BIOST 518

HW #4

1. Methods: The dataset used for this assignment comes from patients with primary biliary cirrhosis enrolled to randomized clinical trial (RCT) of D-penicillamine. We are interested on the association between death from any cause and age, gender and serum bilirubin. It is for note that this dataset comes from patients with different levels of hepatic function measured by bilirubin levels. And it is expected that bilirubin levels in mg/dl would be related to death rate in a multiplicative association; arbitrarily I use below 1, between 1 inclusive and 2, between 2 inclusive and 4, between 4 inclusive and 8, between 8 inclusive and 16 and finally between 16 inclusive and 28 inclusive.

Per each level of bilirubin categorized descriptives for continuous variables (mean, standard deviation, minimum and maximum) are computed and frequencies are used to gender, death status and trial arm. As time to death is a censored variable Kaplan-Meier estimates are used to obtain the 10th, 20th and 50th (median) percentile survival time and 2, 5 and 10 years survival probabilities per each level of bilirubin categorized.

Originally time to death or censoring was in days. It has been converted to years by dividing by 365.25.

Results: The dataset contains 418 records (refer to table 1). Gender was missing information in 106 (25.4%) records and treatment arm on 108 (25.8%) records. Bilirubin levels at enrollment in mg/dl varied between 0.3 and 28.0, had mean 3.22 and a standard deviation 4.408 showing a skewness to the right. Age at enrollment in years ranged between 26.3 and 78.4 years, had mean 50.7, standard deviation 10.45 and did not vary appreciably per levels of bilirubin. About two thirds (66.0%) of all sample were females and did not vary appreciable per bilirubin levels.

The 2, 5 and 10 years survival probability was respectively 88.0, 70.3 and 44.2% and important worse pattern when transitioning from lower to higher levels of bilirubin levels. Also the median survival time per increased levels of bilirubin shows similar pattern varying from above follow up time (13.13 years) on below 1mg/dl to 2.16 years in 8-16mg/dl group.

Figure 1 suggests association between male and survival probability and also association between increasing age and survival probability.

It is for note that the female were average 6.99 years old younger than males with means of 49.21 (SD=10.206) and 56.20 (SD = 11.487) years old respectively.

Table 1 – Descriptives per levels of bilirubine



q1_gender.tifq1_agecat.tif

Figure 1 – Survival by gender left and by 10 years age category (right).

2. They managed to ascertain the death outcome on all subjects only up to 1.46 years which is below 10th percentile (1.67years) of follow up time and only 36/161 deaths would have been counted. Up to this point of time a logistic regression would be used to study the association of predictors in interest with all mortality with first 1.46 years of follow up.

After that point we have censored information. Logistic regression can’t manage the censoring information. So the analysis would be invalid.

3.a) Methods: a Cox proportional-hazards regression with **robust standard errors** is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels. Bilirubin is modeled as continuous untransformed variable and the hazard ratio from the model is used to quantify the association and its 95% confidence interval and two-sided p-values computed from Wald statistics are also reported. The significance level is set to 5%.

Results: All 418 subjects in the dataset contributed to 161 events on total 2194.75 person-years. There is 15.2% relative higher (hazard ratio 1.152) instantaneous risk of death associated with each 1mg/dl higher serum bilirubin level at enrollment. The observed hazard ratio wouldn’t be unusual to lie between 1.121 and 1.185 and we reject the null hypothesis of being equal to 1 with p-value below 0.001.

3.b) Methods: a Cox proportional-hazards regression with robust standard errors is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels. Bilirubin is modeled as continuous variable centered at 1. Fitted hazard ratios are computed from the model for each record on the dataset.

Results: The fitted are presented on answer to question 6.

4.a) Although this dataset isn’t from non healthier subjects I still expected that the liver to excrete bilirubin using the same enzymatic process but with different uptake level according to gravity of liver dysfunction. The descriptive of mortality levels on question 1 and table 1 has suggest such multiplicative relationship.

4.b) Methods: a Cox proportional-hazards regression with robust standard errors is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels. Bilirubin is modeled as continuous logarithm base 2 transformed and the hazard ratio from the model is used to quantify the association and its 95% confidence interval and two-sided p-values computed from Wald statistics are also reported. The significance level is set to 5%.

Results: All 418 subjects in the dataset contributed to 161 events on total 2194.75 person-years. There is 98.4% relative higher (hazard ratio 1.984) instantaneous risk of death associated with each doubling of serum bilirubin level at enrollment. The observed hazard ratio wouldn’t be unusual to be found between 1.781 and 2.212 and we reject the null hypothesis of being equal to 1 with p-value below 0.001.

4.c) Methods: a Cox proportional-hazards regression with robust standard errors is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels. Bilirubin is modeled as continuous logarithm base 2 transformed. Fitted hazard ratios are computed from the model for each record on the dataset.

Results: The fitted results are presented on answer to question 6.

5.a) Methods: a Cox proportional-hazards regression with robust standard errors is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels. Both terms untransformed and logarithm of base 2 transformed bilirubin are included on the model simultaneously. The regression coefficients of the model, its 95% confidence interval and two-sided p-values computed from Wald statistics are reported. The specific Wald statistic of the logarithm of base 2 transformed bilirubin coefficient is used to test the null hypothesis of being 1 (in hazard ratio scale). The significance level is set to 5%.

Results: Table 2 shows the coefficients of regression and the global Wald-test p-value. The Wald-test for the transformed term is 54.89 corresponding to a p-value < 0.0001. Wherefore the null hypothesis of an hazard ratio of 1 for the transformed variable is rejected i.e. there is a non-linear relationship between the hazard ratio and bilirubin.

Table 2 – Cox proportion hazard regression of time to death from any cause with bilirubin untransformed and logarithm base 2 predictors

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Hazard Ratio** | **95% CI** | **p** |
| Total cases in the model | 418 |  |  |
|  |  |  |  |
| Bilirubin untransformed | 0.961 | 0.911 - 1.014 | 0.148 |
| Logarithm base 2 biliribin | 2.271 | 1.828 - 2.821 | < 0.001 |
|  |  |  |  |
| Wald test = 142.12 |  |  | p < 0.0001 |

5.b) Methods: the model fitted on 5a is used to generate a fitted hazard ratios variable that will be used for comparison on question 6.

6. Methods: fitted values computed on 3, 4 and 5 are displayed in scatter plot: x-axis representing bilirubin levels in mg/dl and relative hazard ratios on y-axis. Please note all logarithms are on base 2.

Results: Figure 2 shows the plot.

q6.tif

Figure 2 – Fitted values of relative hazard ratio regression with untransformed bilirubin, logarithm bilirubin and both untransformed bilirubin and logarithm bilirubin predictors.

There isn’t a large difference between the models including a logarithm term. These two regressions are highly similar below 15 mg/dl bilirubin serum levels. It is for note that above 15mg/dl we have sparse data. Linearity is found on the alone logarithm bilirubin predictor and slightly by the both untransformed and logarithm bilirubin.

Linearity is found by the alone logarithm bilirubin predictor and slightly by the both untransformed and logarithm bilirubin. The alone untransformed bilirubin hasn’t linearity with relative hazard ratio.

7.a) About age at enrollment. On question 1 we found that age is associated with mortality (figure 1) in this sample but not with bilirubin at enrollment (table 1). Age is precedent of death.

About gender. First ¼ of our subjects have no information on gender. Figure 1 is suggestive of some association between gender and mortality but table 1 doesn’t suggest association with bilirubin levels at enrollment on this sample. Gender is a characteristic that never changes.

By these facts I find no evidence of confounding.

7.b) About age at enrollment. On question 1 we found that age is associated with mortality (figure 1) in this sample but not with bilirubin at enrollment (table 1). Age is precedent of death.

About gender. First ¼ of our subjects have no information on gender. Figure 1 is suggestive of some association between gender and mortality but table 1 doesn’t suggest association with bilirubin levels at enrollment on this sample. Gender is a characteristic that never changes.

This suggests that they (age and gender) are precision variables.

7.c) Methods: a Cox proportional-hazards regression with robust standard errors is used to compare the distribution of time to any cause of death across groups defined by serum bilirubin levels at enrolment adjusted to gender and age at enrolment. Bilirubin is included after being transformed in logarithm of base 2. Hazard ratio from the model is used to quantify the association and its 95% confidence intervals and two-sided p-values computed from Wald statistic are also reported. The significance level is set to 5%.

Please note that given that ¼ of sample doesn’t contain gender information two models are fit. One with missing gender information and the other considering the missing values as a third. A model excluding these missing was fitted also fitted to compare the effect.

Results: Table 3 shows results of the regression including the missing values on gender and table 4 shows the same regression excluding the missing values on gender. The hazard ratios of the logarithm base 2 of serum bilirubin levels are quite similar despite the large standard error on the reduced dataset. Given the gender and age are hold constant a two-fold increase of serum bilirubin at enrollment was associated with a 2.12 times higher instantaneous risk of death and it wouldn’t be surprise to find this hazard ratio between 1.840 and 2.416. We reject the null hypothesis of null of instantaneous hazard ratio of 1 with p-vale < 0.001.

The adjusted hazard ratio (2.12) is slightly greater than the non adjusted (1.984) suggesting either confounding or precision effect of age and gender on mortality from any cause and bilirubin levels.

Table 3 – Cox proportional-hazards regression of time to death from any cause of death by serum bilirubin levels log-transformed adjusted for age and sex (including the missing values on gender).

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Hazard Ratio** | **95% CI** | **p** |
| Total cases in the model | 418 |  |  |
|  |  |  |  |
| Logarithm base 2 of serum bilirubin levels | 2.021 | 1.795 - 2.275 | < 0.001 |
|  |  |  |  |
| Gender |  |  |  |
| Male | 1.000 | - |  |
| Female | 0.973 | 0.572 - 1.657 | 0.920 |
| Missing | 0.913 | 0.497 - 1.676 | 0.769 |
|  |  |  |  |
| Age (years) | 1.045 | 1.026 - 1.063 | < 0.001 |
|  |  |  |  |
| Wald test = 141.53 |  |  | p < 0.0001 |

Table 4– Cox proportional-hazards regression of time to death from any cause of death by serum bilirubin levels log-transformed adjusted for age and sex (excluding the missing values).

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Hazard Ratio** | **95% CI** | **p** |
| Total cases in the model | 312 |  |  |
|  |  |  |  |
| Logarithm base 2 of serum bilirubin levels | 2.108 | 1.840 - 2.416 | < 0.001 |
|  |  |  |  |
| Gender |  |  |  |
| Male | 1.000 | - |  |
| Female | 0.934 | 0.552 - 1.581 | 0.799 |
|  |  |  |  |
| Age (years) | 1.039 | 1.018 - 1.059 | < 0.001 |
|  |  |  |  |
| Wald test = 120.69 |  |  | p < 0.0001 |

8. Ignoring the treatment arm depends upon the fact of the D-penicilamine whether effectively reduce mortality or not. Without effect we could ignore it. But if there is some effect of D-penicilamine we would miss a precision variable for the association between enrollment bilirubin levels and mortality from all causes.

It is for note that including the treatment variable we would have to reduce our sample ¼ of records.

Appendix

Stata code

clear

set more off

/\*

infile age albumin alkphos ascites bili cholest edema edmadj hepmeg ///

obstime platelet protime sex ///

sgot spiders stage status treatmnt triglyc urinecu ///

using liver.txt

drop in 1

\*/

lab var bili "Serum bilirubin (mg/dl)"

lab define sex 1 F 0 M

lab value sex sex

lab define tto 1 Drug 2 Placebo

lab value treatmnt tto

// Question 1

// death, age, sex, serum bilirubin, D-penicillamine treatment (treatmnt)

misstable pattern age sex treatmnt bili obstime

misstable pattern age sex bili obstime

stset obstime, failure(status) scale(30.4375)

stset obstime, failure(status) scale(365.25)

egen bilicat = cut(bili), at(0 1 2 4 8 16 32) icodes label

ctabstat age, stat(mean sd min max n) by(bilicat) ///

col(stat) long format(%5.3f)

tab sex bilicat, miss col

tab status bilicat, miss col

tab treatmnt bilicat, miss col

sum bili

sts list, at(2 5 10) by(bilicat)

sts list, at(2 5 10)

stci , p(10) by(bilicat)

stci , p(20) by(bilicat)

stci , p(50) by(bilicat)

// Gender

recode sex (1 = 1) (2 = 2) (miss = 3), gen(gender)

lab define sex 3 "Miss gender", add

lab value gender sex

sts graph, by(gender) ///

/// risktable(, order(1 "Male" 2 "Female" 3 "Miss gender") righttitles) ///

xtitle(Time after enrolment (years)) ///

ytitle(Survival probability) ylabel(0(0.1)1, angle(horizontal) format(%2.1f)) ///

xlabel(0 (1) 13) ///

legend(order(1 "Male" 2 "Female" 3 "Miss gender") rows(1))

graph export q1\_gender.tif, replace

egen agecat = cut(age), at(10 (10) 80) icodes label

sts graph, by(agecat) ///

/// risktable(, order(1 "Male" 2 "Female" 3 "Miss gender") righttitles) ///

xtitle(Time after enrolment (years)) ///

ytitle(Survival probability) ylabel(0(0.1)1, angle(horizontal) format(%2.1f)) ///

xlabel(0 (1) 13) ///

legend(order(1 "[20 - 30[" 2 "[30 - 40[" 3 "[40 - 50[" ///

4 "[50 - 60[" 5 "[60 - 70[" 6 "[70 - 80["))

graph export q1\_agecat.tif, replace

// Question 3 - a

stset obstime, failure(status) scale(365.25)

stcox bili, robust

lincom 10\*bili, eform // This is equivalent to bill\_beta power 10

// Question 3 - b

gen cbili = bili - 1

stcox cbili, robust

predict fithrA

// Question 4 - b

stset obstime, failure(status) scale(365.25)

gen log2bili = log(bili)/log(2)

gen logbili = log(bili)

stcox log2bili, robust

predict fithrB

/\* OR

// b1\*log(bili) = b1\*log(2)\*logbase2(bili)

di log(2)

stcox logbili, robust

lincom .69314718\*logbili, eform

\*/

// Question 5

gen bilisq = bili^2

gen logbili = log(bili)

stcox bili, robust nolog

stcox bili log2bili, robust nolog

testparm log2bili

predict fithrC

// Question 6

stcox bili c.bili#c.bili, robust nolog

predict fithrD

scatter fithrA fithrB fithrC fithrD bili, ///

xtitle(Serum bilirubin levels (mg/dl)) ///

ytitle(Relative Hazard) ///

ylabel(0(5)50, angle(horizontal) format(%2.0f)) ///

yline(0(5)50, lcolor(ltblue)) ///

xlabel(0 (5)30) ///

xline(0(5)50, lcolor(ltblue)) ///

legend(order(1 "Untransformed bilirubin" ///

2 "Logarithm transformed bilirubin" ///

3 "Both Untransformed and Logarithm transformed bilirubin" ///

4 "Both Untransformed and squared transformed bilirubin") cols(1))

scatter fithrA fithrB fithrC bili, ///

xtitle(Serum bilirubin levels (mg/dl)) ///

ytitle(Relative Hazard) ///

ylabel(0(5)50, angle(horizontal) format(%2.0f)) ///

yline(0(5)50, lcolor(ltblue)) ///

xlabel(0 (5)30) ///

xline(0(5)50, lcolor(ltblue)) ///

legend(order(1 "Untransformed bilirubin" ///

2 "Logarithm transformed bilirubin" ///

3 "Both Untransformed and Logarithm transformed bilirubin") cols(1))

graph export q6.tif, replace

// Question 7

stcox log2bili i.gender age, robust nolog

stcox log2bili i.sex age, robust nolog