis. `https://www.nhs.uk/conditions/asthma/diagnosis/`, 2016. [Online; accessed 2017-11-05].
[19] M Palmqvist, G Persson, L Lazer, J Rosenborg, P Larsson, and J Lotvall. Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *European Respiratory Journal*, 10(11):2484–2489, 1997.
[20] Stuart J. Pocock, Susan E. Assmann, Laura E. Enos, and Linda E. Kasten. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practiceand problems. *Statistics in Medicine*, 21(19):2917–2930, 2002.
[21] Ph Quanjer, M D Lebowitz, I Gregg, Martin Miller, and Ole Pedersen. Peak expiratory flow: Conclusions and recommendations of a working party of the european respiratory society. 24:2S–8S, 03 1997.
[22] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2017.
[23] Klaus F. Rabe, Rudolf Jrres, Dennis Nowak, Nikolaus Behr, and Helgo Magnussen. Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. *American Review of Respiratory Disease*, 147(6_pt_1):1436–1441, 1993. PMID: 8503554.
[24] Stephen Senn. Testing for baseline balance in clinical trials. *Statistics in Medicine*, 13(17):1715–1726, 1994.
[25] Stephen Senn. Change from baseline and analysis of covariance revisited. *Statistics in Medicine*, 25(24):4334–4344, 2006.
[26] JA van Noord, JJ Smeets, JA Raaijmakers, AM Bommer, and FP Maesen. Salmeterol versus formoterol in patients with moderately severe asthma: onset and duration of action. *European Respiratory Journal*, 9(8):1684–1688, 1996.
[27] Andrew J. Vickers. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Medical Research Methodology*, 1(1):6, Jun 2001.
[28] Andrew J Vickers and Douglas G Altman. Analysing controlled trials with baseline and follow up measurements. *BMJ*, 323(7321):1123–1124, 2001.
[29] Hadley Wickham. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2009.
[30] World Health Organisation. Asthma Fact Sheet. `http://www.who.int/mediacentre/factsheets/fs307/en/`, 2017. [Online; accessed 2017-11-02].

Appendix A. R code

The R code that was used to explore the data and run statistical analysis is detailed below.

```
1
2  ##################
3  #Preamble
4  ##################
5
6  Asthma_Data <- read.table("asthma.txt", header = TRUE)
7
8  colnames(Asthma_Data)[3] <- "Treatment" # Used to make default Legends on plots have capital "
       T" for treatment.
9
10 levels(Asthma_Data$Treatment) <- c("Formoterol", "Salbutamol") # Used to make default Legends
       on plots used full names for treatment groups
11
12 library("dplyr")
13 library("ggplot2")
14 library("car")
15
16 ############################
17 # Exploratory Analysis
18 ############################
19
20 nrow(Asthma_Data) # No. of subjects.
21 ncol(Asthma_Data) # No. of variables.
22
23 summary(Asthma_Data) # Summary statistics for the data.
24
25 hist(Asthma_Data$baseline) # Histogram of baseline PEF.
26 hist(Asthma_Data$response) # Histogram of response PEF.
27 hist(Asthma_Data$age) # Histogram of Age.
28
29
30 Asthma_Data$change_score <- Asthma_Data$response - Asthma_Data$baseline
31 # Calculate the change scores
32
33 hist(Asthma_Data$change_score) # Histogram of change scores.
34
35
36 Salbutamol <- filter(Asthma_Data, Asthma_Data$treatment == "S")
37 # Create a subgroup for patients treated with Salbutamol.
38
39 Formoterol <- filter(Asthma_Data, Asthma_Data$treatment == "F")
40 # Create a subgroup for patients treated with Formoterol.
41
42
43 summary(Salbutamol) # Summary statistics for the Salbutamol group.
44 summary(Formoterol) # Summary statistics for the Formoterol group.
45
46
47 hist(Salbutamol$response, xlim= c(50, 500)) # Histogram of baseline PEF for the Salbutamol
       group.
48 hist(Formoterol$response, xlim= c(50, 500)) # Histogram of baseline PEF for the Formoterol
       group.
49
50 hist(Salbutamol$baseline, xlim= c(50, 500)) # Histogram of baseline PEF for the Salbutamol
       group.
51 hist(Formoterol$baseline, xlim= c(50, 500)) # Histogram of baseline PEF for the Formoterol
       group.
52
53 hist(Salbutamol$change_score, xlim= c(-150, 150), ylim = c(0.00001, 6)) # Histogram of change
       scores for the Salbutamol group.
54 hist(Formoterol$change_score, xlim= c(-150, 150), ylim = c(0.00001, 6)) # Histogram of change
       scores for the Formoterol group.
55
```

```r

cor(Asthma_Data$baseline, Asthma_Data$change_score)
# Pearson correlation of baseline PEF and change score for all groups.

cor(Salbutamol$baseline, Salbutamol$change_score)
# Pearson correlation of baseline PEF and change score for Salbutamol

cor(Formoterol$baseline, Formoterol$change_score)
# Pearson correlation of baseline PEF and change score for Formoterol.


cor(Asthma_Data$baseline, Asthma_Data$response)
# Pearson correlation of baseline PEF and response PEF score for all groups.
cor(Salbutamol$baseline, Salbutamol$response)
# Pearson correlation of baseline PEF and response PEF score for Salbutamol.
cor(Formoterol$baseline, Formoterol$response)
# Pearson correlation of baseline PEF and response PEF score for Formoterol.


ggplot(Asthma_Data, aes(x = Treatment, y = response)) +
  geom_boxplot() + coord_flip() + ggtitle("Summary statistics and outliers for the two treatment groups") +
  ylab("Response PEF") + xlab("Treatment Group")
# Used to visually summarise the data for each treatment group and to identify possible outliers.

# Removing the outliers:

Asthma_Data_new <- Asthma_Data[-15, ]

ggplot(Asthma_Data_new, aes(x = treatment, y = response)) +
  geom_boxplot() + coord_flip()

Salbutamol_new <- Salbutamol[-7, ]
Formoterol_new <- Formoterol


# Check it is appropriate to use an Analysis of Covariance:

ggplot(Asthma_Data_new, aes(x=baseline,  group = Treatment, colour = Treatment, fill= Treatment)) +
  geom_density(alpha = 0.7) + xlab("Baseline PEF") +
  ylab("Density") + ggtitle("Density plots of the Baseline PEF for each group")
# Density plot to visualise the baseline imbalance between groups.


ggplot(Asthma_Data_new, aes(x = baseline, y = change_score, group = Treatment, colour = Treatment, shape = Treatment)) +
  geom_point() + xlab("Baseline PEF")  + ylab("Change Score") + ggtitle("Plot of the relationship between Baseline PEF and Change Scores")
# Plot to show the relationship between baseline PEF and change scores.



##########################################
# Testing the assumptions of ANCOVA
##########################################

# 1) Homoscedasticity (Homogeneity of Variances between groups).

# Use Fishers F-Test to compare the variances in response for the two treatment groups:

Var_check <- var.test(Salbutamol_new$response, Formoterol_new$response)

Var_check

```

```
# p-value = 0.5926
# Testing at the 5% significance level we can comfortably say that there is not enough evidence in the data to suggest that the variance of the each treatment groups response variable are different.


#Or could use Levene's test from package "car":

Levene_var_check <- leveneTest(Asthma_Data_new$response, Asthma_Data_new$treatment)
Levene_var_check

# p-value = 0.6738
# Testing at the 5% significance level we can comfortably say that there is not enough evidence in the data to suggest that the variance of the each treatment groups response variable are different.




# 2) Normality Testing for each treatment groups response variable.

# Use a QQ-plot to see if the data differs greatly from a normal distribution:

qqplot_asthma <- ggplot(Asthma_Data_new, aes(sample = response, group = Treatment, colour = Treatment, shape = Treatment)) +
  geom_qq() + xlab("Normal theoretical quantiles") + ylab("Response PEF") + ggtitle("Normal QQ-plot for Response PEF for each treatment")

qqplot_asthma

# Salbutomol almost certainly has normal distribution.
# Formoterol isn't as obvious since we have so few observations but it is reasonable to assume it has a normal distribution as the line is fairly straight.

# We could also use a formal Normallity test.
# Use the Shapiro-Wilks test to test for within group normal distributions:

S_shapiro <- shapiro.test(Salbutamol_new$response)
S_shapiro
# p-value =  0.7195
# At the 5% Sig. level we can comfortably assume the distribution is normal.

F_shapiro <- shapiro.test(Formoterol_new$response)
F_shapiro
# p-value =  0.1954
# At the 5% Sig. level we can comfortably assume the distribution is normal.




#3) For each independent variable, the relationship between the dependent variable (response) and the covariate (baseline) is linear.

# Plot baseline PEF against response PEF and look for a linear relationship:

ggplot(Salbutamol_new, aes(x= baseline, y = response)) + geom_point()
# Definitely looks linear apart from one outlier.

cor(Salbutamol_new$baseline, Salbutamol_new$response, method = "pearson")
# Pearson correlation of 0.6589

ggplot(Formoterol_new, aes(x= baseline, y = response)) + geom_point()
# Looks reasonably linear, more outliers but very small population so difficult to say.

cor(Formoterol_new$baseline, Formoterol_new$response, method = "pearson")
# Pearson correlation of 0.5897

```

```r
ggplot(Asthma_Data_new, aes(x= baseline, y = response, group = Treatment, shape = Treatment, colour = Treatment)) + geom_point()
```

```r
# 4) The lines expressing these linear relationships are all parallel (Homogenity of regression slopes).

# Fit linear models for the data:

Treatment_LM <- lm(Asthma_Data_new$response ~ Asthma_Data_new$baseline + Asthma_Data_new$treatment)

Treatment_LM

levels(Asthma_Data_new$treatment) # Used to see the order in which the treatment groups are assessed.

# The model is
# Response = 172.7519 + 0.5426*Baseline - 26.6253*I_Treatment
# Where I_Treatment = 0 if F and 1 if S

S_LM <- lm(Salbutamol_new$response ~ Salbutamol_new$baseline)
S_LM

#The model is
# Response = 133.8587 + 0.5904*Baseline

F_LM <- lm(Formoterol_new$response ~ Formoterol_new$baseline)
F_LM

#The model is
# Response = 191.7625 + 0.4825*Baseline


ggplot(Asthma_Data_new, aes(x= baseline, y = response, group = Treatment, shape = Treatment, colour = Treatment)) + geom_point() +
  geom_abline(colour = "Blue", alpha = 0.7, intercept = S_LM$coefficients[1], slope = S_LM$coefficients[2]) +
  geom_abline(colour = "Red", alpha = 0.7, intercept = F_LM$coefficients[1], slope = F_LM$coefficients[2]) +
  xlab("Baseline PEF") + ylab("Response PEF") + ggtitle("Plot of the regression slopes for each treatment")

# A plot to compare the regression slopes for the two groups.
```

```r
##################
# ANCOVA
##################

# Since all the assumptions are satisfied we can now perform an ANCOVA.
# Using our linear models from before:

ANCOVA_results <- anova(Treatment_LM)

ANCOVA_results

# We can see that the p-value for Treatment is 0.256876
# Testing at the 5% significance level we can say
# that there is not enough evidence to support the idea
# that there is a difference in the efficacy of the two treatments
```

```r
# Or using the "car" package to test with different types of sum of squares:

ANCOVA_t2_results <- Anova(Treatment_LM, type = "II")

ANCOVA_t2_results


# We can see that the p-value for Treatment is 0.25688
# Testing at the 5% significance level we can say
# that there is not enough evidence to support the idea
# that there is a difference in the efficacy of the two treatments


ANCOVA_t3_results <- Anova(Treatment_LM, type = "III")

ANCOVA_t3_results


# We can see that the p-value for Treatment is 0.25688
# Testing at the 5% significance level we can say
# that there is not enough evidence to support the idea
# that there is a difference in the efficacy of the two treatments
```