1. Group 23 (Group LGL)

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2. Background knowledge, feedback, and eyes on the field:

***a.***

In this study, we aim to explore the relationship between renal dysfunction and mortality rates among patients with heart failure. The question was firstly studied by Ahmad et al utilizing survival analysis. In their work, Ahmad mainly identified potential risk factors for mortalities among patients with heart failure. Renal dysfunction (measured by serum creatinine) is reported as an important risk factor. A 1-unit increment in serum creatinine is associated with 2.24 hazard ratio with p-value less than 0.05. Chicco et al. re-analyzed the dataset using machine learning methods and reported that the serum creatinine level is the most important predictor for these patients’ mortalities. Analogous research topic has been studied by Van Domburg et al., using estimated glomerular filtration rate (eGFR) as the indicator of renal dysfunction in patients with known or suspected coronary artery disease. Van Domburg et al. employed a multivariable adjusted Cox model and found the hazard ratios were 1.33, 1.67, and 3.38 among patients with mild, moderate, and severe renal impairment compared to their peers with normal renal function. In summary, although extent literature employed different biomarkers for renal dysfunction, increasing hazard ratios were reported for impaired renal function among patients with cardiovascular diseases.

After reviewing existing literature, we aimed to use a multivariable adjusted Cox model as the main model as it is commonly used in this research area. However, previous studies reported less on the modification of other biomarkers such as eject fraction. Potential effect modification by other biomarkers may reveal heterogenous effects of renal dysfunction among different subgroups. Thus, we aim to fill this gap in the current project.

***b.***

We appreciate very much the teaching stuff and out peers’ comments. These comments point out several very important questions which we will incorporate in our analysis. We aggregate the comments and show our response and analysis plan below (***for question i and ii***).

* In the primary question, you want to explore the association between serum creatine and mortality rates for patients with heart failure. I think the question is not specific enough. What kind of association you expect they should have? Which aspects you aim to explore?

Response: It is a very helpful question. We will make it more clear that there are three sperate models and the association definitions are different: 1) The association between the expected survival time and serum creatine: the association is on average how many days will increase if the patient’s serum creatinine level decreased by 10% in a linear model; 2) the association between death by 30 days and serum creatine: the association is the average odds ratio of death by 30 days if the patient’s serum creatinine level decreased by 10% in a logistic model; and 3) the association between mortality rates and serum creatine: the association is the average hazard ratio if the patient’s serum creatinine level decreased by 10% in a Cox model. In model fitting part, how about add a model selection process that is to fit different models that we mentioned in class, can compare which models fit better?

* The dataset is small. Overfitting is easier to happen. Simper model may be better.

Response: We appreciate this comment. As a solution to limited sample size, we aim to utilized a lasso regression in the linear model to help us identify key covariates influencing patients’ morality. Based on the sparse results of the lasso regression, we will decide which covariate to be included in our main analysis. In this study, we prefer lasso regression as a prediction selection method to stepwise regression, since stepwise regression is somehow time-consuming and may get inconsistent predictor sets when utilizing different step directions. To determine the optimal penalty parameter in lasso, , we will utilize a cross-validation process, or let the function in nlmet package to automatically decide the optimal parameter. After getting the most suitable covariate sets, we will delete and add a covariate according to the lasso results, and assess the goodness-of-fit in the model with these three different covariate sets. Detailed analysis please see the methods section below.

* My only advice is about your data set. Even though your dataset has only 299 cases, the dataset seems clean and complete, so after you check if there is any missing data and if it’s not too much, maybe using complete cases with all data entries would be both easier and more accurate, Or at least the variable you are interested in is complete. If the missing data reduce the number of cases a lot, the mice function in R could also be helpful.

Response: We appreciate this comment. Actually, after checking the dataset, luckily, we found there were no missing values. Thus, we think it is no need to make imputations. Thanks for the comment, and thanks for sharing this important package *MICE.*

***iii.***

As the research problem has been widely discussed in previous medicine literature, though the quantitative results are relatively limited, its clinical meaning is relatively clear. Given the limited time of this project and our limited resources, we admit we fail to contact a clinician expert during the past 1 month. We will keep reading related literature and try our best to seek domain experts’ help.

3. Analysis Plan:

We make several amendments to our original analysis plan. We highlight these changes below.

1. Data cleaning: although the dataset has been elaborated by Davide Chicco, we will check any potential missing data in the outcome, exposure, and covariates. We will report the number of missing data and if the number is less than 10%, we will consider including a “missing” indicator for categorical variables and imputing continuous variables. We will report the final number of patients included in our study.
2. Checking the exposure:

The primary exposure, serum creatine, which is continuous, is usually categorized into two different levels (≤ 1.5 mg/dL for the normal level vs, and > 1.5 mg/dL for the abnormal level). In this project, we will first treat the serum creatine as a continuous variable and calculate its sample mean, standard deviation, median, and range. Also, to make it comparable with previous studies, we will assess the serum creatine as a binary variable and report the proportion of normal and abnormal levels in patients.

1. Checking the outcomes:

We will calculate the average person-time until death in the normal serum creatinine group and the abnormal serum creatinine group. We will also calculate the Death 30-day in these two groups. Survival plots will be made to visualize the mortality rates in serum creatinine groups. Chis-squared tests will be applied to test the difference of Death 30-day in these groups.

1. Checking other covariates.

In this project, age (continuous), sex (male vs. female), anemia (yes vs. no), diabetes (yes vs. no), ejection fraction (≤ 30, 31-44, and ≥ 45), smoking (yes vs. no), platelets (continuous, kilo platelets/mL), and serum sodium (continuous, mEq/L) will be considered as covariates.

The proportions for categorical covariates and mean (standard deviations) for continuous covariates in normal serum creatinine group and abnormal serum creatinine group will be calculated and compared using chi-squared tests and t-tests respectively.

1. Modeling analysis

First, we will do a simple linear regression do assess the association between survival time and serum creatine level in patients who died by the end of the study. The serum creatine level will model as a continuous and a categorical variable respectively. Second, the probability of Death 30-day will be modeled by logistic models. Third, we combine the incidence of deaths and time at risk among patients with identical covariate patterns. Poisson regression models will be employed to model the association between serum creatine level and incidence rate of deaths. Last, in our main analysis, we will perform survival analysis. A Kaplan-Merrier plot will be made for patients stratified by serum creatine (normal vs. abnormal). Then Cox proportional-hazards model will be performed, with outcome to be the survival time with the event (0 for censored and 1 for death). In addition to serum creatine level (both continuous and categorical variable will be assessed), appropriate covariates will be adjusted for.

1. Model selection

Considering the relatively small sample in this project, we decide to make our model as parsimonious as possible. In this sense, we will utilize lasso regressions to help us determine the optimal covariate sets in the models mentioned in subsection e), except the Poisson regression. To justify the selection by the lasso regression, based on the covariates automatically determined by the software, we decide to include and delete a covariate. Thus, in each model we will have three sets of covariates. We will perform models using these three covariates sets and compare the model performance based on AIC.

1. Subgroup analysis for potential effect modification.

To check whether effect modification exists, we will include an interaction term between serum creatine and potential covariates finally decided by our model selection in subsection f) in the Cox model. The p-value of the interaction term as well as variance-deviance analysis will be used to determine if there are any effects modifications.

1. Checking nonlinearity

To check whether the relationship between serum creatine and mortality risks is linear, we will replace the linear term in the fully adjusted model with a natural spline of serum creatine. The knots and degrees of freedom of the spline will be determined during the following analysis.

4. Missing Data

After checking the data clearly, there was no missing in this dataset, partly because Davide Chicco et al. have elaborated the original dataset before uploading to the archive. Here we would like to answer the following questions as required supposing we did have some missing data in serum creatine and smoking.

1. Because the biomarkers information was retrieved from blood reports. Smoking and drinking information was retrieved from clinicians’ notes. If we had some missing data in serum creatine and smoking, it would be more likely be Missing Completely at Random (MCAR) as these documents should be recorded in the reports. The missing is more likely to be caused by system errors or notes missing. We can test the assumption by regression the chance of missing event on known covariates. If there are no significant associations, we deem it is a MCAR, or we will deem it as Missing at Random (MAR). Missing Not at Random (MNAR) is not taken into account because of the underlying data generating process. If the data is MCAR, we will just use the complete cases in our analysis. If the data is MAR, complete case analysis is fine, but we will consider using a missing indicator.
2. Please check the diagram below.

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Figure 1. Flow chart for handling missing data

***It is worth noting that although we discussed the potential scenarios that we had missing values in the data, actually there is no missing data in the actual dataset***. Thus, the steps above are just discussed and will not be used in the real data analysis process.

***5. Modeling***

***a. Linear, flexible/additive or other methods (LASSO, ridge) from this topic:***

***Originally, the linear regression is not our most interested method because we assess the survival time for patients with heart failure. We generated a new variable which fits the linear regression framework.***

* New variable: the death time for people who died by the end. There were 96 people who actually died by the end of the study period. Their time to death can serve as a linear outcome in the linear model.
* We will assess the association between death time and the baseline serum creatine level among those who died by the end of the study (n = 96). It is a quite small sample size.

We first fitted a crude a linear model

* The fitted results showed that the is 0.6687, with a p-value of 0.879. The results suggested that in the crude model there was no significant association between serum creatine level and death time among people who died by the end.
* We then decided to fit a multivariate linear model adjusting for other covariates. Since the sample size is relatively small, we utilized a Lasso to help determine the covariate sets. Unfortunately, according to the results of Lasso, it suggested that we should only include intercept term in our model which is not very helpful.

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Figure 2. Cross-validation results of LASSO when choosing the most appropriate penalty parameter in the linear regression.

* Then, we just adjusted for patients’ age and sex based on the background knowledge
* Again, the results showed that there was no significant association between serum creatine level and death time, with equal to 1.1688, the p-value equal to 0.80.
* Based on this result, we performed some model diagnostics, using studentized residuals.

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Figure 3. QQ-plot testing the normality of residuals

* Clearly the residuals were not normally distributed, violating the normality assumption underlying the linear regression. They were also linearly associated with the outcome (death time), indicating there were some unmeasured important factors determining the death time.

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Figure 4. Scatter plot for outcome against residuals

* In summary, among patients who finally died at the end of the study, their death time was not associated with their baseline serum creatine level.

***b. Logistic, multinomial, ordinal:***

***Originally, the logistic regression is not our most interested method because we assess the survival time for patients with heart failure. We generated a new variable which fits the logistic regression framework.***

* We modeled the association between death by 30 days (“Death 30-day”, binary variable) and serum creatine level. A total of 6 participants were lost to follow-ups before the 30 days. We excluded them from the data set in this analysis.
* Let denote the probability of Death 30-day

In a crude model,

* The estimated OR for a 1 mg/dL increment in serum creatine level for Death 30-day is 1.44 (95% CI 1.12 to 1.89). It meant that high level of serum creatine value is harmful for patients with heart failure.
* In multivariate adjusted logistic models, we first used Lasso regression to help us determine the appropriate covariate sets.

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Figure 5. Cross-validation results of LASSO when choosing the most appropriate penalty parameter in the logistic regression.

* According to the cross-validation results using (MSE as the metric), the optimal number of covariates was 6 and were listed in the table below. To justify the lasso results, along the axis of , we put a covariate into the covariate set and exclude a covariate out of the set. Then we perform logistic regressions for those three covariates sets and compare their results.

Table 1. Logistic regressions for the associate between baseline serum creatine level and deaths by 30 days from the baseline (N= 283)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Model 1 | Model 2 | Model 3 |
| OR for 1 mg/dL in serum creatine | 1.32 (0.98, 1.78) | 1.32 (0.98, 1.78) | 1.32 (0.98, 1.78) |
| Adjusted variables | age (continuous) + anemia (binary) + ejection fraction (continuous) + platelets (continuous) + serum sodium (continuous) | + sex (binary) | -platelets (continuous) |
| AIC | 198.6465 | 198.6465 | 198.6465 |

* According to the results of those three logistic regressions, 1 mg/dL increment in serum creatine is associated with 1.32 (95% CI 0.98 to 1.78) OR as death by 30-days. The point estimated OR suggests that the serum creatine is harmful for patients’ survival. However, since the 95% Confidence Interval contains the null association, we need more samples to reach the conclusion.
* We will do some diagnostics. The line of residuals against predictors in the model are approximately horizontal, with p-values > 0.05 from the t-test, suggesting the assumption underlying the logistic models are meeting.

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Figure 6. Plots for residuals against predictors in the logistic regression

* Because most predictors in the model are linear, there is hard to evaluate the model by calibration. We will evaluate its goodness to fit by discrimination. The AUC is 0.7629, which is somewhat acceptable.

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Figure 7. ROC curve for the logistic regression

***c. Poisson***

***Originally, the Poisson regression is not our most interested method because we assess the survival time for patients with heart failure. We generated a new variable which fits the Poisson regression framework.***

* We will use a Poisson regression to assess the incidence rate to death during the whole follow-up period. The original dataset includes individual observations. To assess the “count” in the data, we collapse the data into group data with the total cases of death and total time at risk based on the covariate’s combinations above.
* We categorized persons by serum creatine (normal vs. abnormal), age (<65 vs >= 65), sex (male vs. female), eject fraction (≤ 30, 31-44, and ≥ 45), platelets (lower than the median vs. higher than the median), and serum sodium (lower than the median vs. higher than the median). After collapse, we had 126 combinations i.e., 126 rows in the group dataset.
* We applied a Poisson regression to test the association between incidence rate of death and serum creatine level. The offset was the total time at risk in that group.

Let denote the incidence rate of death, t denotes the mean incidence cased of death during the risk at time.

Xj,i represents the adjusted variables (including dummy variables) above.

ti represents the total risk at time in that group

* According to the results of the quasi-Poisson regression, compared to people with normal level of serum creatine, the incidence rate ratio of death among people with abnormal serum creatine level is 2.60 (95% 1.68 to 4.00).
* To account for potential over-dispersion in the variance which may violate the Poisson assumption, we conducted a quasi-Poisson regression. In the quasi-Poisson regression’s assumption, the variance is . The point estimate stayed the same, but the 95% CI was wider from 1.27 to 5.52.

***d. Survival analysis***

**i.** We decide to take survival analysis into out project. Actually it will be the main analysis.

**ii.** The outcome is the survival time with the end point status for the patients with heart failure during the follow-up period. The interested exposure is the patients’ serum creatine value. The adjusted covariates are age (continuous), anemia (binary), ejection fraction (continuous), platelets (continuous), serum sodium (continuous). The covariate set is just the same as in the logistic regression and Poisson regression. In the next stage of analysis, we will use a lasso regression in the survival analysis framework.