**Statistical Analysis Plan (SAP)**

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| **Title** | Investigating the Relationship Between Baseline Potassium Levels and the Digitalis Investigation Group Trial Results |
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| **Acronyms** | *CHF* | *Congestive Heart Failure* |
|  | *DIG* | *Digitalis Investigation Group* |
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# Study Overview

Background/Introduction: Digoxin is a treatment for heart failure that has been given to patients for a long time. It is also one of the most commonly given drugs to treat heart failure. The DIG main trial was a randomized, double-blind, placebo controlled trial to study the effects of Digoxin on mortality and hospitalization due to worsening heart failure. This trial was important, as the effects of Digoxin on preventing death and worsening heart failure were largely unknown prior to this trial. While the trial did not find a statistically significant reduction in deaths in the treatment group over the placebo group, it did find a trend in decrease in overall hospitalizations and hospitalizations due to worsening heart failure.

It is possible that the effect of Digoxin on patients is altered by serum potassium levels. Hyperkalemia, which is high blood potassium levels, can cause heart arrhythmia and can even lead to heart attacks if it is not treated. Due to these effects, the potential effect that blood potassium levels have on the Digoxin treatment in terms of improving patient outlook for heart failure should be investigated. Other baseline characteristics (such as age, race, BMI, diuretics) that were measured from patients in the DIG main trial could also contribute to varying blood potassium levels. This study will be a secondary analysis that will investigate these follow-up questions for the DIG Main Trial of Digoxin, using the results of the DIG Trial.

## Study Aim

Investigate the association between Baseline Potassium Levels and the DIG Trial Results.

## Study Hypothesis

There is an association between Baseline Potassium Levels and the DIG Trial Results.

## Primary Hypothesis

Higher baseline potassium levels will be associated with increased odds of hospitalization for worsening heart failure.

## Secondary Hypothesis

Higher baseline potassium levels will be associated with baseline subject characteristics and derived variables age, BMI, NSYM, and TRTMT.

## Secondary Hypothesis

Baseline potassium levels will have a significant interaction with Digoxin treatment to change the effect of treatment on hospitalization for worsened heart failure.

# Study Population

## Inclusion/Exclusion Criteria

The list of exclusion criteria for the DIG trial can be found in Table 1 in their paper1 describing the rationale, design, implementation, and baseline characteristics of patients for the trial. The dataset used only contains data from the main DIG trial, which was performed on patients who fit the exclusion criteria, and whose left ventricular ejection fraction was less than or equal to 0.45. Patients who fit the exclusion criteria and had a left ventricular ejection fraction that was less than or equal to 0.45 will be referred to as “eligible patients” in the cohort diagram below.

Data used for this secondary analysis of the DIG trial is the baseline and outcome data from the main DIG trial2. Patients who are missing data for any of the variables in Section 3 of this Statistical Analysis Plan will not be included in the analyses. Only patients with complete and plausible serum potassium levels (between 2.5 and 10 mmol/L) will be included in the analyses. Having a serum potassium level below 2.5 can be classified as severe hypokalemia and could be life threatening, and above 10 would be severe hyperkalemia, thus these observations will not be considered.

**Cohort Diagram:**

Eligible patients

(n = 6800)

Randomization

Placebo

(n = 3403)

Digoxin

(n = 3397)

Secondary Analysis

Main Trial Patients

(n = 6800)

Excluded: missing data for variables

(n = 1444)

Patients with complete data (n = 5356)

Excluded: KLEVEL > 10 or < 2.5

(n = 32)

Final Dataset

(n = 5324)

## Data Acquisition

|  |  |
| --- | --- |
| Study design | This study is a retrospective, cohort study. |
| Data source/how the data were collected | Data was collected from the DIG main trial for the effect of Digoxin on mortality and morbidity in patients with heart failure2.  Information for the dataset can be found at:  <https://biolincc.nhlbi.nih.gov/media/teachingstudies/digdoc.pdf?link_time=2021-03-01_01:18:49.171599> |
| Contact information for team member responsible for data collection and acquisition | Dr. Beth Hauser (elizabeth.hauser@duke.edu) |
| Date or version (if downloaded, provide date) | The DIG trial was published in 1997. The data was taken directly from the trial results. |
| Where dataset is stored | /Users/costastavrianidis/Desktop/School/Graduate School/Spring 2022/BIOS 706/Final Exam |

Notes: *There is data not discussed in the DIG trial paper included in this dataset such as body mass index, serum creatinine, etc. To protect patient confidentiality, most variables have been permuted over the set of patients within treatment group. It should be noted that this dataset could reproduce the results of the paper, however it should not be used for other publication purposes.*

# Outcomes, Exposures, and Additional Variables of Interest

## Primary Outcome(s)

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| --- | --- | --- | --- |
| **Outcome** | **Description** | **Variable Name and Source** | **Specifications** |
| Hospitalization for Worsening Heart Failure | Patient hospitalization due to worsening heart failure. | WHF: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: no event  1: first event |

## Primary Exposure(s)

|  |  |  |  |
| --- | --- | --- | --- |
| **Exposure** | **Description** | **Variable Name and Source** | **Specifications** |
| Serum Potassium Level | Serum potassium level of patient measured at baseline in mmol/L. | KLEVEL: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |

## Secondary Exposure(s)

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Description** | **Variable Name and Source** | **Specifications** |
| Treatment | Patient received either Digoxin or placebo for the trial. | TRTMT: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: assigned to placebo group  1: assigned to treatment group |
| Age | Age of patient in years at date of randomization. | AGE: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Body Mass Index | Body mass index, measured as weight in kg/height in meters2. | BMI: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| NSYM | Number of symptoms of CHF. Sum of questions 13-20 on patient baseline form. | NSYM: taken from the DIG trial results dataset. | Coded as a numeric, discrete variable.  Coded as a factor variable due to observations being only from 0-4. |
| Sex | Sex of patient. | SEX: taken from the DIG trial results dataset. | Coded as a binary variable where:  1: Male  2: Female |
| Race | Race of patient. | RACE: taken from the DIG trial results dataset. | Coded as a binary variable where:  1: White  2: Nonwhite |
| Chest X-Ray | CT-ratio for patient. | CHESTX: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Ejection Fraction Percent | Ejection fraction value as a percent. | EJF\_PER: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Ejection Fraction Method | Ejection fraction method used for measurement. | EJFMETH: taken from the DIG trial results dataset. | Coded as a categorical variable where:  1: Radionuclide  2: Angiography  3: 2-D echo |
| Serum Creatinine Level | Serum creatinine level at baseline of the patient. Measured in mg/dl. | CREAT: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Congestive Heart Failure Duration | Duration of congestive heart failure in the patient in months. | CHFDUR: taken from the DIG trial results dataset. | Coded as a numeric, discrete variable. |
| Heart Rate | Heart rate of the patient at baseline measured in beats per minute. | HEARTRTE: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Blood Pressure | Blood pressure of the patient at baseline measured in mmHg. | SYSBP: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |
| Current NYHA Functional Class | New York Heart Association class of patient at baseline. | FUNCTCLS: taken from the DIG trial results dataset. | Coded as a categorical variable where:  1: Class I  2: Class II  3: Class III  4: Class IV |
| Etiology of CHF | The etiology of CHF for the patient at baseline. | CHFETIOL: taken from the DIG trial results dataset. | Coded as a categorical variable where:  1: Ischemic  2: Hypertensive  3: Valvular  4: Idiopathic  5: Alcohol related  6: Other |
| Previous Myocardial Infarction | Patient had a previous myocardial infarction at baseline. | PREVMI: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Current Angina | Patient has angina at baseline. | ANGINA: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| History of Diabetes | Patient has a history of diabetes at baseline. | DIABETES: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Hypertension | Patient has a history of diabetes at baseline. | HYPERTEN: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Use of Digoxin | Patient has used Digoxin within one week prior to randomization. | DIGUSE: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Potassium-Sparing Diuretics | Patient is using potassium-sparing diuretics at baseline. | DIURETK: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Other Diuretics | Patient is using other diuretics at baseline. | DIURET: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Potassium Supplement | Patient is using a potassium supplement at baseline. | KSUPP: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Ace Inhibitor | Patient is using an ace inhibitor at baseline. | ACEINHIB: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Nitrates | Patient is using nitrates (oral or paste) at baseline. | NITRATES: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Hydralazine | Patient is using hydralazine at baseline. | HYDRAL: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Vasodilaters | Patient is taking some other vasodilator(s) at baseline. | VASOD: taken from the DIG trial results dataset. | Coded as a binary variable where:  0: No or unknown  1: Yes |
| Dose of Digoxin/Placebo | Dose given to patient for Digoxin or Placebo in trial. | DIGDOSE: taken from the DIG trial results dataset. | Coded as a numeric, continuous variable. |

# Note: *variables for questions 13-20 in the baseline form (*[*https://biolincc.nhlbi.nih.gov/media/teachingstudies/digdoc.pdf?link\_time=2021-03-01\_01:18:49.171599*](https://biolincc.nhlbi.nih.gov/media/teachingstudies/digdoc.pdf?link_time=2021-03-01_01:18:49.171599)*) were not included in the analysis because the variable NSYM is the sum of those questions (which have binary responses).*

# Statistical Analysis Plan

The DIG trial dataset will be cleaned according to the cohort diagram in Section 2.1, where only patients with complete data and plausible (between 2.5 and 10 mmol/L) KLEVEL will be included in the analyses. Table 1 will be a descriptive table, showing the number of observations for each subgroup for categorical variables, and descriptive statistics for continuous variables.

## Demographic and Clinical Characteristics (“Table 1”)

|  | WHF No Event (N=3666) | WHF Event (N=1658) | Overall (N=5324) |
| --- | --- | --- | --- |
| **KLEVEL** |  |  |  |
| Mean (SD) | 4.35 (0.428) | 4.34 (0.439) | 4.35 (0.432) |
| Median [Min, Max] | 4.30 [2.60, 6.30] | 4.30 [2.90, 5.60] | 4.30 [2.60, 6.30] |
| **TRTMT** |  |  |  |
| 0 | 1713 (46.7%) | 935 (56.4%) | 2648 (49.7%) |
| 1 | 1953 (53.3%) | 723 (43.6%) | 2676 (50.3%) |
| **AGE** |  |  |  |
| Mean (SD) | 63.4 (10.9) | 63.5 (10.7) | 63.4 (10.9) |
| Median [Min, Max] | 65.0 [21.0, 91.0] | 64.0 [22.0, 92.0] | 64.5 [21.0, 92.0] |
| **BMI** |  |  |  |
| Mean (SD) | 27.1 (5.12) | 27.2 (5.31) | 27.1 (5.18) |
| Median [Min, Max] | 26.5 [14.4, 58.3] | 26.5 [15.1, 62.7] | 26.5 [14.4, 62.7] |
| **NSYM** |  |  |  |
| 0 | 38 (1.0%) | 15 (0.9%) | 53 (1.0%) |
| 1 | 82 (2.2%) | 34 (2.1%) | 116 (2.2%) |
| 2 | 250 (6.8%) | 115 (6.9%) | 365 (6.9%) |
| 3 | 338 (9.2%) | 144 (8.7%) | 482 (9.1%) |
| 4 | 2958 (80.7%) | 1350 (81.4%) | 4308 (80.9%) |
| **SEX** |  |  |  |
| 1 | 2873 (78.4%) | 1272 (76.7%) | 4145 (77.9%) |
| 2 | 793 (21.6%) | 386 (23.3%) | 1179 (22.1%) |
| **RACE** |  |  |  |
| 1 | 3248 (88.6%) | 1382 (83.4%) | 4630 (87.0%) |
| 2 | 418 (11.4%) | 276 (16.6%) | 694 (13.0%) |

## Analyses Plan for Primary Hypothesis

To test our primary hypothesis that higher baseline potassium levels will be associated with increased odds of hospitalization for worsening heart failure, we can perform a logistic regression due to the binary nature of the outcome variable, WHF. The logistic regression will have KLEVEL as the exposure variable and WHF as the outcome variable. Our statistical null hypothesis will be that the coefficient for KLEVEL in the model will be equal to zero, and the alternative will be that it is not equal to zero. From this coefficient, we will be able to make inference on the odds of hospitalization for worsening heart failure for every unit increase in serum potassium level, and be able to test this coefficient for significance using the Wald z-statistic. Before running the logistic regression, we will check our assumptions first.

1. The outcome variable must be binary, which we know is the case as that is how it is coded in the results of the trial.
2. The observations being used for the logistic regression model must all be independent of each other, which they are, as they are taken from the DIG clinical trial results where each observation was a different patient.
3. To check for outliers, we will use a Cook’s distance plot, and then a decision will be made on whether to keep them in the model or remove them.
4. To check for a linear relationship between the exposure variable and the log-odds of the outcome variable, we will examine the relationship visually by plotting.
5. Sample size (N = 5324) is sufficiently large to fit a logistic regression model.

## Analyses Plan for Secondary Hypotheses

To test our secondary hypothesis that higher baseline potassium levels will be associated with baseline subject characteristics and derived variables age, BMI, NSYM, and TRTMT, we will utilize a LASSO regression model that performs variable selection and regularization. Using a variable selection is appropriate due to the high number of baseline characteristics and derived variables as predictors. LASSO regression will utilize penalization and can send coefficients in the regression model to 0, thus leaving us with the “best” predictors that we can to either support our hypothesis or not support it. We can then interpret the coefficients of the variables left in the model to see its linear association with KLEVEL. Before running the LASSO regression, we will check our assumptions first.

1. There must be linearity between the independent and dependent variables.
2. Multicollinearity should be checked, however LASSO regression can handle multicollinearity well.
3. The observations being used for the regression model must all be independent of each other, which they are, as they are taken from the DIG clinical trial results where each observation was a different patient.
4. Homoscedasticity will be checked by fitting a scale-location plot.
5. Residuals of the model must be normally distributed, which will be checked with a Q-Q plot.

To test our secondary hypothesis that baseline potassium levels will have a significant interaction with Digoxin treatment to change the effect of treatment on hospitalization for worsened heart failure, we will fit a logistic regression model with an interaction term. The outcome of the logistic regression will be WHF, and we will fit an interaction term (KLEVEL \* TRTMT) to see if the terms have a significant interaction with each other to change the effect of treatment on the odds of hospitalization for worsened heart failure. Our statistical null hypothesis will be that the coefficient for the interaction term in the model will be equal to zero, and the alternative will be that it is not. The same assumptions of the primary hypothesis will be checked before fitting the logistic regression model. The Wald z-statistic can be used to check for significance of the interaction term in the model against our null.

# Statistical Analysis Results

## Primary Hypothesis

When comparing two patients, a patient with 1 mmol/L higher baseline serum potassium level has 3% lower odds of being hospitalized due to worsening heart failure.

The logistic regression model for this hypothesis had a coefficient of -0.03129 for KLEVEL with a p-value of 0.6484 from the Wald test. So while we can interpret the odds ratio as we did above, it must be noted that the coefficient for KLEVEL as a predictor in this model did not meet statistical significance. Due to this, we would fail to reject our null that the coefficient of KLEVEL in the model is equal to zero. This is equivalent to saying that we would fail to reject the null that baseline potassium levels are associated with increased risk of hospitalization for worsening heart failure.

## Secondary Hypothesis (1.2.2)

The LASSO regression sent all coefficients for the variables to zero except for the coefficient for CREAT, which is the serum creatinine level of the patient at baseline in mg/dl. We can interpret coefficient as: every one mg/dl increase in serum creatinine level of a patient is associated with a 1.22e-17 mmol/L increase in serum potassium level.

## Secondary Hypothesis (1.2.3)

Using the odds ratios of the model, we can say that the odds of being hospitalized due to worsening heart failure increases by 1% for every 1 mmol/L increase in serum potassium level for patients that received the placebo. The odds of being hospitalized due to worsening heart failure decreases by 7% for every 1 mmol/L increase in serum potassium level for patients that received the Digoxin treatment.

The coefficient of the interaction term is -0.08119 with a p-value of 0.556 from the Wald test. So while we can interpret the odds ratios of the model above, the interaction term is not significant, thus we would fail to reject our null that the coefficient of the interaction term is equal to zero. This is equivalent to stating that we would fail to reject the null that baseline potassium levels interact with Digoxin treatment to change the effect of treatment on hospitalization for worsening heart failure.

# Discussion/Addendum for Additional Analyses

## Primary Hypothesis

Looking at the results for the primary hypothesis, we were not able to find a significant association between baseline potassium levels and risk of hospitalization for worsening heart failure. A drawback of this analysis that should be noted is that this logistic regression has the assumption that baseline potassium level is linearly associated with the log-odds of hospitalization due to worsening heart failure. Thus, this analysis is only testing for this linear association. It is entirely possible that there is a non-linear relationship between these variables, however this test would not capture that. Further testing for this potential non-linear association would be a good follow up to these results.

## Secondary Hypothesis (1.2.2)

The secondary hypothesis results had the LASSO regression only keep the coefficient for baseline serum creatinine levels, and it was nearly zero. While this coefficient can be interpreted to explain a potential linear association between serum creatinine and potassium level, it is somewhat impractical to do it due to how close to zero that coefficient is. The LASSO regression is performing variable selection and selecting coefficients that would explain a linear association between these predictors and potassium level, but it is possible that there exists other types of non-linear relationships between these variables and potassium levels. A good follow up to this analysis would be to explore those potential relationships. Also, if one wanted fit a multiple linear regression model with all of the predictors and KLEVEL as the outcome, you could then be given coefficients for all of the predictors along with p-values. However, based on the results of the LASSO regression, this model likely would have insignificant p-values associated with these coefficients, and potentially a very small R2 value.

## Secondary Hypothesis (1.2.3)

The secondary hypothesis results were not able to find a significant interaction between baseline potassium level and treatment that changes the effect of treatment on hospitalization for worsening heart failure. From a standpoint of the original DIG clinical trial, this finding is positive. If there were a significant interaction and baseline potassium levels did change the effect of treatment on hospitalization due to worsening heart failure, which was one of the outcomes of the trial, that would introduce bias in the results if not accounted for. However, with this test, it should be noted again that this analysis is only testing for this linear association between the predictors and the log-odds of the outcome. If there is a non-linear association between predictors and the log-odds of the outcome, this model may have not captured it. A follow up analysis should explore the potential of non-linear relationships between these variables.

# Appendix

# References

**1.** The Digitalis Investigation Group. “Rationale, Design, Implementation, and Baseline Characteristics of Patients in the Dig Trial: A Large, Simple, Long-Term Trial to Evaluate the Effect of Digitalis on Mortality in Heart Failure.” *Controlled Clinical Trials*, vol. 17, no. 1, 1996, pp. 77–97., https://doi.org/10.1016/0197-2456(95)00065-8.

**2.** “The Effect of Digoxin on Mortality and Morbidity in Patients with Heart Failure.” *New England Journal of Medicine*, vol. 336, no. 8, 1997, pp. 525–533., https://doi.org/10.1056/nejm199702203360801.