Investigation of Repeated measurement data using Mixed Models

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Abstract

Objective: This study investigates repeated measurements data of different subjects using the heart rate (RR-Interval) as response. The study seeks to explore the use of Mixed models with the objective of observing which methods leads to depleted standard errors.

Methodology: Various mixed models were fit and carried out taking baseline correctional methods into account. Six different types covariance structures were taken into account as a working correlation to investigate the models.

Results, Discussions and Conclusion: It was observed that Model D1 was the most efficient model out of all the other four models as it had the least standard errors in all cases. Again Model D1 coming from the unstructured covariance structure was the best model as it had the smallest AIC. It was found out that ECG measurements taken longer before the up-take of treatment tend to have smaller standard errors.

Keywords: RR-interval, compound symmetry, unstructured, covariance, Akaike Information Criterion (AIC), mixed models

Declaration

I, the undersigned, hereby declare that the work contained in this research project is my original work, and that any work done by others or by myself previously has been acknowledged and referenced accordingly.

Finhai Mupfuti, 14 May 2020

Contents

1. Introduction

1.1 Background of the Study

In a clinical trial, especially a thorough QT/QTc Study (TQT), the main emphasis is to study and monitor the heart muscle repolarization. By doing so, it gives an opportunity of assessing a drug's potential to delay the cardiac repolarization. This entails on wanting to observe how a drug does affect subjects either in a positive or negative way and seeing the extent of the severity. According to the ICH-E14 guideline, it is there essential to determine and examine how different drugs will change the cardiac system and its repolarization. Of great importance is to observe the significance of a prolonged RR-interval which if not given greater attention can result in a more deadly and fateful condition. This is initiated by a heart rhythm condition causing fast and uncontrollable heart patterns and because of this, at times the heart may take long to regenerate between these heart beats which can prove fatal.

A more objective way should therefore be adopted in the need of wanting to carrying out a crossover experiment. This is aided by most drugs available possessing a short and half life. A crossover study will ensure that both pharmacodynamic and pharmacokinetic effects are met and satisfied. Therefore, the use of different statistical methods is very useful in solving the ICH-E14 guideline and ensuring that all is met. Since the guideline is centralized on the measuring the QTc prolongation, it is important to note that the RR-interval will be a key variable in determining and estimating the risk changes so as to align with the objectives of the study.

The heart rate is known to be a cardiac process that involves the enumeration of an individual's heart beat per minute. The difference in heart rate are of interest since there is an availability of rich information and variety of inferences that can be drawn. The motivation for this is that variability that tends to be large in heart rate is identified with a healthful individual where on the other hand, a decreased variability indicates irregularity for an individual. A common degree to measure this will be incorporate the RR-interval which evaluates the differences in heart beats which will be useful in a study with repeated measurements.

Since the RR-interval is a key parameter in the analysis of cardiac repolarization, most and past research studies have put much emphasis on both the QT and the QTc-interval (corrected-heart rate interval). These studies involve baseline corrections and covariance structures separately in which situation analysis is desired for the relationship between the drug and a subject. During the past years, a large number of papers have been published on both baseline corrective and covariance methods of adjusting the prolongation of the heart muscle. Methods based on these utilization are found in (Sethuraman and Sun, 2009) and (Schall and Ring, 2010) to mention a few. (Sethuraman and Sun, 2009), using a TQT study compared the impact of four baseline correctional methods which were time-matched before each period, time-averaged, time-matched baseline before the first period and pre-dose in terms of the QT-interval prolongation induced by moxifloxacin. Results showed that the predose baseline had the highest variability in comparison to the other baseline methods. (Schall and Ring, 2010) compared a number of more sophisticated covariance structures for modeling QT data uncorrected for the heart rate.

To present, statistical mixed models have emerged as a powerful tool to analyse clinical trial studies and dataset that are of the nature of repeated measurements. The main reason for this is that the repeated nature of these data taken from an electrocardiogram (ECG) will enable to measure effects of pharmacodynamics with conclusions and meaningful information being derived for it. This study seeks

to explore the use of statistical methods in the form of mixed models to analyse data with repeated measurements with an objective to come up with a different methodology that assesses treatment's potential to affect the cardiac system of a subject.

1.2 Aims and Objectives

The aim of this study is to find a method which leads to small standard errors for the estimates of interest and compare the results with methods of selection using the heart rate as the response. On the other hand, the objectives of the study is to investigate and compare:

1. Different definitions of the baseline when analysing the "change from baseline" of the RR-intervals.
2. Various structures of the covariance matrices to describe the repeated measurements.
3. The interaction of the baseline correction methods and covariance matrices
4. A comparison of results coming from R and SAS software.

1.3 Project Layout

This project consists of five chapters.

Chapter 1 gives a historical background (Introduction), aims and objectives of the study. It gives an overview of the study as a collective.

Chapter 2 gives a literature review on the statistical methods related to the RR-interval, the data used for the study, the five baseline correction methods, covariance structures associated with repeated measurements, the method of Analysis of Covariance (ANCOVA), exploration and appreciation of the method of QT correction (corrected-heart rate (QTc-interval)).

Chapter 3 gives the methodology used in the analysis and describes how the study was carried out, that is the methods applied and the mathematical models for the five different baseline correctional methods.

Chapter 4 provides the actual statistical analysis and results obtained.

Chapter 5 gives a brief outline of the discussion, summary and conclusions of the findings from study.

2. Literature Review

2.1 Introduction

In this chapter we seek to review the theoretical background on mixed models and the medical background of the study. Mixed models consists of an array of methods for a statistical model containing both fixed effects and random effects. It Studies various correlated measurements coming from each unit of interest. We take a look at some related literature on different types of baseline correctional methods that have an influence upon the objectives of the studies.

2.2 Relationship between QT-interval and the RR-interval (heart rate)

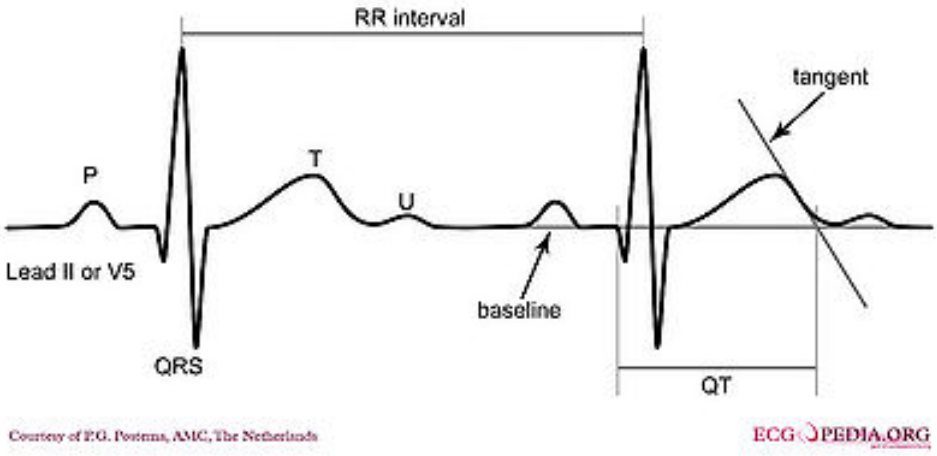

Figure 2.1: Representation of a ECG Trace

The QT interval is defined to be a measure taken from the electrocardiogram and is used to assess risks associated by the heart. In essence, QT interval shows the longevity for ventricular depolarization and from figure 2.1 it can be seen that the QT interval is nested within the RR interval. It is measured from the beginning of the QRS complex interval up to the end of the T-wave. However, the QT-interval should be corrected for the heart rate inorder to compare results. On the other hand, the RR interval is the time between QRS complex interval which is considered as the distance between two heart beats.

It is a measure of the ventricular rate and is measured by the length separated by two consecutive R waves. The idea is to enumerate the number of RR intervals between the two R waves and then propagate by 10 to get the actual reading representing the RR-interval. Moreover, there is a high dependence between the QT interval and the heart rate which shows that an inverse relationship. It is represented in the context of RR interval being short only when the heart rate interval is much longer and the QT interval being short-lived. Because of this, necessary adjustments has to be made to the QT interval.

Interval	Description	Range
QT-Interval	Duration of ventricular depolarization	up to 0.43 seconds (must be corrected for heart rate)
R-R Interval	Time between beats	0.6 -1 sec (heart rate: 60-100 bpm)

Table 2.1: Key Variables

2.3 Repeated Measures

Repeated measures are data developed by observing different individuals under various experimental conditions whereby subjects are assumed to be a random sample from a population of interest. For this crossover study, the RR-interval was measured from the ECG reading coming from a hierarchical order. The RR-interval was measured at different time points per treatment period. For each time point, subjects went threw different various ECG data points which consisted of four wave forms per ECG. This gave rise to numerous RR-interval measurements per subject at various time points. Furthermore, these various RR-interval measurements will be in the form of a repeated time points which will be averaged to assess for potential prolongation.

The repeated measures are observations that are ordered by time. Because of these repeated measures, there is an occurrence of some serial correlation as measurements taken at an hourly basis in time tend to be similar. The analysis of repeated measurements can be done by reduction of the stream of data into summary measures to be subsequently analysed by statistical methods as though they are raw data, or by analysis of the entire data set. Summary measures that can be employed as the area under a curve (AUC), rate of change, average response, depending on the data structure.

These summary measures are appropriate in our study as there is no missingness in the data and each subject is contributing the same amount of information. However, Mixed models were employed to tackle these repeated measurements taken from the ECG diagram. This a common technique for analysing repeated measures data taking into account the whole data set as well as capturing the correlation. Mixed models can be linear or non-linear depending on the outcome measured and its trajectory.

Consider the figure below which describes the setup of the study;

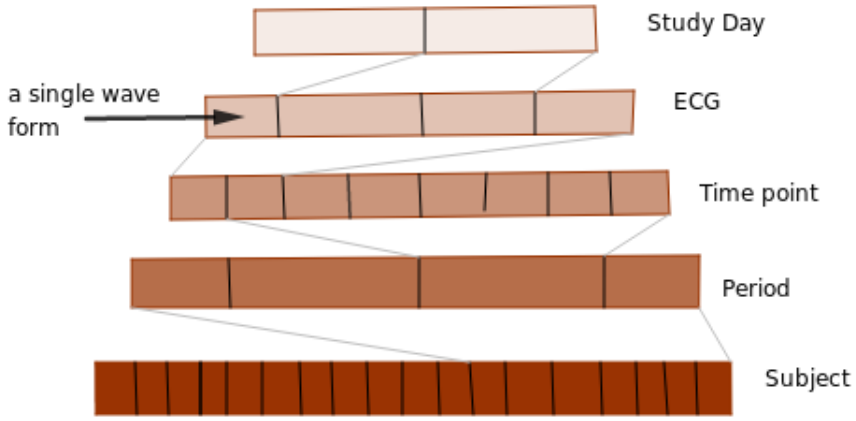

Figure 2.2: Data Structure of the study with RR-interval measured per wave form

The study consists of 40 patients who underwent a randomized and double-blinded experiment from a crossover trial. Measurements were taken at baseline at various time points and thereafter at designated hourly time interval. The study consisted of four treatments namely a high-dose, low-dose, active control treatment and a placebo drug. More importantly, subjects received all 4 treatments in different sequences and before the start of each treatment, patients undertook a minimally of 7-day washout period between treatments. In addition, patients were also fasted before the uptake of any treatment.

For the crossover design, a two level study day (Day -1 that is baseline day and Day 1 that is the post-baseline) is prominent and vital as a day of prolongation assessment. There are 8 levels of time points where ECG data are taken with respect to treatment uptake. Generally from the time points, there are 3 ECGs consisting of measurements taken from 3 wave forms which intuitively shows 12 RR and QT interval measurements per repeated time points that are averaged (Sethuraman and Sun, 2009).

Subject	Periods	Time Points	ECG per Time point	Wave forms	Wave forms per subject
40	4	8	3	3	288

Table 2.2: Data Points in the study

2.3.1 Summary Measures.

When subjects were taken in a repeated way from the ECG measurements, the observations tend to be similar for each subject therefore the presence of correlation. Also, measurements taken close in time seem to be more correlated than those further apart. Since the measurements were taken on an hourly basis, for the study to obtain reliable inference there is need for an analytical tool that takes into account this correlation. As earlierly indicated from section 2.3, summary measures are methods used to avoid this correlation between measurements and the multivariate nature of longitudinal studies. Summary measures tend to consider individuals as a group which in turn avails the dependent-variable for each individual subject to one number which summarizes an individual's response curve. This method reduces the data to univariate response of independent measurements, on which classical techniques can be applied.

The decision to use summary measure solely depends on the type of data available as well as the research question to be answered. In our case, there should be a smart method to capture the RR interval in ECG. One way will be to apply (Sethuraman and Sun, 2009) way which involve taking the average of ECG wave form data. This whereby the average of 12 RR interval measurements per subject, treatment, study day and time point is taken into account. By taking the average of the wave form, it results in having one value on the ECG level.

2.3.2 Mixed Models.

Mixed models are defined to be a regression model that explicitly include both random and fixed effects. For fixed effects, the idea is that the different levels associated to an effect are all assumed to be well-known and defined whereas for random effects, the associated levels of the effects are well assumed random. Mixed models are especially favourable when the data is dependent and in most instances when independent variable is not completely characterized. Moreover, they accommodate the evaluation of variability coming from various factors and the benefit being that they are not too much affected by effects of outliers which is attributed by the shrinkage effects.

Mixed models with repeated measures are appealing because of the adjustable covariance structure that incorporates for no uniform correlation upon different subjects and for data that is not balanced. The advantage of using mixed models is that the responses of all subjects come to an identical mathematical form where parameter estimates differ among subjects. Furthermore, unlike analysis of variance, mixed models solves the problem of having data that is unbalanced mainly in the case of time points that is unevenly spaced. This gives rise to correlated error terms which is defined as a variance that is heterogeneous and mixed models thereby uses the data to yield valid standard errors and adequate statistical tests.

The motivation of using mixed models is the nature of the data that spans over time. There are special variables such as the period that will have subjects that will experience many treatments over time and time variable which has repeated measures which will have correlation. Because of this (Schall and Ring, 2010) proposed the application of fixed and random effects for a univariate data given by;

$$y_{ij} = \alpha + P_m + \tau_j + S_i + \epsilon_{ij} \tag{2.3.1}$$

Where the y_{ij} was the QT measurement for subject i having treatment j, α is the intercept, P_m is the m^{th} period effect, τ_j is the treatment effect of j, S_i is the random effect of subject i.
Furthermore, by applying the ICH E14 guideline (Shah, 2005), the model is expanded by having baseline as an independent variable to yield;

$$y_{ij} = \alpha + b_i + P_m + \tau_j + S_i + \epsilon_{ij} \tag{2.3.2}$$

2.3.3 Analysis of Covariance (ANCOVA).

In the study, the response variable ($\triangle RR$) underwent analysis of covariance for the repeated measurements with each patient's baseline correction value as a covariate. Proposing an ANCOVA model using change from time-matched baseline as the dependent variable whereby time-matched baseline, pre-dose baseline and period specific average baseline as covariates.

Past studies have been carried out to assess the various covariance structures in crossover studies. (Lu, 2013) proposed an analysis of covariance model with change from time-matched baseline as response and considering time-matched baseline, day-average baseline for both the current treatment and

across treatment as covariates inorder to adjust for within-subject variation. On the other hand, (Ring et al., 2006) compared the performance of time series analysis of the E14 endpoints by assuming six different covariance structure and later using the AIC to select the best model.
To present, limited and few studies have been dedicated to addressing the dependence of covariance structure for the change of RR-interval from baseline on the baseline correction method. For our case, emphasis will be to solve this issue by assuming the dependence and thereby incorporating different covariance structures namely unstructured, toeplitz, autoregressive and compound symmetry. For the case of repeated measure, the covariance structure is defined as (Pinheiro et al., 2007);

$$y_i = X_i\beta + Z_i\mu_i + \epsilon_i; \quad i = 1, 2, \cdots, N \tag{2.3.3}$$

$$\mu_i \sim N\Big(0, \omega_i\Big), \quad \epsilon_i \sim N\Big(0, \sum_i\Big)$$

Where in matrix form, N are the number of subjects with n_i repeated measures in each i^{th} subject. β is the p-dimensional vector of fixed effects, μ_i is the q-dimensional vector of random effects and ω_i as the correlation between random effects while X_i is the fixed effect covariates and Z_i is the between-subject random effect covariates.

2.4 Baseline Correction Methods

The ICH E14 guideline specifically requires a thorough QT (TQT) study to be conducted to assess a drug's potential to prolong the QT interval (Shah, 2005). For this study, because the objectives are centralized on the heart rate, thus focus will be on the RR-interval variable. It will be of great interest to explore what is learnt in the corrected-heart rate and explore it to the RR-interval. Baseline corrective methods will be very crucial to get unbiased results. This is attributed by underlying factors that a subject may have misleading results of prolongation of the RR-interval due to external factors not related to pharmacodynamics. These factors may include daily exercise, food ingestion and etc and therefore baseline correction will be essential in removing variability not related to the treatment in the study. The analysis will include the response being the change from baseline and thereby having a baseline value as a covariate.

As described by (Dmitrienko and Smith, 2003), the change from baseline measurement is defined as;

$$\triangle y = y - \phi y_0 \tag{2.4.1}$$

Where y is the post-dose measurement, y_0 is the baseline RR-interval measurement and;

$$\phi = \begin{cases} 1, & \text{Evaluating the change from baseline} \\ 0, & \text{Evaluate the post-dose measurement} \end{cases}$$

By adopting the statistical model for the QTc interval proposed by (Ring, 2010) as shown belowe

$$y_i = \alpha + \beta x_i + \epsilon_i \tag{2.4.2}$$

Adjusting the above equation to incorporate the baseline value as a covariate, yields;

$$\triangle y = \alpha + \beta x + \gamma y_0 + \epsilon \tag{2.4.3}$$

Recall, $\triangle y = y - \phi y_0$ thus;

$$y - \phi y_0 = \alpha + \beta x + \gamma y_0 + \epsilon \tag{2.4.4}$$

Yielding;

$$y = \alpha + \beta x + \Big(\gamma + \phi\Big) y_0 + \epsilon \tag{2.4.5}$$

Where $\Big(\gamma + \phi\Big)$ is the baseline RR-interval measurement effect. For this TQT study, there were 5 baseline correction methods that were used, namely the time-matched baseline, time-matched average baseline, pre-dose baseline, time-matched from a separate baseline profile and the absolute RR-interval method.

2.4.1 Change from Period-Specific Time-Matched Baseline - Model D1.

The time-matched baseline method is defined as the change from baseline that is the difference between the post-dose RR-interval measurement and the baseline RR-interval measurement.

$$y_{ijt} - b_{ijt} \tag{2.4.6}$$

Where y_{ijt} is the post-dose RR-interval measurement for subject i receiving treatment j at a particular time point t and b_{ijt} is the baseline RR-interval measurement for subject i receiving treatment j.

2.4.2 Change from Period-Specific Mean of Baseline - Model D2.

Time-matched baseline is defined as the difference between all post-dose RR-interval values and all pre-dose RR-interval baseline measurement time points that are averaged out just before the uptake of a treatment. This is shown as;

$$y_{ijt} - b_{ij\bar{t}} \tag{2.4.7}$$

Where $b_{ij\bar{t}}$ is the average of all the pre-dose RR-interval baseline measurements time point for subject i receiving treatment t.

2.4.3 Time-Matched from Separate Baseline Profile - Model C.

Time-matched from separate baseline is defined as the difference between all post-dose RR-interval values and all baseline RR-interval from a separate particular period baseline measurement time points that are taken just before the treatment. This is shown as;

$$y_{ijt} - b_{ij\bar{t}} \tag{2.4.8}$$

2.4.4 Change from Pre-Dose Baseline - Model B.

The change of pre-dose baseline method is defined as the difference between all post-dose RR-interval values and all pre-dose RR-interval baseline measurements just before the uptake of a treatment. This is shown as;

$$y_{ijt} - b_{ijt} \tag{2.4.9}$$

Where b_{ijt} is the pre-dose RR-interval baseline measurements for subject i before receiving treatment j at a pre-dose time point t.

2.4.5 Absolute RR-interval Method - Model A.

This the case when there is no baseline adjustment. For this instance, the post-dose RR-interval is analyzed without adjusting for the effect of the RR-interval measurements for each subject taken before dosing. More importantly, this adjustment is not recommended for a thorough QT Trial (Sethuraman and Sun, 2009). In the model, the response variable is shown as;

$$y_{ijt} \tag{2.4.10}$$

Where y_{ijt} is the post-baseline QT measurement for subject i, in treatment j at time t.

2.5 QT Correction Methods

2.5.1 Method for heart rate correction.

There are considerable publications and mathematical models based on the most appropriate method for heart rate correction. The most commonly used methods are the correctional formulae from Fridericia and Bazett as indicated by (Dmitrienko and Smith, 2003). It is important to observe that the methodology from Fridericia was based on cubic root function for the QT-RR relationship basing from a linear regression whereas for Bazett based a square-root function on the QT-RR relationship for the heart rate.

However for this study, the correctional method will be based on a statistical concept made known by (Ring, 2010) rather than applying a mathematical model. The reason is that a statistical method incorporates a multilevel hierarchical nature of the data in this study and considers the error structure term.

From equation 2.4 and using the parabolic model (linear on the log-scale) given in the form of

$$y = \alpha + \beta x \tag{2.5.1}$$

But letting $y = \log(\text{QT})$ and $x = \log(\text{RR})$ and substituting we obtain;

$$\log(\text{QT}) = \alpha + \beta \log(\text{RR}) \tag{2.5.2}$$

By choosing α parameter be equal to the heart rate corrected QT interval as QTc. Since there is use of a log-scale; we have

$$\log(\text{QT}) = \log(\text{QTc}) + \beta \log(\text{RR}) \tag{2.5.3}$$

This can also be written in another way as;

$$\log(\text{QT}) = \log(\text{QTc}) + \log(\text{RR})^{\beta} \tag{2.5.4}$$

$$QT = QTc \times RR^{\beta} \tag{2.5.5}$$

$$\text{QTc} = \frac{QT}{RR^{\beta}} \tag{2.5.6}$$

QTc is the corrected heart-rate QT interval formulae that will be used in the study and β is the slope that will be used for the corrected heart rate QT interval.

3. Methodology

This chapter seeks to explain how this study was carried out with the objective of obtaining efficient results in the analysis. The following sections would describe in detail how data was analyzed and the tools used. This entails subsections on the data applied, the nature of the data, associated tests and the software applied in the study.

3.1 Exploratory Data Analysis

Exploratory data analysis was done to get an insight into the data prior to the analysis. Graphs were created to explore the patterns relevant to the scientific question, that is it may help to identify scientifically relevant variables to include in the proposed model. Furthermore, by exploring the data, this may help to identify unusual observations. The data were explored to see the individual profiles, the mean structure and the variance structure.

3.1.1 Individual Profile.

This exploration was conducted to gain some rough picture about how patients would evolve as well as to provide indication in terms of the between and within-subject variability. Moreover, this exploration provided an idea about what random effects would be associated. Connecting the repeated measurements for each subject over time shows you whether there is a discernible pattern common to most subjects (Patetta, 2005). This may be done by smoothing using spline routine (Patetta, 2005) or by calculating average at each time point for balanced data.

3.1.2 Mean Structure.

This exploration was aimed at describing the average evolution of the RR-interval, overall and according to different subgroups. It is important to observe that from such exploration, we can obtain some indications about the functional form of the evolution. By showing the average evolution in subgroups, it was useful in illustrating the relation between the response and covariates over time and thereby making necessary conclusion on whether or not there was any possible differences in the groups. The average RR-interval level was calculated at each time of measurement plotted.

3.1.3 Variance Structure.

This stage was aimed at getting an insight about how the variation between time points evolves. This gave indications in terms of how the variance was to be modelled so that valid inference can be made (Verbeke and Molenberghs, 2000). Again this exploration was conducted overall and according to different subgroups. Squared residuals were calculated at each time of measurement, and then plotted.

3.1.4 Correlation Structure.

For the reason that a mixed model takes into account correlation between measurements, we explore the correlation structure in order to have an idea on how to model it. We calculated the correlation matrix summarizing correlations between measurements taken at different time points. For the reason that a mixed model takes into account correlation between measurements, we explore the correlation

structure in order to have an idea on how to model it. We calculated the correlation matrix summarizing correlations between measurements taken at different time points.

3.2 Mixed Model

From the data made available for the study and because of the nature of repeated measure, equation 2.3.2 is extended by integrating multiplication of fixed effects and random effects (Schall and Ring, 2010). Therefore, for the analysis, there were various models proposed depending on the particular baseline correction method.

3.2.1 Change from Period-specific Time-Matched Baseline - Model D1.

The model was given by;

$$y_{ijkm} = \mu + B_{ijk} + P_j + T_k + D_m + TD_{km} + TP_{jk} + \theta_i\Big(P_j\Big) + \epsilon_{ijkm} \tag{3.2.1}$$

Where y_{ijkm} is the RR-interval measurement for subject i from period j coming from treatment group m at time point k, TD_{km} is the interaction effect of time k and treatment m, TP_{jk} is the interaction effect of the time and period, T_k is the time effect coming from time point k, P_j is the period effect coming from j ,B_{ijk} is the baseline effect for subject i from period j from time point k,$\theta_i\Big(P_j\Big)$ is the random subject nested in the period effect and ϵ_{ijkm} is the error (residual variability) term.

3.2.2 Change from Period-specific Mean of Baseline - Model D2.

The model used in the analysis was defined as;

$$y_{ijkm} = \mu + B_{ij} + P_j + T_k + D_m + TD_{km} + TP_{jk} + \theta_i\Big(P_j\Big) + \epsilon_{ijkm} \tag{3.2.2}$$

Where B_{ij} is the baseline effect for subject i from period j.

3.2.3 Change from Pre-Dose Baseline - Model B.

The model used in the analysis was;

$$y_{ijkm} = \mu + B_{ij} + P_j + T_k + D_m + TD_{km} + TP_{jk} + \theta_i\Big(P_j\Big) + \epsilon_{ijkm} \tag{3.2.3}$$

Where B_{ij} is the baseline effect for subject i from period j.

3.2.4 Time-Matched from Separate Baseline Profile - Model C.

The model was given by;

$$y_{ijkm} = \mu + B_{ik} + P_j + T_k + D_m + TD_{km} + TP_{jk} + \theta_i\Big(P_j\Big) + \epsilon_{ijkm} \tag{3.2.4}$$

Where B_{ik} is the baseline effect for subject i from time k.

3.2.5 Absolute RR-interval Method - Model A.

This is the model of there not being any baseline adjustment. The mathematical model used in the analysis was;

$$y_{ijkm} = \mu + P_j + T_k + D_m + TD_{km} + TP_{jk} + \theta_i\Big(P_j\Big) + \epsilon_{ijkm} \tag{3.2.5}$$

3.3 Model Selection

A model selection was carried out by application of the Akaike Information Criterion (AIC) for model assessment. The AIC comes after a Japanese statistician Hirotugu Akaike, who formulated it. The formulae is given by;

$$\text{AIC} = -2L + 2p, \tag{3.3.1}$$

Where L is the log-likelihood and it depends on type of likelihood selected. What it means is that when the Likelihood is a maximum likelihood (ML) method then p is the total number of parameters in the model. If the Likelihood is restricted maximum likelihood (REML), then p is the total number of variance component parameters in the model. Of great paramount effect is that the model with the smallest value of AIC represents the best model and the AIC penalizes models with large numbers of parameters. This means that if a model with a sizeable number of parameters has little effect in likelihood then the AIC for the models will propose the more parsimonious model is the best model. The term 2p means that the AIC value goes higher by 2 for each parameter estimated.

As compared to other model selection criterion (for example, BIC $= -2L + \ln(N).p$ and DIC), AIC is a good metric that has to be used. AIC does not depend on sample size as compared to the BIC. As shown, AIC outlines the problems of overfitting, whereas BIC has a problem of underfitting. In the study, there is a high sample size which is tolerant with AIC rather than BIC. Moreover, the AIC hopes to get the best approximating model converging to the true model whereby BIC converges as N approaches to infinity and the DIC is applied in a Bayesian setup.

3.4 Software

Analysis of the data was carried out in both SAS *version 9.4* and R *version 3.6.3*. This involved data manipulation and cleaning. R was also used for exploratory data analysis. For the Mixed model analysis for the corrected heart-rate, this carried out both in R and SAS. The significance level used in this study was 5%. R packages used in this study were *nlme, lme4, lsmeans, ggplot* and *lmerTest*.

3.4.1 SAS code for Crossover Data.

```
proc mixed data = mean_model_D1;
    CLASS SUBJNO Treatment Period Reltime;
    MODEL mean_RR = mean_RR_mbas Treatment Period RELTIME
    Treatment*RELTIME Period*RELTIME / DDFM = kr;
    RANDOM RELTIME / SUBJECT = SUBJNO;
    REPEATED RELTIME / SUBJECT = SUBJNO*Treatment TYPE = un R;
    lsmeans Treatment*RELTIME / PDiff;
    ods output LSMEANS = lsmeans DIFFS = diffs
```

```
               (WHERE RELTIME = _RELTIME);
run;
```

3.4.2 R code for Crossover Data.

```
D1.form <- formula(mean_RR ~ mean_RR_mbas RELTIME + Period
                   + Treatment + RELTIME:Treatment + RELTIME:PERIOD)
fm.D1 <- gls(D1.form, weights = varPower(form = ~RELTIME),
             correlation = corCompSymm(form = ~1|SUBJNO),
             data = mean_model_D1)
```

4. Results

In this chapter the results are obtained by making use of a statistical analysis. We will initially subdivide our analysis into two parts namely the exploratory data analysis and mixed model results. In the exploratory data analysis, we will also explore the subject's profile analysis, the mean structure of the overall patients together with their subgroups of interest and together with the variance structure of these patients. On the other hand, results will be obtained for the mixed models to best describe and answer the question.

4.1 Exploratory Data Analysis

4.1.1 Individual Profiles.

Figure 6.1 (in Appendix A) depicts RR-interval level of evolution of all 40 patients from the beginning to the end of the study. The profiles of these subjects shows a high variation between the evolution of the heart rate of individuals over time. This big variation occurs in different trends of the RR-interval level of evolution over time. This thereby gave a rise to random intercepts and slopes being included in the model as there was a wide variation to both between and within subjects over time. This was evident from the different starting points and from the different stopping points, showing no balance. Moreso, this difference in starting points of subjects from one another is attributed by the time to occurrence of heart beats differing from each subject.

4.1.2 Mean Structure.

Figure 4.1 clearly shows the relationship between the RR-interval over time. The mean structure show that the heart rate is very high from the beginning of the study but then drops drastically until time 4 then immediately shows a slow increase up to time 5. This is accompanied by a further decrease in level until time 6 where there is a sudden surge of rise up to the end of the study (time 8). This suggests more or less a non-linear relationship making smooth ups and downs.

Mean structure of different subgroups was considered with an objective to get a further insight of the data available. The motive for this was to examine whether or not interest groups may have a significant effect to the heart rate of an individual. However from the different subgroups (Treatment and Period), a similar evolution pattern to that of the overall mean structure is observed. In figure 4.2,Treatment B has a consistency and higher level compared to other treatments in terms of the heart rate of individuals. For the period, it appears that the evolution are similar as seen from figure 4.3, though period 4 seems to be highest. Based on these observations, we could therefore expect that maybe treatment and period may have a significant effect on the evolution.

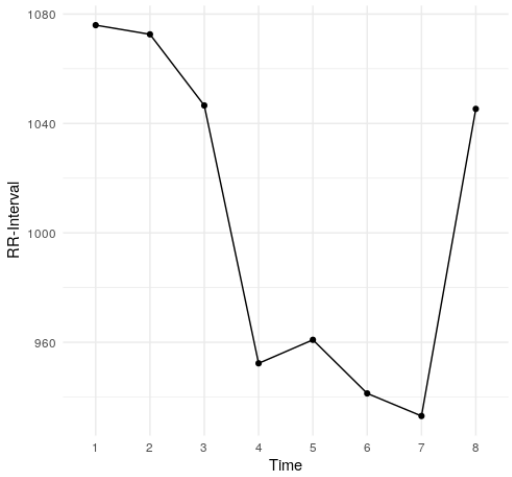

Figure 4.1: *Overall Mean Structure*

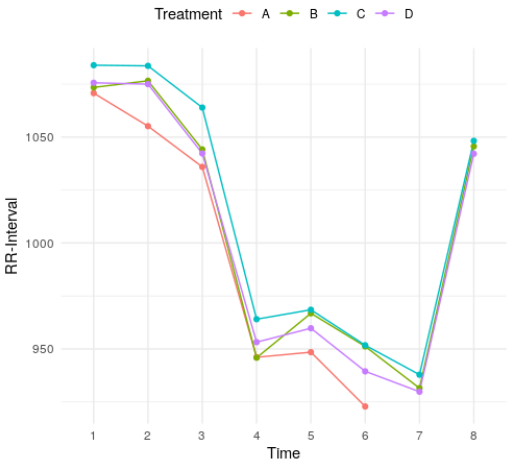

Figure 4.2: *Mean Structure by Treatment*

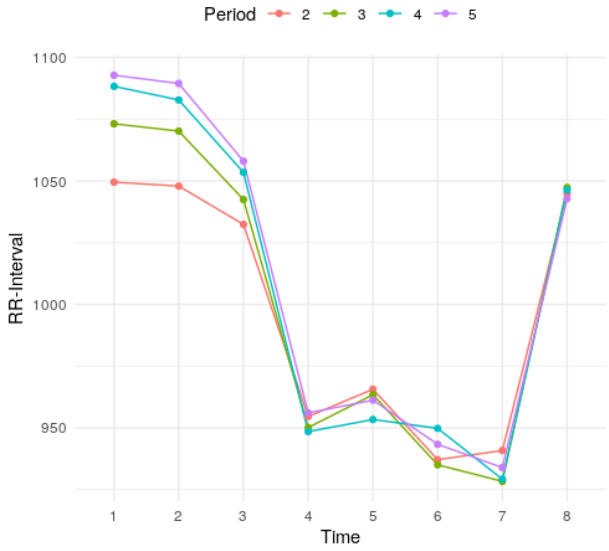

Figure 4.3: *Mean Structure by Period*

4.1.3 Variance Structure.

Similarly, figure 4.4-4.6 shows the variance structure of the RR-interval level from the beginning to the end of the study period. It shows the structure for the overall and for different subgroups. There is a consideration that the variance function does not look stable over time nor is there a clear visible trend in it.

The figure shows the variability being high at the start and as the time passed, the variability dropped. There was a flactuation of a mixture of upward and downward thrust of trend throughout the study. Just as in the mean structure, the variance structure has a similar relationship representing some non-linear functional form. It can observed that all treatments and periods tends to have a similar variation in their respective subgroups. Overall and by subgroups, the variance seems relatively stable and therefrom, a constant variance model could be a plausible starting point.

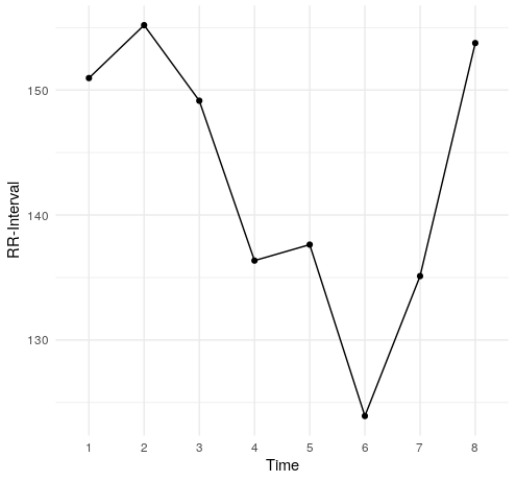

Figure 4.4: *Overall Variance Structure*

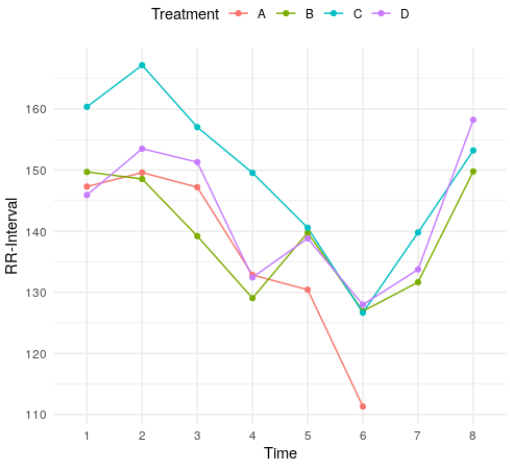

Figure 4.5: *Variance Structure by Treatment*

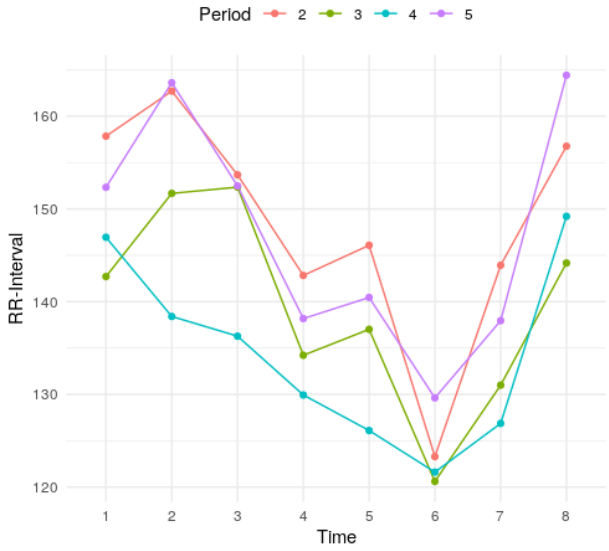

Figure 4.6: *Variance Structure by Period*

4.1.4 Heart Beat Patterns of the RR-intervals by Period.

The individual heart patterns of the RR-interval measurements were observed for each period. The main focus was to examine the behaviour of the heart rhythms from both perspectives of the baseline and post-baseline day cases. Clearly from figures 4.7 – 4.10, it was seen that there was a stability between baseline and post-baseline day. The change was slow over time and could be concluded that there was no significant different at baseline day but some considerable change only at post-baseline day.

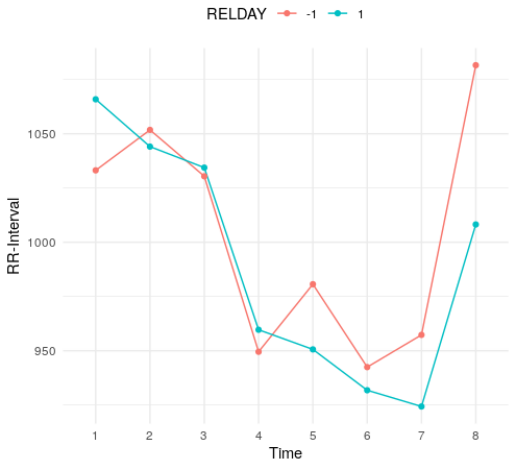

Figure 4.7: *RR-interval plot for Period 2*

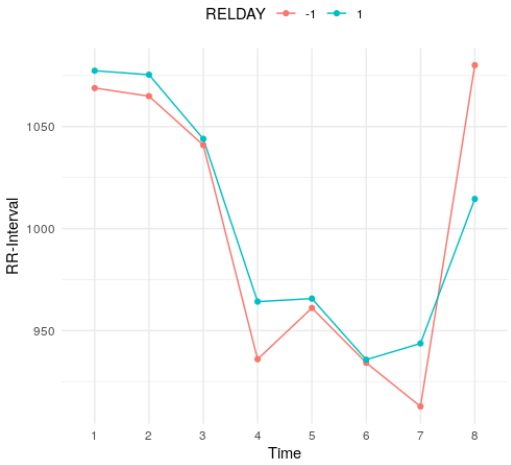

Figure 4.8: *RR-interval plot for Period 3*

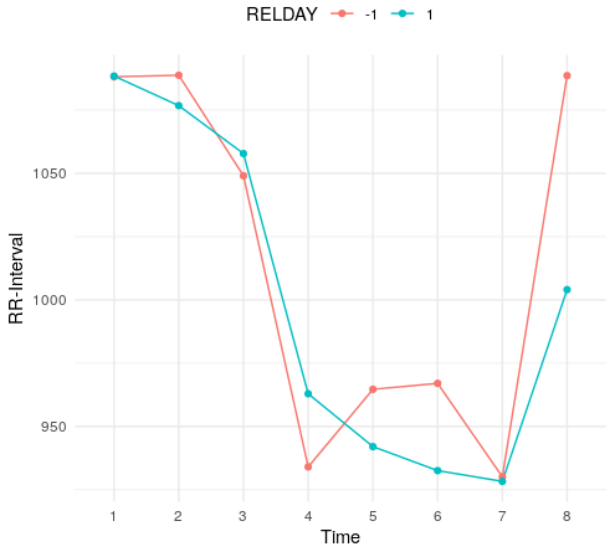

Figure 4.9: *RR-interval plot for Period 4*

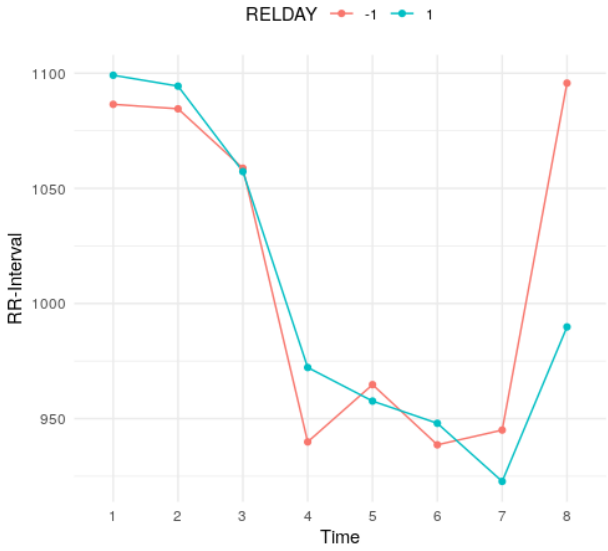

Figure 4.10: *RR-interval plot for Period 5*

4.2 Change from Baseline Plots

Figure 4.11 – 4.14 shows plots for change from baseline were derived from 4 models. These models were namely model D1, D2, C and B with their respective baselines. Model A was not plotted as it was the only model without any baseline covariate in its model. The objective of these plots was to examine and explore the maximum changes of the heart rate induced by different treatments which were exhibited over time. It is worth mentioning that three of the four models had its maximum changes in the heart in the beginning of the study that is at time 1 and gradually decreases as time increases. Interestingly, just as the overall mean structure and the mean structure of different subgroups from the section 4.1, there is a similar functional path of the average of the heart rate that is followed.

Model D1, D2 and C have a high change in the beginning of the study and gradually decrease at a certain time and then slowly increases. On the other hand, for Model D1, the maximum change is observed at time 4 and unlike the three other models, there is some flacuations of ups and downward thrust. The major difference between model D1 and the other three models is that after reaching its maximum there is a constant decrease in the RR-interval whereas for the other three, the is decrease but towards the end of the study there an increase in the RR-interval.

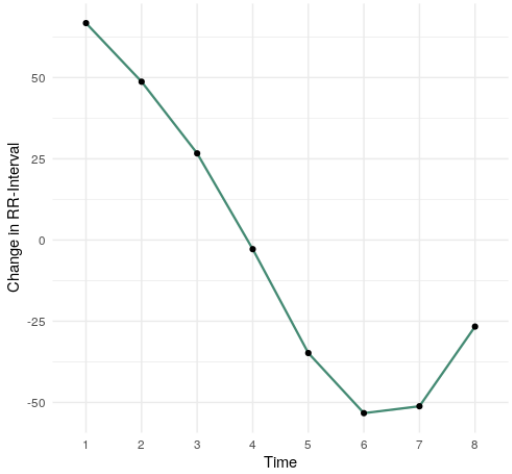

Figure 4.11: *Change from Baseline for Model C*

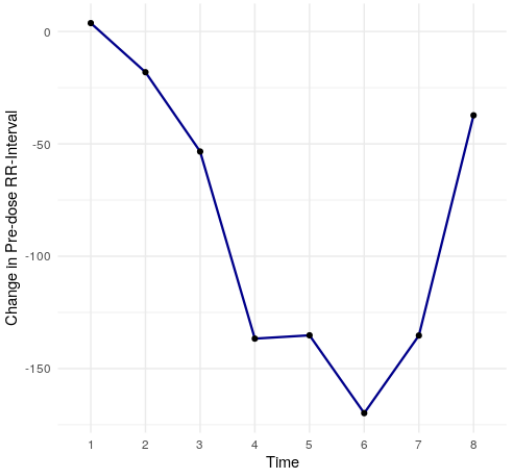

Figure 4.12: *Change from Baseline for Model B*

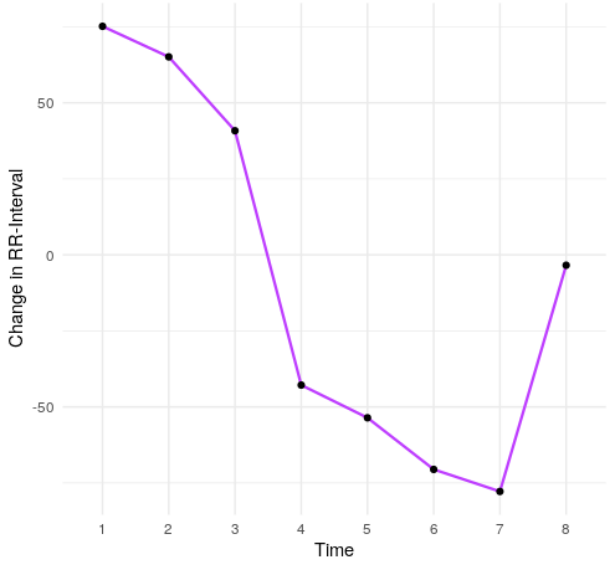

Figure 4.13: *Change from Baseline for Model D2*

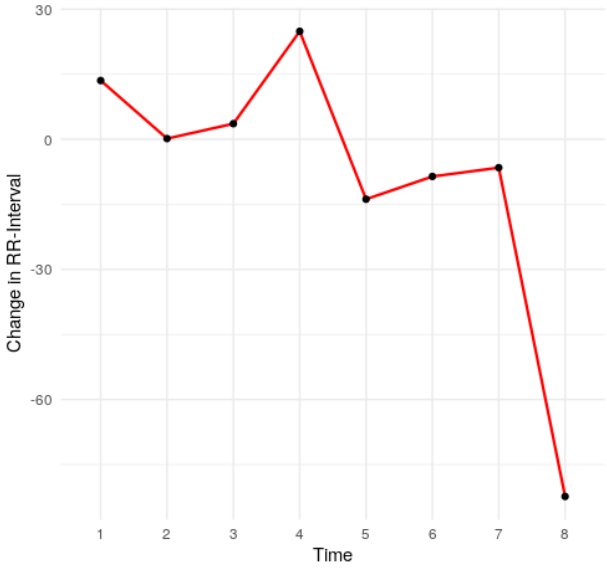

Figure 4.14: *Change from Baseline for Model Model D1*

4.3 Mixed Model

Based on the findings of the exploratory analysis and study design, a linear mixed model was fitted by applying one likelihood method namely the REML. Results of parameter estimates by treatment and time point for the 5 models under different covariance structure as the working correlation were observed and displayed in Appendix B. The parameters of the models was estimated using R and SAS;

and a comparison of results was done. Parameter estimates, their empirically corrected standard errors (SE) for the different baseline correctional methods with different working correlation are as tabulated in the following tables. Table 4.1 displays the summary statistics for comparison of the two softwares estimates of repeated measurement analyses. The results presented show the average of all data columns in Appendix B of the different models. More importantly, 5 Model were fitted and only one that is Model D2 was not displayed because it broke down under all 6 covariance structures. This is because the model had an infinite likelihood and hence stopped.

the parameter estimate and the difference estimate only considered the average of the absolute values.

Table 4.1: Summary Statistics for a comparison of SAS and R estimates of repeated measurement analyses for different models and covariance structures

		SAS Results				R Results			Difference	
Model	Covariance	Est.	Std.Err	AIC	Time	Est.	Std.Err	AIC	Est.	Std.Err
D1	Compound	33.2	13.92	12871.5	24 sec	36.42	14.73	12930.92	3.32	0.81
A	Compound	1009.91	21.24	13076.8	19 sec	1010.25	21.2	13124.13	0.57	0.04
B	Compound	76.66	21.2	13080.2	20 sec	76.81	21.2	13132.55	0.42	0.01
C	Compound	58.12	21.3	13082.9	17 sec	57.94	21.2	13132.46	0.23	0.01
D1	Unstructured	31.29	12.86	12803.1	1:04h	N/A	N/A	N/A	N/A	N/A
A	Unstructured	1008.8	12.86	12989.5	1:01h	N/A	N/A	N/A	N/A	N/A
C	Unstructured	62.27	21.04	12995.3	4:49h	N/A	N/A	N/A	N/A	N/A
D1	Autoregressive	33.16	14.3	12820.8	34 sec	35.	14.7	13078.41	2.81	0.3
A	Autoregressive	1010.72	21.3	12997.8	22 sec	1011	21.2	13084.11	0.28	0.01
B	Autoregressive	76.75	21.31	13003.4	15 sec	77.11	21.2	13098.62	0.36	0.01
C	Autoregressive	66.58	21.3	13003.8	14 sec	66.39	21.2	13092.4	0.18	0.01
D1	Toeplitz	32.71	13.96	12807.4	4:54m					
A	Toeplitz	1009.91	21.31	13005.7	8:01m					
B	Toeplitz	76.75	21.32	13013.2	7:18m					
C	Toeplitz	57.92	21.32	13011.7	8:46m					
D1	Variance	33.22	13.93	12869.5	1:01m					
A	Variance	1009.9	21.24	13074.8	16 sec					
B	Variance	76.66	21.25	13078.2	16 sec					
C	Variance	58.12	21.26	13080.9	20 sec					
D1	ARMA	33.5	14.13	12814.8	1 sec	36.48	14.1	12926.4	2.98	0.03
A	ARMA	1009.91	21..33	12998.8	1 sec	1010.25	21.2	13074.6	0.34	0.01
B	ARMA	76.75	21.33	13004.4	1 sec	77.13	21.2	13092.8	0.38	0.01
C	ARMA	61.14	21.33	13004.8	1 sec	61.35	21.2	13090.3	0.21	0.01

From the above table, we can observe that the results yielded by both SAS and R seem to be similar but are different in some way. Firstly we notice that in terms of the paramater estimates, results from R tend to be higher than those from SAS. This is evident from the 4 models fit across different covariance structures with values slightly above and this is consistent throughout. This may perharps tells us that R somewhat overestimates compared to SAS. Noteably, the absolute difference of the estimates is minimal and on average is less that 1 representing similarity in results irrespective of the software package used.

Of great paramount are the standard errors of the models across the covariance structures for the two softwares applied. It can be seen that correct to 1 decimal place, the results are exactly the same and little can be said otherwise. The only alarming bells came in two instances of Model D1 under

the compound symmetry and autoregressive covariance structure. There was a comparative and major difference in standard errors between SAS and R. In those two cases, R overestimated the results and this is seen by a major upsurge in a difference with 0.81 and 0.3 respective. Overall, there is similar standard errors between R and SAS.

The Akaike Information Criteria (AIC) was used to compare the 5 models under six different covariance structures to ascertain the best model. Again this was carried out in SAS and R to examine for consistency of results and conclusions. It is worth pointing out that again when in R, the results of AIC tend to much more inflated than those in SAS. But remarkably, it can be seen that despite that the same conclusions can be drawn. This evident of the fact that in SAS, the best model was Model D1 under the unstructured covariance structure with an AIC of 12803.1. This model was the best because it had the smallest value making it the most appropriate model to consider in this case.

On the other hand, in R there was a different result observed and the best model to consider was Model D1 but coming from the compound symmetry covariance structure with an AIC value of 12930.92. The difference in conclusion can be based by R not yielding any single result for the 5 models under the unstructured covariance structure. Using the unstructured in R was time consuming and all models broke down as the likelihood function evaluation limit reached without any convergence being achieved. Lastly the time output was only displayed in the SAS software.

Table 4.2 displays the summary statistics of the treatment contrast for the different models under different covariance structures. This contrast was only conducted in SAS and therefore there was no comparison of results in R and SAS as in other results. Full results of the parameter estimates of treatment contrast over time can be seen in Appendix C and therefore the table gives the averag

Table 4.2 displays the summary statistics of the treatment contrast for the different models under different covariance structures. This contrast was only conducted in SAS and therefore there was no comparison of results in R and SAS as in other results. Full results of the parameter estimates of treatment contrast over time can be seen in Appendix C and therefore the table gives the averag

There was no comparison to be made by R and SAS because R never gave a mechanism of displaying the time output unlike in SAS. It can be observed that most covariance structures were quick in fitting respective models except for the unstructured case. In the unstructured case, it took hours for each model and interestingly, in R, though it was not possible to get the time output and the fact that all models broke down, the same conclusion can be derived in that it took a long time to run.

Table 4.2: Summary Statistics of treatment contrasts of repeated measurement analyses for different models and covariance structures using SAS

Model	Covariance	Mean Contrast A-D		Mean Contrast B-D		Mean Contrast C-D		Overall Average	
		Est.	Std.Err	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
D1	Compound	35.62	19.7	43.49	19.70	41.48	19.73	40.2	19.70
A	Compound	65.56	30.03	71.50	30.05	74.32	30.05	70.46	30.04
B	Compound	65.27	30.05	63.25	30.07	73.92	30.07	70.01	30.06
C	Compound	65.70	30.05	71.67	30.07	74.50	30.07	70.63	30.07
D1	Unstructured	33.11	18.97	41.04	18.98	38.61	19.01	37.58	18.43
A	Unstructured	65.56	30.93	71.50	30.95	74.33	30.95	70.46	29.38
C	Unstructured	65.69	30.92	71.67	30.94	74.48	30.95	70.61	30.94
D1	Toeplitz	34.91	19.76	42.83	19.76	40.71	19.79	39.48	19.77
A	Toeplitz	65.56	30.13	71.50	30.15	74.31	30.15	70.46	30.14
B	Toeplitz	65.40	30.15	71.14	30.17	74.10	30.16	70.21	30.16
C	Toeplitz	65.45	30.13	71.35	30.17	74.20	30.17	70.33	30.16
D1	Autoregressive	34.91	19.76	42.83	19.76	40.71	19.79	42.03	20.26
A	Autoregressive	65.56	30.13	71.50	30.14	74.33	30.14	70.46	30.14
B	Autoregressive	65.41	30.14	71.16	30.16	74.12	30.15	70.23	30.15
C	Autoregressive	65.44	30.13	71.33	30.15	74.18	30.15	70.32	30.14
D1	Variance	35.61	19.69	43.49	19.69	41.48	19.73	40.19	19.70
A	Variance	65.56	30.03	71.50	30.05	74.33	30.05	70.46	30.04
B	Variance	65.27	30.06	70.86	30.07	73.92	30.07	70.02	30.07
C	Variance	65.70	30.06	71.68	30.08	74.50	30.07	70.63	30.07
D1	ARMA	36.05	19.99	43.89	19.99	41.95	20.03	40.63	20.00
A	ARMA	65.56	30.15	71,50	30.17	74.33	30.17	70.46	30.16
B	ARMA	65.41	30.17	71.58	30.18	74.11	30.18	70.37	30.18
C	ARMA	65.44	30.16	71.33	30.19	74.18	30.18	70.32	30.18

Table 4.2 displays the summary statistics of the treatment contrast for the different models under different covariance structures. This contrast was only conducted in SAS and therefore there was no comparison of results in R and SAS as in other results. Full results of the parameter estimates of treatment contrast over time can be seen in Appendix C and therefore the table gives the average of all data column in the Appendix.

It can be observed that the standard error of the treatment contrast are similar but increases consistently for every time point (see Appendix C, Table 6.6) that is for the compound symmetry covariance whereas on the other hand in Table 6.7 showing treatment contrast for unstructured covariance, there is a consistently reduction in standard error for every time point.

From the above table, it is typical that Model D1 gives smaller standard errors in comparison to other models with respect to any covariance structure used. Earlier using SAS, it was observed that the best model was Model D1 from the unstructured covariance and this does not come as a surprise when observing that on average, Model D1 - unstructured yields the smallest standard errors on assessing treatment contrast.

This may be due to the periodic-specific time matched baseline ECG that is taken shortly before the first post-dose time points and then the difference between pre-dose and post-dose measurements which do increase towards the time points. Another important phenomena is that standard errors tend to

smallest in the average contrast class of A-D as compared to the other treatment contrast class. Upon observing the overall average of the standard error of the models, it can be examined that there is a similar trend and correlation of standard errors irrespective of the covariance structure used. Although in this study, we are mainly interested in the model and method that output standard errors that are the smallest.

5. Discussion and Conclusion

In this study, we wanted to analyse repeated measurements of data using mixed models and be able to come up with meaning medical conclusions. A longevity in the heart rate can possibly result in problems affecting a subject in a negative way and in death in other cases. Therefore it was of great importance to investigate how different drugs could potentially affect the heart rate by use of mixed models in an experiment with repeated measurements of data.

Inorder to obtain unbiased and satisfying results, there was need to do some baseline corrective methods to the available data that was available. The motivation of this was to rectify and remove external variability to the heart rate caused by external factors such as daily exercise and food ingestion which could affect the analysis.

An investigation in different baseline corrective models were fit and carried out using mixed models. Various models namely change from period-specific Time-Matched baseline (Model C), change from period-specific mean baseline (Model D2), change from separate baseline profile (Model D1), change from pre-dose baseline (Model B) and an absolute RR-interval model (Model A) were fitted so as to observe the best model and standard errors for estimates of interest. Inorder to define these five models, necessary adjustments in form data management was exploited by transposing the dataset that had 28800 observation so to get two new columns RR_{bas} (RR-interval values at baseline - study day at -1) and RR_{trt} (RR-interval values at post-baseline – study day at 1). By doing this, all other other identifiers where kept and more importantly the number of rows were halved. This new dataset then became the main data for the analysis to be carried out.

Model D1 was derived by getting the difference between the new columns that is the RR_{trt} and RR_{bas}. This difference became the time-matched change and also the response of this model. Model A used the new dataset for the analysis but the only difference was that this model never put into perspective the baseline covariate in its mathematical model. This meant that the response was described by the RR-interval value at post-baseline. For Model B, there was a creation of a new baseline column by the name $RR_{prebase}$ which took RR-interval values only at baseline and at time component 8 for all the same periods. This meant that there was omition of RR-values at baseline and replacing them by a single value which became the pre-dose baseline. Its dependent variable became the difference between the RR-interval values at post-baseline and RR-interval values at pre-dose baseline. Model C made use of the time-matched baseline from period 2 to all other periods. Basically this meant that throwing away the baselines from period 3 to period 5 and replacing them all with baseline at period 2 (time-matched). This baseline is not period specific and was defined to be RR_{sepbas}. The response variable was therefore defined as the difference between the Rr-interval at post-baseline and RR_{sepbas}. Lastly as for Model D2, this was similar to Model B in that there was a derivation of a single value as a baseline value. In this case, the baseline was the mean of all the RR_{bas} at each period and this was a baseline which was period-specific defined to be RR_{mbas}. The response variable was the difference between the RR-interval value at post-baseline and the respective baseline RR_{mbas}. It is important to take note that all Models except for Model A had their respective defined baseline as covariate in the model when analysing data in Mixed Models.

Inorder to determine the aims and objectives of te study, there was a consideration of applying six different covariance structures as the working correlation for the different models. The covariance structures considered in this study were namely; Compound Symmetry, Unstructured, Toeplitz, ARMA, Autoregressive structure and the variance component. The motivation for using all these correlation was to run models and try to observe which had small standard errors for the estimates of interest. Since this

study had repeated measurements, it is believed that if numerous measurements are taken for the same individual, there is some occurrence of some sort of correlation. The idea is to try to solve with when there is repeatedness of measure and by applying these six working correlation, addressed this issue. What was of great importance, was that the method of change from period-specific Time-Matched baseline adjustment (Model D1) seems to be the best model when comparing to other four models irrespective of the covariance structure that was used. It was observed that from Model D1, it also had the least standard errors of the treatment estimates. In comparing all the six covariance structures applied, it was observed that there was no important difference in the standard errors between Model A, Model B and Model C. This proves that these three models were least efficient as they had large standard errors. Model D1 consistently having smaller standard errors could be attributed by measurements of subjects being taken a day before the treatment and this is representative in all six cases. In terms of the calculation time, it is worth pointing out that it was possible to only to comprehend the time of that of SAS software and not in R. The unstructured covariance structure took a lot of considerable time with Model C having the highest time of 4 hours. This is because of there were many covariance parameters to calculate which were 37 and this was the most out of all the other five covariance structures.

More importantly upon differentiating the AIC of all the models coming from all six working correlations, it was observed that Model D1 with an unstructured covariance structure had was the best model as it had the least AIC value among all models that were fit. It was found that Model D2 that is the model with the change from period specific mean of baseline adjustment showed no results in all cases of the covariance structures that were put under the test. It was observed that Model D2 did not converge and it broke down mainly because of it having infinite likelihood.

A comparison of results between software packages R and SAS was done. It is important to mention that results were similar and consistent in both R and SAS. There are two main issues to highlight when studying the results. Firstly since most results were similar, the only major difference came on comparing the standard errors of Model D1 coming from the compound symmetry covariance structure comparing the result in SAS and R. There was a major difference as in SAS, Model D1 had an average standard error of 13.9 and in R, an average of 14.7. Thereafter comparing with other models with their working correlation, seemed to be close to each other both in terms of the parameter estimate and its standard errors with a major difference was a difference of a value of 0.1.

Secondly; when comparing the results of these two software packages, It was observed that in R the covariance structures are limited and this may be attributed that there are necessary improvements to this software package. In R, the idea was to apply 4 covariance structures so as to compare these results with results of SAS. The reason for 4 was because in R, it was not possible to use the Toeplitz and variance component structures. Amazingly, for the unstructured covariance in R there was no result obtained for even a single Model whereas in SAS, only 3 Models namely; D1, A and C yielded results. The reason for not getting any result in R was because the models broke down as the model evaluation limit was reached without any convergence. In conclusion, Model D1 consistently produced small standard errors regardless of the covariance structure that was used. Overall, it was Model D1 from the unstructured covariance structure that had the best model together with appropriate standard errors which were small.

6. Appendix

6.1 Appendix A

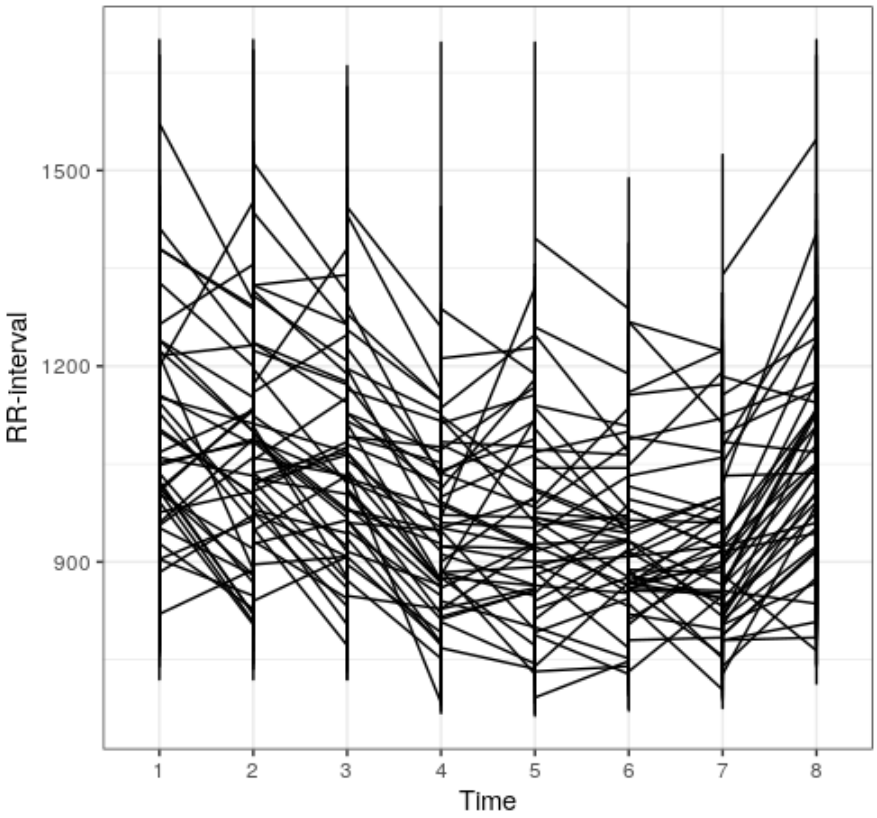

Figure 6.1: Individual Profile of the heart rate of different Subjects

6.2 Appendix B

Table 6.1: Comparison of SAS and R estimates by treatment and time point - Model D1 with compound symmetry covariance

	Model D1 in SAS		Model D1 in R		Diff. of SAS and R	
Effect	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
Trt A * Time 1	35.73	13.92	39.44	14.7	-3.71	-0.78
Trt A * Time 2	8.62	13.92	12.17	14.7	-3.55	-0.78
Trt A * Time 3	6.32	13.87	8.39	14.7	-2.07	-0.83
Trt A * Time 4	-23.57	13.94	-27.65	14.8	4.08	-0.86
Trt A * Time 5	-62.11	13.87	-64.41	14.7	2.3	-0.83
Trt A * Time 6	-56.55	13.97	-61.18	14.8	4.63	-0.83
Trt B * Time 1	50.35	13.92	53.72	14.7	-3.37	-0.78
Trt B * Time 2	51.82	13.93	55.38	14.7	-3.56	-0.77
Trt B * Time 3	24.66	13.87	26.68	14.7	-2.02	-0.83
Trt B * Time 4	-15.80	13.97	-20.22	14.8	4.42	-0.83
Trt B * Time 5	-15.92	13.89	-18.63	14.7	2.71	-0.81
Trt B * Time 6	-36.32	13.91	-39.43	14.7	3.11	-0.79
Trt C * Time 1	47.34	13.96	51.67	14.8	-4.33	-0.84
Trt C * Time 2	42.21	13.97	46.73	14.8	-4.52	-0.83
Trt C * Time 3	30.40	13.92	34.36	14.7	-3.96	-0.78
Trt C * Time 4	-11.84	13.91	-14.91	14.7	3.07	-0.79
Trt C * Time 5	-26.98	13.87	-29.06	14.7	2.08	-0.83
Trt C * Time 6	-36.42	13.91	-39.52	14.7	3.1	-0.79
Trt D * Time 1	56.75	13.91	59.97	14.7	-3.22	-0.79
Trt D * Time 2	41.35	13.94	45.15	14.8	-3.8	-0.86
Trt D * Time 3	34.54	13.86	35.94	14.7	-1.4	-0.84
Trt D * Time 4	1.27	13.97	-3.25	14.8	4.52	-0.84
Trt D * Time 5	-38.07	13.88	-40.43	14.7	2.36	-0.82
Trt D * Time 6	-41.87	13.94	-45.76	14.8	3.89	-0.86
Average	33.2	13.92	36.42	14.73	3.32	0.82

Table 6.2: Comparison of SAS and R estimates by treatment and time point - Model A with compound symmetry covariance

	Model A in SAS		Model A in R		Diff. of SAS and R	
Effect	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
Trt A * Time 1	1071.33	21.23	1071	21.2	0.33	0.03
Trt A * Time 2	1042.97	21.23	1043	21.2	-0.03	0.03
Trt A * Time 3	1029.48	21.23	1029	21.2	0.48	0.03
Trt A * Time 4	953.23	21.23	953	21.2	0.23	0.03
Trt A * Time 5	928.11	21.23	928	21.2	0.11	0.03
Trt A * Time 6	916.08	21.23	916	21.2	0.08	0.03
Trt B * Time 1	1083.35	21.24	1083	21.2	0.35	0.04
Trt B * Time 2	1086.25	21.24	1086	21.2	0.25	0.04
Trt B * Time 3	1047.41	21.24	1055	21.2	-7.59	0.04
Trt B * Time 4	958.43	21.24	958	21.2	0.43	0.04
Trt B * Time 5	971.20	21.24	971	21.2	0.20	0.04
Trt B * Time 6	947.82	21.24	948	21.2	-0.18	0.04
Trt C * Time 1	1087.59	21.24	1088	21.2	-0.41	0.04
Trt C * Time 2	1083.89	21.24	1084	21.2	-0.11	0.04
Trt C * Time 3	1063.94	21.24	1064	21.2	-0.06	0.04
Trt C * Time 4	972.57	21.24	973	21.2	-0.43	0.04
Trt C * Time 5	964.88	21.24	965	21.2	-0.12	0.04
Trt C * Time 6	947.77	21.24	948	21.2	-0.23	0.04
Trt D * Time 1	1088.58	21.24	1089	21.2	-0.42	0.04
Trt D * Time 2	1077.55	21.24	1078	21.2	-0.45	0.04
Trt D * Time 3	1052.66	21.24	1053	21.2	-0.34	0.04
Trt D * Time 4	974.71	21.24	975	21.2	-0.29	0.04
Trt D * Time 5	951.72	21.24	952	21.2	-0.28	0.04
Trt D * Time 6	936.34	21.24	936	21.2	0.34	0.04
Average	1009.91	21.24	1010.25	21.2	0.57	0.04

Table 6.3: Comparison of SAS and R estimates by treatment and time point - Model B with compound symmetry covariance

	Model B in SAS		Model B in R		Diff. of SAS and R	
Effect	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
Trt A * Time 1	-15.81	21.2	-15.32	21.2	-0.49	0.00
Trt A * Time 2	-44.46	21.3	-43.75	21.2	-0.71	0.01
Trt A * Time 3	-57.56	21.2	-57.15	21.2	-0.41	0.00
Trt A * Time 4	-132.93	21.2	-133.19	21.2	0.26	0.00
Trt A * Time 5	-159.28	21.3	-158.60	21.2	-0.68	0.01
Trt A * Time 6	-170.44	21.2	-170.43	21.2	-0.01	0.00
Trt B * Time 1	-3.71	21.3	-3.29	21.2	-0.41	0.01
Trt B * Time 2	0.01	21.3	-0.20	21.2	0.21	0.00
Trt B * Time 3	-38.79	21.3	-39.03	21.2	0.24	0.01
Trt B * Time 4	-127.93	21.3	-128.04	21.2	0.11	0.01
Trt B * Time 5	-115.19	21.3	-115.28	21.2	0.09	0.01
Trt B * Time 6	-137.49	21.3	-138.41	21.2	0.92	0.01
Trt C * Time 1	0.89	21.3	1.04	21.2	-0.15	0.01
Trt C * Time 2	-2.06	21.3	-2.49	21.2	0.43	0.01
Trt C * Time 3	-21.67	21.3	-22.36	21.2	0.69	0.01
Trt C * Time 4	-113.16	21.3	-113.76	21.2	0.6	0.01
Trt C * Time 5	-120.57	21.3	-121.38	21.2	0.81	0.01
Trt C * Time 6	-137.88	21.3	-138.54	21.2	0.66	0.01
Trt D * Time 1	2.62	21.3	2.20	21.2	0.42	0.01
Trt D * Time 2	-8.38	21.3	-8.82	21.2	0.44	0.01
Trt D * Time 3	-32.84	21.3	-33.62	21.2	0.78	0.01
Trt D * Time 4	-111.39	21.3	-111.70	21.2	0.31	0.01
Trt D * Time 5	-134.77	21.3	-134.78	21.2	0.01	0.01
Trt D * Time 6	-149.95	21.3	-150.12	21.2	0.17	0.01
Average	76.66	21.2	76.81	21.2	0.42	0.01

6.3 Appendix C

Table 6.4: Comparison of SAS and R estimates by treatment and time point - Model C with compound symmetry covariance

	Model C in SAS		Model C in R		Diff. of SAS and R	
Effect	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
Trt A * Time 1	69.13	21.2	69.03	21.2	0.1	0.00
Trt A * Time 2	40.48	21.3	40.82	21.2	-0.34	0.01
Trt A * Time 3	27.13	21.2	27.26	21.2	-0.13	0.00
Trt A * Time 4	-49.36	21.3	-48.86	21.2	-0.5	0.01
Trt A * Time 5	-74.16	21.2	-74.15	21.2	-0.01	0.00
Trt A * Time 6	-86.10	21.3	-86.29	21.2	0.19	0.01
Trt B * Time 1	81.22	21.3	81.00	21.2	0.22	0.01
Trt B * Time 2	84.30	21.3	83.81	21.2	0.49	0.01
Trt B * Time 3	45.35	21.3	45.03	21.2	0.32	0.01
Trt B * Time 4	-44.01	21.3	-43.74	21.2	-0.27	0.01
Trt B * Time 5	-31.20	21.3	-31.00	21.2	-0.20	0.01
Trt B * Time 6	-54.61	21.3	-54.36	21.2	-0.25	0.01
Trt C * Time 1	85.44	21.3	85.27	21.2	0.17	0.01
Trt C * Time 2	81.94	21.3	81.46	21.2	0.48	0.01
Trt C * Time 3	61.93	21.3	61.53	21.2	0.40	0.01
Trt C * Time 4	-29.76	21.3	-29.65	21.2	-0.11	0.01
Trt C * Time 5	-37.38	21.3	-37.39	21.2	0.01	0.01
Trt C * Time 6	-54.53	21.3	-54.48	21.2	-0.05	0.01
Trt D * Time 1	86.31	21.3	86.31	21.2	0.00	0.01
Trt D * Time 2	75.38	21.3	75.23	21.2	0.15	0.01
Trt D * Time 3	50.46	21.3	50.35	21.2	0.11	0.01
Trt D * Time 4	-27.78	21.3	-27.43	21.2	-0.35	0.01
Trt D * Time 5	-50.73	21.3	-50.44	21.2	-0.29	0.01
Trt D * Time 6	-66.20	21.3	-65.78	21.2	-0.42	0.01
Average	58.12	21.3	57.94	21.2	0.23	0.01

Table 6.5: Comparison of SAS and R estimates by treatment and time point - Model D1 with Autoregressive Covariance

	Model D1 in SAS		Model D1 in R		Diff. of SAS and R	
Effect	Est.	Std.Err	Est.	Std.Err	Est.	Std.Err
Trt A * Time 1	37.42	14.31	39.44	14.7	-2.02	-0.39
Trt A * Time 2	10.24	14.30	12.17	14.7	-1.93	-0.4
Trt A * Time 3	7.26	14.25	8.39	14.7	-1.13	-0.45
Trt A * Time 4	-25.43	14.33	-27.65	14.8	2.22	-0.47
Trt A * Time 5	-63.16	14.26	-64.41	14.7	1.25	-0.44
Trt A * Time 6	-58.66	14.36	-61.18	14.8	2.52	-0.44
Trt B * Time 1	51.89	14.31	53.72	14.7	-1.83	-0.39
Trt B * Time 2	53.45	14.31	55.38	14.7	-1.93	-0.39
Trt B * Time 3	25.58	14.26	26.68	14.7	-1.1	-0.44
Trt B * Time 4	-17.81	14.35	-20.22	14.8	2.41	-0.45
Trt B * Time 5	-17.16	14.28	-18.63	14.7	1.47	-0.42
Trt B * Time 6	-37.74	14.29	-39.43	14.7	1.69	-0.41
Trt C * Time 1	49.32	14.35	51.67	14.8	-2.35	-0.45
Trt C * Time 2	44.27	14.36	46.73	14.8	-2.46	-0.44
Trt C * Time 3	32.53	14.30	34.36	14.7	-1.83	-0.4
Trt C * Time 4	-13.24	14.29	-14.91	14.7	1.67	-0.41
Trt C * Time 5	-27.93	14.26	-29.06	14.7	1.13	-0.44
Trt C * Time 6	-37.83	14.29	-39.52	14.7	1.69	-0.41
Trt D * Time 1	58.22	14.30	59.97	14.7	-1.75	-0.41
Trt D * Time 2	43.08	14.32	45.15	14.8	-2.07	-0.48
Trt D * Time 3	35.18	14.25	35.94	14.7	-0.76	-0.45
Trt D * Time 4	-0.79	14.36	-3.25	14.8	2.46	-0.44
Trt D * Time 5	-39.15	14.27	-40.43	14.7	1.28	-0.43
Trt D * Time 6	-43.65	14.33	-45.76	14.8	2.11	-0.47
Average	33.16	14.30	35.97	14.7	2.81	0.4

Table 6.6: Treatment contrasts by time point derived by SAS - for all Models with compound symmetry covariance

		Model D1		Model A		Model B		Model C	
Contrast	Time	Estimate	Std.Err	Estimate	Std.Err	Estimate	Std.Err	Estimate	Std.Err
A-D	Time 1	-21.02	19.58	-17.24	30.03	-18.43	30.05	-17.17	30.05
	Time 2	-5.62	19.58	-6.22	30.03	-7.43	30.05	-6.25	30.04
	Time 3	1.19	19.60	96.62	30.03	17.03	30.06	18.68	30.04
	Time 4	34.46	19.87	18.68	30.03	95.59	30.05	96.92	30.06
	Time 5	73.80	19.74	119.61	30.03	118.97	30.05	119.86	30.06
	Time 6	77.60	19.83	134.99	30.03	134.15	30.05	135.33	30.06
B-D	Time 1	-6.40	19.59	-5.23	30.05	-6.33	30.07	-5.08	30.07
	Time 2	9.00	19.59	5.80	30.05	4.68	30.07	5.85	30.06
	Time 3	15.81	19.60	30.69	30.05	29.13	30.08	30.77	30.06
	Time 4	49.08	19.86	108.63	30.05	107.69	30.07	109.01	30.08
	Time 5	88.42	19.73	131.62	30.05	131.07	30.07	131.96	30.08
	Time 6	92.22	19.81	147.01	30.05	146.25	30.07	147.42	30.09
C-D	Time 1	-9.41	19.60	-0.98	30.05	-1.73	30.07	-0.86	30.07
	Time 2	5.99	19.59	10.04	30.05	9.27	30.07	10.07	30.06
	Time 3	12.80	19.62	34.94	30.05	33.73	30.07	34.99	30.06
	Time 4	46.07	19.92	112.88	30.05	112.28	30.07	113.23	30.08
	Time 5	85.41	19.78	135.87	30.05	135.66	30.07	136.18	30.08
	Time 6	89.21	19.88	151.26	30.05	150.84	30.07	151.64	30.09
Average		40.2	19.70	70.46	30.04	70.01	30.06	70.63	30.07

Table 6.7: Treatment contrasts by time point derived by SAS - for all Models with unstructured covariance

		Model D1		Model A		Model C	
Contrast	Time	Estimate	Std.Err	Estimate	Std.Err	Estimate	Std.Err
A-D	Time 1	-21.36	19.58	-17.24	31.99	-17.18	32.01
	Time 2	-5.57	19.41	-6.22	31.93	-6.24	31.93
	Time 3	-0.34	19.18	18.67	31.73	18.68	31.69
	Time 4	29.01	18.35	96.62	30.34	96.89	30.33
	Time 5	69.79	19.07	119.61	30.40	119.84	30.43
	Time 6	72.57	18.27	134.99	29.19	135.30	29.15
B-D	Time 1	-6.50	19.59	-5.23	32.01	-5.09	32.03
	Time 2	9.28	19.42	5.80	31.95	5.84	31.95
	Time 3	14.51	19.18	30.69	31.74	30.76	31.70
	Time 4	43.86	18.34	108.63	30.35	108.98	30.35
	Time 5	84.64	19.07	131.62	30.42	131.93	30.45
	Time 6	87.43	18.26	147.01	29.20	147.39	29.18
C-D	Time 1	-10.15	19.60	-0.98	32.01	-0.87	32.03
	Time 2	5.63	19.42	10.04	31.95	10.06	31.95
	Time 3	10.86	19.20	34.94	31.74	34.98	31.71
	Time 4	40.22	18.40	112.88	30.35	113.20	30.35
	Time 5	80.99	19.12	135.87	30.42	136.15	30.45
	Time 6	83.78	18.32	151.26	29.20	151.61	29.18
Average		37.58	18.43	70.46	29.38	70.61	30.94

Table 6.8: Treatment contrasts by time point derived by SAS - for all Models with toeplitz covariance

		Model D1		Model A		Model B		Model C	
Contrast	Time	Estimate	Std.Err	Estimate	Std.Err	Estimate	Std.Err	Estimate	Std.Err
A-D	Time 1	-21.11	19.64	-17.24	30.13	-17.91	30.15	-17.30	30.14
	Time 2	-5.61	19.64	-6.22	30.13	-6.90	30.15	-6.19	30.14
	Time 3	0.78	19.66	18.68	30.13	17.75	30.16	18.68	30.14
	Time 4	32.99	19.93	96.62	30.13	96.04	30.15	96.39	30.15
	Time 5	72.72	19.80	119.61	30.13	119.25	30.14	119.41	30.15
	Time 6	76.25	19.88	134.99	30.13	134.52	30.14	134.73	30.16
B-D	Time 1	-6.43	19.65	-5.23	30.15	-5.85	30.17	-5.35	30.16
	Time 2	9.07	19.65	5.80	30.15	5.17	30.17	5.76	30.15
	Time 3	15.46	19.67	30.69	30.15	29.81	30.17	30.63	30.15
	Time 4	47.68	19.91	108.63	30.15	108.10	30.16	108.34	30.18
	Time 5	87.40	19.79	131.62	30.15	131.31	30.16	131.36	30.17
	Time 6	90.93	19.87	147.01	30.15	146.58	30.16	146.68	30.19
C-D	Time 1	-9.61	19.66	-0.98	30.15	-1.41	30.16	-1.08	30.16
	Time 2	5.89	19.65	10.04	30.15	9.61	30.16	10.03	30.15
	Time 3	12.28	19.69	34.94	30.15	34.26	30.17	34.90	30.15
	Time 4	44.50	19.98	112.88	30.15	112.54	30.16	112.61	30.18
	Time 5	84.22	19.84	135.87	30.15	135.75	30.16	135.63	30.17
	Time 6	87.75	19.93	151.26	30.15	151.02	30.16	150.95	30.18
Average		39.48	19.77	70.46	30.14	70.21	30.16	70.33	30.16

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