**Biost 518: Applied Biostatistics II**

**Biost 515: Biostatistics II**

Emerson, Winter 2015

**Homework #4**

February 2, 2015

**Written problems:** To be submitted as a MS-Word compatible file to the class Catalyst dropbox by 9:30 am on Monday, February 9, 2014. See the instructions for peer grading of the homework that are posted on the web pages.

*On this (as all homeworks) Stata / R code and unedited Stata / R output is* ***TOTALLY*** *unacceptable. Instead, prepare a table of statistics gleaned from the Stata output. The table should be appropriate for inclusion in a scientific report, with all statistics rounded to a reasonable number of significant digits. (I am interested in how statistics are used to answer the scientific question.)*

***Unless explicitly told otherwise in the statement of the problem, in all problems requesting “statistical analyses” (either descriptive or inferential), you should present both***

* ***Methods: A brief sentence or paragraph describing the statistical methods you used. This should be using wording suitable for a scientific journal, though it might be a little more detailed. A reader should be able to reproduce your analysis. DO NOT PROVIDE Stata OR R CODE.***
* ***Inference: A paragraph providing full statistical inference in answer to the question. Please see the supplementary document relating to “Reporting Associations” for details.***

This homework investigates associations between death from any cause and age, sex, and serum bilirubin in a population of patients with primary biliary cirrhosis who were enrolled in a randomized clinical trial (RCT) of D-penicillamine. The data can be found on the class web page (follow the link to Datasets) in the file labeled liver.txt. Documentation is in the file liver.doc.

1. Provide suitable descriptive statistics pertinent to the scientific questions addressed in this homework.

**Answer:**

**Methods:** The scientific questions are the associations between death from any cause and age, sex, and serum bilirubin in a specific population. Since the question involving censoring distribution, to provide suitable descriptive statistics, I provided Kaplan- Meier curve, with the minimum and maximum observed time, the Kaplan-Meier estimates of the 10th, 20th, and 50th (median) percentiles, the estimates of 5 year and 8 year survival probabilities, and the restricted mean time of follow-up during the observed time (restricted to the largest observed time).

For age and bilirubin, they are continuous variables. Therefore, I presented their range, mean, standard deviation, quartiles and the number of missing values. To investigate the associations between age, bilirubin and death, I categorized age and bilirubin, separately. Age was categorized by common sense: 26 – 40 years old, 40 – 60 years old (including 40) and 60 – 79 years old (including 60). We expect the number of people to act multiplicatively on bilirubin (the number of people will tend to decrease multiplicatively as the bilirubin level increasing). Therefore, serum bilirubin was categorized as: 0.25 – 1 mg/dl, 1 – 4 mg/dl (including 1 mg/dl), 4 – 16 mg/dl (including 4 mg/dl), and greater than 16 mg/dl (including 16 mg/dl). Within these categories, Kaplan-Meier estimates of survival were calculated and graphed (just as I mentioned above).

For sex, it is a binary variable. I just presented the number and percentage. And to investigate the associations between sex and death, I dichotomized the data by sex. Within the two groups, Kaplan-Meier estimates of survival were calculated and graphed (just as I mentioned above).

**Inference:**

**For all the subjects:**

For the all 418 subjects, the estimate Kaplan-Meier curve is presented as below. Among these subjects and during the study time, the smallest survival time is 0.11 year, while the largest survival time is 13.13 years (which is censored). The Kaplan-Meier estimates of the 10th, 20th, and the 50th percentiles are 1.67 years, 3.15 years and 9.30 years, respectively. The Kaplan-Meier estimates of 5 year and 8 year survival probabilities are 70.3% and 56.9%, respectively. The restricted mean estimate of the subjects’ survival time is 8.35 years.



Figure 0 Kaplan-Meier curves for all subjects

**For the association between death and age:**

First, the 418 subjects are all with available age. The mean age among the data is 50.74 years old, with standard deviation: 10.45 years and the range are 26.28 to 78.44 years old. Table 1 below presents the Kaplan-Meier estimates of the survival distribution within the strata defined by age and for all the subjects. And Figure 1 presents the Kaplan-Meier curves for all the groups.

From the following table and figure, it is easy to find that there is a trend that younger people tend to have larger percentile of survival (see the table 1 for 10th, 20th and 50th percentile of survival). And the survival probability at 5 years and 8 years are both higher for younger people. In Figure 1, we also can find this trend. The blue line (representing the youngest group) is always higher than the red one (representing the middle age group) and the red line is always higher than the green one (representing the elder group).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Age (years) | | | All Subjects |
|  | 26 – 40 | 40 (included) - 60 | 60 (included) - 79 |
| N Subjects | 69 | 261 | 88 | 418 |
| N Deaths | 13 | 101 | 47 | 161 |
| 5 Year Survival Probability | 86.4% | 71.0% | 56.4% | 70.3% |
| 8 Year Survival Probability | 74.2% | 57.5% | 43.8% | 56.9% |
| 10th Percentile of Survival (year) | 3.91 | 1.51 | 0.95 | 1.67 |
| 20th Percentile of Survival (year) | 7.36 | 3.00 | 2.13 | 3.15 |
| 50th Percentile of Survival (year) | NA1 | 9.43 | 6.85 | 9.30 |
| Restricted mean of Survival (year) | 10.26 | 8.22 | 6.44 | 8.35 |

Table 1 Kaplan-Meier estimates for groups defined by age

1 Based on the Kaplan-Meier estimates computed within strata defined by age, NA indicates that the percentile is not estimable with the available data. (The 50th percentile of survival for group 26 – 40 years old is not available since there are not 50% uncensored data in this group.)



Figure 1 Kaplan-Meier curves for groups defined by age

**For the association between death and sex:**

First, among the 418 subjects, there are 312 subjects with available sex. I just omitted the 106 subjects and dichotomized the 312 subjects by sex. Among the 312 subjects, there are 276 females and 36 males, i.e. 88.5% female and 11.5% male. Table 2 below presents the Kaplan-Meier estimates of the survival distribution within the groups defined by sex and for all the subjects. And Figure 2 presents the Kaplan-Meier curves for the two groups.

From the following table and figure, it is easy to find that there is a trend that female tends to have larger percentile of survival than male (see the table 2 for 10th, 20th and 50th percentile of survival). And the survival probability at 5 years and 8 years are both higher for female. In Figure 2, we also can find this trend. The red line (representing females) is higher than the blue one (representing males) almost everywhere (except a short period at beginning).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Sex | | All Subjects with Available Sex |
|  | Male | Female |
| N Subjects | 36 | 276 | 312 |
| N Deaths | 22 | 103 | 125 |
| 5 Year Survival Probability | 52.2% | 73.5% | 71.1% |
| 8 Year Survival Probability | 39.2% | 60.1% | 57.3% |
| 10th Percentile of Survival (year) | 1.67 | 1.90 | 1.90 |
| 20th Percentile of Survival (year) | 2.74 | 3.38 | 3.20 |
| 50th Percentile of Survival (year) | 6.53 | 9.39 | 9.30 |
| Restricted mean of Survival (year) | 6.73 | 8.35 | 8.14 |

Table 2 Kaplan-Meier estimates for groups defined by sex



Figure 2 Kaplan-Meier curves for group defined by sex

**For the association between death and serum bilirubin:**

First, the 418 subjects are all with available serum bilirubin. The mean age among the data is 3.22 mg/dl, with standard deviation: 4.41 mg/ dland the range are 0.3 to 28.0 mg/dl. Table 3 below presents the Kaplan-Meier estimates of the survival distribution within the strata defined by serum bilirubin and for all the subjects. And Figure 3 presents the Kaplan-Meier curves for all the groups.

From the following table and figure, we can find that there is a trend that people with lower serum bilirubin level tend to have larger percentile of survival (see the table 3 for 10th, 20th and 50th percentile of survival). And the survival probability at 5 years and 8 years are both higher for people with lower bilirubin level. In Figure 3, we also can find this trend. The blue line (representing the group with bilirubin level 0.3 – 0.9 mg/dl) is always higher than the red one (representing the group with bilirubin level 1.0 – 3.9 mg/dl) and the red is always higher than the green (representing the group with bilirubin level 4.0 – 15.9 mg/dl) and the green is always higher than the yellow (representing the group with bilirubin level 16.0 – 28.0 mg/dl).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Serum Bilirubin (mg/dl) | | | | All Subjects |
|  | 0.3 – 0.9 | 1.0 – 3.9 | 4.0 – 15.9 | 16.0 – 28.0 |
| N Subjects | 142 | 185 | 75 | 16 | 418 |
| N Deaths | 20 | 74 | 52 | 15 | 161 |
| 5 Year Survival Probability | 93.1% | 72.8% | 31.6% | 6.3% | 70.3% |
| 8 Year Survival Probability | 83.3% | 54.1% | 19.9% | NA1 | 56.9% |
| 10th Percentile of Survival (year) | 5.72 | 2.08 | 0.57 | 0.21 | 1.67 |
| 20th Percentile of Survival (year) | 9.81 | 3.93 | 1.10 | 0.59 | 3.15 |
| 50th Percentile of Survival (year) | NA1 | 8.82 | 3.26 | 2.34 | 9.30 |
| Restricted mean of Survival (year) | 10.80 | 8.13 | 4.25 | 2.20 | 8.35 |

Table 3 Kaplan-Meier estimates for groups defined by bilirubin

1 Based on the Kaplan-Meier estimates computed within strata defined by bilirubin, NA indicates that the percentile and survival probability are not estimable with the available data.



Figure 3 Kaplan-Meier curves for group defined by bilirubin

1. In prior homeworks using the Cardiovascular Health Study datasets, we were able to use logistic regression to investigate associations between mortality and various covariates. Why might such an approach not seem advisable with these data? (Consider the extent to which such analyses might be confounded and/or lack precision.)

**Answer:**

Since the observed time in this dataset is censored, we don’t know anything about the censoring subjects. Therefore, we are unable to use logistic regression to investigate associations between mortality and other covariates for the whole dataset. In homework 1, we can investigate associations between 4-year all-cause mortality and other covariates, since the minimum observed time for censoring data is more than 4 years. However, in this dataset, the minimum observed time for censoring data is 1.46 years and the number of data with observed time less than 1.46 years is 36. Therefore, we can only investigate 1.46-year all-cause mortality and other covariates. But it will be lack of precision because of the small sample size and if we estimate precision by the standard error, the precision will be around 0.3 of the previous.

1. Perform a statistical regression analysis evaluating an association between serum bilirubin and all-cause mortality by comparing the instantaneous risk (hazard) of death over the entire period of observation across groups defined by serum bilirubin modeled as a continuous variable.
   1. Include a full report of your inference about the association.

**Answer:**

**Methods:** I used proportional hazard regression to compare the distributions of the time to all-cause death across groups defined by serum bilirubin, and modeled serum bilirubin as a continuous variable. Based on Wald statistics and Huber-White sandwich estimator, we can get the estimate of the hazard ratio from the regression model with robust standard error, a confidence interval and two-sided p-value. We can use these values to quantify the association between all-cause mortality and serum bilirubin.

**Inference:** The dataset has 418 subjects in total, and they all have available serum bilirubin. The mean of the level of serum bilirubin among the 418 subjects is 3.22 mg/dl (standard deviation: 4.41 mg/dl, range: 0.3 mg/dl – 28.0 mg/dl). There are 161 subjects died during the follow-up time, which is 38.5% of the whole dataset. From the results of a proportional hazard regression, we estimate that the instantaneous risk of death is a relative 15.2% higher (hazard ratio 1.152) for each 1 mg/dl higher serum bilirubin level at baseline. According to a 95% confidence interval based on robust standard error, this observed hazard ratio would not be judged unusual if the true instantaneous risk of death were between 12.1% and 18.5% higher for each 1 mg/dl higher serum bilirubin level (i.e. 95% CI for hazard ratio is (1.121, 1.185)). Based on the fact that the two-sided p value is less than 0.001, we can with high confidence reject the null hypothesis that the risk of all-cause death is not associated with serum bilirubin level. Instead, we will prefer that there is a tendency for higher mortality with higher serum bilirubin levels.

* 1. For each population defined by serum bilirubin value, compute the hazard ratio relative to a group having serum bilirubin of 1 mg/dL. (This will be used in problem 6). If *HR* is the hazard ratio (use the actual hazard ratio estimate) obtained from your regression model, this can be effected by the Stata code

gen fithrA = *HR ^ (bili* – 1)

It could also be computed by creating a centered bilirubin variable, and then using the Stata predict command

gen cbili = bili – 1

stcox cbili

predict fithrA

**Answer:** Fitted values are to be displayed in problem 6.

1. Perform a statistical regression analysis evaluating an association between serum bilirubin and all-cause mortality by comparing the instantaneous risk (hazard) of death over the entire period of observation across groups defined by serum bilirubin modeled as a continuous logarithmically transformed variable.
   1. Why might this analysis be preferred *a priori?*

**Answer:**

I would prefer this analysis a priori, because I would expect the risk of all-cause death act multiplicatively on serum bilirubin level. Therefore, it would be better to defined the groups by serum bilirubin modeled as a continuous logarithmically transformed variable.

* 1. Include a full report of your inference about the association.

**Answer:**

**Methods:** I used proportional hazard regression to compare the distributions of the time to all-cause death across groups defined by serum bilirubin modeled as a continuous logarithmically transformed variable (with base 1.1). Based on Wald statistics and Huber-White sandwich estimator, we can get the estimate of the hazard ratio from the regression model with robust standard error, a confidence interval and two-sided p-value. We can use these values to quantify the association between all-cause mortality and serum bilirubin.

**Inference:** The dataset has 418 subjects in total, and they all have available serum bilirubin. The mean of the level of serum bilirubin among the 418 subjects is 3.22 mg/dl (standard deviation: 4.41 mg/dl, range: 0.3 mg/dl – 28.0 mg/dl). There are 161 subjects died during the follow-up time, which is 38.5% of the whole dataset. From the results of a proportional hazard regression on continuous logarithmically transformed bilirubin, we estimate that the instantaneous risk of death is a relative 9.88% higher (hazard ratio 1.0988) for each 10% higher serum bilirubin level at baseline. According to a 95% confidence interval based on robust standard error, this observed hazard ratio would not be judged unusual if the true instantaneous risk of death were between 8.26% and 11.5% higher for each 10% higher serum bilirubin level (i.e. 95% CI for hazard ratio is (1.0826, 1.115)). Based on the fact that the two-sided p value is less than 0.001, we can with high confidence reject the null hypothesis that the risk of all-cause death is not associated with serum bilirubin level. Instead, we will prefer that there is a tendency for higher mortality with higher serum bilirubin levels.

* 1. For each population defined by serum bilirubin value, compute the hazard ratio relative to a group having serum bilirubin of 1 mg/dL. (This will be used in problem 6). If *HR* is the hazard ratio (use the actual hazard ratio estimate) obtained from your regression model, this can be effected by the Stata code

gen logbili = log(bili)

stcox logbili

fithrB = *HR ^ (logbili*)

(Note that the log(1) = 0 when using any base, so there is no need to rescale by the bilirubin values. Note also that you might want to use a different base in your logarithmic transformation in order to facilitate more natural reporting of effects.)

**Answer:** Fitted values are to be displayed in problem 6.

1. One approach to testing to see whether an association between the response and the predictor of interest is adequately modeled by an untransformed continuous variable is to add some other transformation to the model and see if that added covariate provides statistically significant improved “fit” of the data. In this case, we could test for “linearity” of the bilirubin association with the log hazard ratio by including both the untransformed and log transformed bilirubin. (Other alternatives might have been bilirubin and bilirubin squared, but in this case our *a priori* interest in the log bilirubin might drive us to the specified analysis.)
   1. Provide full inference related to the question of whether the association is linear.

**Answer:**

**Methods:** I used a proportional hazard regression to compare the distributions of the time to all cause death across groups defined by serum bilirubin. To see whether an association between all-cause death and serum bilirubin level is adequately modeled by an untransformed continuous variable (serum bilirubin level) or not, we can include both the untransformed and log transformed bilirubin in our test. We mainly use the estimates of the coefficients corresponding to log transformed bilirubin from the regression to make a conclusion. Based on Wald statistics and Huber-White sandwich estimator, we can get the estimate of the hazard ratio (corresponding to log transformed bilirubin), a 95% confidence interval and two-sided p value.

**Inference:** The dataset has 418 subjects in total, and they all have available serum bilirubin. The mean of the level of serum bilirubin among the 418 subjects is 3.22 mg/dl (standard deviation: 4.41 mg/dl, range: 0.3 mg/dl – 28.0 mg/dl). There are 161 subjects died during the follow-up time, which is 38.5% of the whole dataset. From the results of a proportional hazard regression on both untransformed and logarithmically transformed bilirubin, we estimate that the instantaneous risk of death (corresponding to logarithmically transformed bilirubin) is a relative 11.9% higher (hazard ratio 1.119) for each10% higher serum bilirubin level at baseline. According to a 95% confidence interval based on robust standard error, this observed hazard ratio would not be judged unusual if the true instantaneous risk of death were between 8.65% and 15.3% higher for each 10% higher serum bilirubin level (i.e. 95% CI for hazard ratio is (1.0865, 1.153)). Based on the fact that the two-sided p value is less than 0.001 and the two-sided p value for the coefficient corresponding to untransformed bilirubin (which is 0.148), we can with high confidence say that with added logarithmically transformed bilirubin, the “fit” of the data has been statistically significantly improved.

* 1. Again, save the fitted values from this model by obtaining the estimated HRs relative to a group with bilirubin of 1 mg/dl. (This will be used in problem 6.)

**Answer:** Fitted values are to be displayed in problem 6.

1. Display a graph with the fitted hazard ratios from problems 3 - 5. Comment on any similarities or differences of the fitted values from the three models.

**Answer:**

Figure 4 is the graph with the fitted hazard ratios from problems 3 – 5. All the three line have positive trends. It means that the three models all predict a trend that it predominantly upward with higher serum bilirubin. For bilirubin level ranges from 0 to around 16 mg/dl, the predict values from logarithmically transformed model and the combined model (untransformed and logarithmically) are quite close. There obvious differences between the lines from the two models and the line from the linear model (untransformed bilirubin). The greatest differences between the models occur at the largest of the bilirubin level, where our data is relatively sparse. Since there are only a few people with bilirubin level higher than 16 mg/dl (in our dataset there are only 14 out of 418) and the more interpretability of the logarithmically transformed model than the combined model, I would prefer to use the log transformed model to predict the hazard ratio. From the figure, we can see that the linear fit is really different from other models, and it gets really big values of estimates when the bilirubin gets larger. I would not choose the linear fit to predict the hazard ratios.



Figure 4 Fitted hazard ratios from the three regression models

1. We are interested in considering analyses of the association between all cause mortality and serum bilirubin after adjustment for age and sex.
   1. What evidence is present in the data that would make you think that either sex or age might have confounded the association between death and bilirubin? (In real life, we would ideally decide whether to adjust for potential confounding in our pre-specified statistical analysis plan (SAP)).

**Answer:**

**Methods:** To have a sense about the association between sex, age and bilirubin, I stratified the dataset by sex and age, separately, and then calculated the mean bilirubin level for all the categories. To have a sense about the association between sex, age and all-cause death, we can just look at the descriptive statistics in problem 1.

**Inference:** Among the 312 subjects with available sex, the mean bilirubin level for male is 2.87 mg/dl, and the mean bilirubin level for female is 3.31 mg/dl. Among the 418 subjects with available age, the mean bilirubin for subjects from 26 – 40 years old is 2.61 mg/dl, the mean bilirubin for subjects from 40 (included) – 60 years old is 3.53 mg/dl, and the mean bilirubin for subjects from 60 (included) – 79 years old is 2.78 mg/dl. Therefore, we can see an association between sex and bilirubin and an association between age and bilirubin, too. And from the descriptive statistics in problem 1, we know that sex and age are all associated with the response, all-cause death. Female and younger people tend to have lower instantaneous risk of death.

According to the definition of confounder, a confounder must be associated with the predictor of interest in the sample, and it needs to be causally associated with the response and not in the causal pathway of interest. In this case, I think that both age and sex confounded the association between death and bilirubin. But if I can only choose one, I would choose age, since the difference of mean bilirubin is larger across age groups.

* 1. What evidence is present in the data that would make you think that either sex or age might have added precision to the analysis of the association between death and bilirubin? (In real life, we would ideally decide whether to adjust in our pre-specified SAP).

**Answer:**

**Methods:** I mainly look at the same descriptive statistics as part a, with the Kaplan-Meier estimates in problem 1. And I did proportional hazard regression on log transformed bilirubin (base of 1.1) with robust standard error for every stratum defined by sex and age, separately, to find that whether age or sex is an effect modifier.

**Inference:** We know that the association between response and predictor of interest differs in strata defined by effect modifier. From the results of the proportional hazard regression, we get the estimate of the hazard ratio for each 10% increase in bilirubin is 1.0462 for male and 1.115 for female. And according to the two-sided p value calculated based on Wald statistics, the result for male is not statistically significant, while the result for female is statistically significant. Similarly, I did proportional hazard regression within groups defined by age, and found that the estimate of hazard ratio for each 10% increase in bilirubin is 1.110 for 26 – 40 group, 1.112 for 40 – 60 group and 1.076 for 60 – 79 group. These estimates of hazard ratios are quite close to each other. And according to the two-sided p values, the results are all statistically significant. Based on these results, I would think that sex might have added precision to the analysis of the association between death and bilirubin.

* 1. Provide full inference regarding an association between death and bilirubin after adjustment for sex and age.

**Answer:**

**Method 1:** I adjust the analysis for sex and age by stratified the dataset by the two variables. That is I divided the whole dataset into six groups, which is male 26 – 40 group, male 40 – 60 group, male 60 – 79 group, female 26 – 40 group, female 40 – 60 group and female 60 – 79 group. To compare the distributions of the time to all-cause death across groups defined by serum bilirubin in each stratum, I did proportional hazard regression and modeled serum bilirubin as a continuous logarithmically transformed variable (base of 1.1). Based on Wald statistics and Huber-White sandwich estimator, we can get the estimate of the hazard ratio from the regression model with robust standard error, a confidence interval for the estimate and two-sided p-value. We can use these values to quantify the association between all-cause mortality and serum bilirubin in each stratum.

**Inference:** First, there are only 3 subjects in the male 26 – 40 group, the analysis in this group would not make any sense. I just ignored it.

For male 40 – 60 group, there are 18 subjects and the estimate of the hazard ratio for each 10% increase in serum bilirubin is 1.0937, with a 95% confidence interval (1.00427, 1.191), and the two-sided p value is 0.04. The result is statistical significant.

For male 60 – 79 group, there are 15 subjects and the result is not statistical significant, I just ignored it.

For female 26 – 40 group, there are 55 subjects and the estimate of the hazard ratio for each 10% increase in serum bilirubin is 1.121, with a 95% confidence interval (1.0396, 1.208), and the two-sided p value is 0.03. The result is statistical significant.

For female 40 – 60 group, there are 179 subjects and the estimate of the hazard ratio for each 10% increase in serum bilirubin is 1.125, with a 95% confidence interval (1.100, 1.150), and the two-sided p value is less than 0.001. The result is statistical significant.

For female 60 – 79 group, there are 42 subjects and the estimate of the hazard ratio for each 10% increase in serum bilirubin is 1.0922, with a 95% confidence interval (1.0408, 1.146), and the two-sided p value is less than 0.01. The result is statistical significant.

We can see that in each stratum with enough number of subjects, there is a trend that the hazard ratio gets larger with the increase of the serum bilirubin level.

**Method 2:** To compare the distributions of the time to all-cause death across groups defined by serum bilirubin in each stratum, we could just do a proportional hazard regression on serum bilirubin level modeled serum bilirubin as a continuous logarithmically transformed variable (base of 1.1), with age and sex. Based on Wald statistics and Huber-White sandwich estimator, we can get the estimates of the hazard ratios from the regression model with robust standard error, confidence intervals for the estimates and two-sided p-values. We can use these values to quantify the association between all-cause mortality and serum bilirubin with the adjustment for age and sex.

**Inference:** The dataset has 418 subjects in total, and they all have available serum bilirubin, but only 312 subjects with available sex. There are 161 subjects died during the follow-up time, which is 38.5% of the whole dataset. From the results of a proportional hazard regression on logarithmically transformed bilirubin level with adjustment of age and sex, we estimate that the instantaneous risk of death is a relative 10.80% higher (hazard ratio 1.1080) for each 10% higher serum bilirubin level for males with the same age. A 95% confidence interval for this estimate is from 1.0874 to 1.129. Based on the two-sided p value (< 0.001), this result is statistical significant. And the instantaneous risk of death is 6.60% lower (hazard ratio 0.934) for female than male with the same bilirubin level and age. A 95% confident interval for this estimate of hazard ratio is from 0.552 to 1.581, and according to the two-sided p value (0.799), this result is not statistical significant. For age, we estimate that the instantaneous risk of death is a relative 3.86% higher (hazard ratio 1.0386) for each 1 year older. Based on a 95% confidence interval (1.0183, 1.0593), the data would not be unusual if the true hazard ratio is in this interval. In conclusion, we would prefer that there is a trend for higher mortality with higher serum bilirubin with the adjustment of age and sex.

1. Note that in the above analyses, we completely ignored the intervention in the RCT? What impact could this have had on our results?

**Answer:**

If the intervention is associated with the predictor of interest (i.e. serum bilirubin level) and it is causally associated with mortality but not in the causal path of interest, the intervention is a confounder, and we need to adjust our analysis for it. If we don’t, we might not be able to answer our scientific question correctly.

If we consider the intervention in the RCT and there is difference between the associations between bilirubin and mortality for groups defined by intervention, the intervention can be considered as an effect modifier. If we do not adjust our analysis for it, we might get wrong answer, such as wrong trend.