Biostat 518

HW 04

1. **Methods:** Of 418 patients, 106 were missing values for sex, presence of spider angiomata, presence of hepatomegaly, and stage of disease but were included in analyses that did not involve those variables. 108 patients were missing data on assignment to treatment with D-penicillamine or placebo, but likewise will be included in analyses not based on that variable. Descriptive statistics were stratified based on serum bilirubin measurements in mg/dL taken at study enrollment. Stage of disease, presence of spider angiomata, and indication of hepatomegaly were included to help visualize any association between bilirubin levels and disease severity. Fifty-nine point five percent of patients fell within the normal range of serum bilirubin levels (.3-1.9mg/dL). Other strata were based on consecutive doublings of the upper limit of that range.

**Inference:** Bilirubin levels in our sample ranged from .3to 28mg/dL, with a mean of 3.22(4.41). Average age was roughly equal across groups defined by bilirubin level, though the lower end of the age range increased with bilirubin levels. Sex was very unevenly distributed across groups, and could potentially lead to confounding. Stage of disease and hepatomegaly both increased with serum bilirubin, and spider angiomata also increased across groups with the exception of a drop in the 3.8-7.6 bilirubin range. Time to observation of censored event also varied across groups, with shorter times in the upper ranges. This, combined with the observed increases in other symptoms, makes a strong case for elevated serum bilirubin levels indicating disease severity, and in turn may help us estimate time to mortality for patients suffering from primary biliary cirrhosis (PBC). The percentage of patients enrolled in treatment with d-Penicillamine was roughly equivalent for the three lowest groups, but dropped significantly for the groups with 4 times normal bilirubin levels and above.

From Figure 1 below, we see that the p50 survival time for the sample is 9.3 years, and that the overall mortality rate appears to have been fairly constant over the time-period. When groups are categorized by serum bilirubin levels (Figure 2), we see decreased survival with increased bilirubin, though beyond 7.6mg/dL it might not matter as much: mortality doesn’t appear much worse for the group with levels above 15.2 when compared to the 7.6-15.2 group. Over half of the sample in the group with bilirubin levels below 1.9 were still alive by the end of the study period, but P50 for groups in order of the rest of the groups from lowest to highest bilirubin levels were: 6.27 years, 3.70 years, 2.16 years, 2.34 years. More analyses will be needed to determine if this is a statistically significant trend, but the rise in symptoms of disease, coupled with this trend in decreasing mortality certainly lends credence to the hypothesis that serum bilirubin measurements can be used to predict mortality in PBC patients.

**Table 1:** Descriptive statistics for patients stratified by baseline level of serum bilirubin in mg/dL. Hard brace(]) indicates inclusive.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline serum bilirubin mg/dL (n)** | | | | | |
|  | <=1.9 (249) | (1.9-3.8](76) | (3.8-7.6] (50) | (7.6-15.2](27) | >15.2 (16) | Total (418) |
| **Age\*(yrs)** | 50.92(10.22) 26.28-76.7 | 50.08(11.41) 29.56-71.89 | 51.00(11.53)  30.86-78.44 | 50.75(10.14) 33.18-70.56 | 50.23(6.37) 41.95-65.88 | 50.74(10.45)  26.28-78.44 |
| **Sex(% male)** | 8.81 | 18.03 | 20.04 | 5.95 | 06 | 11.57 |
| **Stage of disease\*** | 2.82(.90)  1-41 | 3.20(.75)  2-43 | 3.38(.77)  1-44 | 3.65(.61)  2-45 | 3.38(.77)  2-46 | 3.03(.88)  1-47 |
| **Spider angiomata(%)** | 16.01 | 48.53 | 354 | 47.15 | 76.96 | 28.87 |
| **Hepatomegaly (%)** | 36.51 | 62.33 | 754 | 88.25 | 84.66 | 51.37 |
| **Time to censoring event\*(yrs)** | 6.30(2.85) .52-13.14 | 4.50(2.73) .26-12.2 | 3.58(2.40)  .11-10.52 | 2.59(2.15) .12-8.65 | 2.21(1.62)  .11-6.21 | 5.25(3.03)  .11-13.14 |
| **Treatment(%)** | 51.42 | 52.43 | 57.54 | 35.35 | 30.86 | 50.68 |

\* clinically normal level of bilirubin is between .3 and 1.9 mg/dL (Wikipedia, accessed February 6, 2015 from: <http://en.wikipedia.org/wiki/Bilirubin>)

1Based on 181 observations (68 patients missing data)

2Based on 179 observations (70 patients missing data)

3Based on 61 observations (15 missing)

4Based on 40 observations (10 missing)

5Based on 17 observations (10 missing)

6Based on 13 observations (3 missing)

7Based on 312 observations (106 missing)

8Based on 310 observations (108 missing)

**Figure 1:** Kaplan-Meier survival curve for all patients.



**Figure 2:** Kaplan-Meier survival curves stratified by serum bilirubin mg/dL as measured at baseline. Groups are inclusive on the upper bound of bilirubin levels.



2. This dataset involves right-censored data with variable observation times, which cannot be analyzed with logistic regression without making unnecessary assumptions and adjustments. When analyzing mortality with logistic regression, it is best to pick one follow-up time and analyze mortality as a Bernoulli variable, but then we wouldn’t be using all of the data that’s available to us. It would be better to use proportional hazards regression, which was designed specifically to deal with this sort of analysis.

3. a. **Methods**: A Cox proportional hazards regression with a Breslow method for ties was run on 418 subjects, with 161 observed deaths and robust SE. Wald-based estimates were used for a 95% CI and two-sided P-value.

**Inference:** We estimate that for each 1 mg/dL increase in serum bilirubin, the risk of death is increased 15.2%. However, this is a statistically significant finding (p<.001) and a 95% CI estimates that the true risk for a group with 1 more mg/dL of bilirubin lies between 12.1% and 18.5% higher than a group with lower bilirubin.

b. **Methods:** Our estimated hazard ratio obtained with the above analyses was used to calculate the average hazard ratio for groups stratified by serum bilirubin mg/dL levels relative to a group with a bilirubin level of 1mg/dL.

**Inference:** As a bilirubin level of 1mg/dL falls within the healthy range of bilirubin levels, it is unsurprising that the estimated hazard ratio for our group of subjects with healthy levels of serum bilirubin (<=1.9mg/dL) is very nearly 1 when compared to this group. The Hazard ratio generally increases with serum bilirubin, indicating a higher instantaneous risk of death with higher levels of bilirubin.

**Table 2:** Mean hazard ratio estimates for groups stratified by serum bilirubin compared to a group with 1 mg/dL of serum bilirubin.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline serum bilirubin mg/dL (n)** | | | | | |
|  | <=1.9 (249) | (1.9-3.8](76) | (3.8-7.6] (50) | (7.6-15.2](27) | >15.2 (16) | Total (418) |
| **Mean hazard ratio (mean (SD))** | .999(.011) | 1.05(.016) | 1.13(.033) | 1.33(.087) | 1.67(.180) | 1.07(.153) |

4. a. Many biological factors are best described with a multiplicative model. From our descriptive statistics, we know we have a heavily skewed distribution for serum bilirubin with a long right tail, with a range from .3 to 28 mg/dL. With such a broad range, and with healthy values extending only to 1.9mg/dL, it would seem more appropriate to examine doublings of bilirubin levels rather than absolute differences.

b. **Methods:** Serum bilirubin levels were transformed with a log base 2. A Cox proportional hazards regression with a Breslow method for ties was run on 418 subjects, with 161 observed deaths and robust SE. Wald-based estimates were used to generate a 95% CI and two-sided P-value.

**Inference:**  We estimate that for each doubling in serum bilirubin, the risk of death is increased 98.4%. This is a statistically significant finding (p<.001) and a 95% CI estimates that the true risk for a group with twice the mg/dL of bilirubin lies between 1.781 and 2.212 times higher than a group with lower bilirubin.

**c.** **Methods:** Our estimated hazard ratio obtained with the above analyses was used to calculate the average hazard ratio for groups stratified by serum bilirubin mg/dL levels relative to a group with a bilirubin level of 1mg/dL.

**Inference:** As a bilirubin level of 1mg/dL falls within the healthy range of bilirubin levels, it is unsurprising that the estimated hazard ratio for our group of subjects with healthy levels of serum bilirubin (<=1.9mg/dL) is very nearly 1 when compared to this group (Table 3). The Hazard ratio generally increases with serum bilirubin, indicating a higher instantaneous risk of death with higher levels of bilirubin.

**Table 3:** Mean hazard ratio estimates for groups stratified by serum bilirubin compared to a group with 1 mg/dL of serum bilirubin.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline serum bilirubin mg/dL (n)** | | | | | |
|  | <=1.9 (249) | (1.9-3.8](76) | (3.8-7.6] (50) | (7.6-15.2](27) | >15.2 (16) | Total (418) |
| **Mean hazard ratio (mean (SD))** | .994(.018) | 1.04(.009) | 1.08(.009) | 1.11(.010) | 1.14(.009) | .95(.046) |

**5.** a. **Methods:** A Cox proportional hazards regression with a Breslow method for ties was run on 418 subjects, with 161 observed deaths and robust SE. Serum biliribun and log-base 2 transformed bilirubin were both used as predictors. Wald-based estimates were used to generate a 95% CI and two-sided P-value.

**Inference:** The linear model did not differ significantly from 0, (p<.148) but our log-transformed model did: p<.001. This indicates that the serum bilirubin does not follow a simple linear model. Perhaps our log-transformation was a good choice.

b. **Methods:** Our estimated hazard ratios obtained with the above analyses were used to calculate the average hazard ratio for groups stratified by serum bilirubin mg/dL levels relative to a group with a bilirubin level of 1mg/dL.

**Inference:** As a bilirubin level of 1mg/dL falls within the healthy range of bilirubin levels, it is unsurprising that the estimated hazard ratio for our group of subjects with healthy levels of serum bilirubin (<=1.9mg/dL) is very nearly 1 when compared to this group (Table 4). The Hazard ratio generally increases with serum bilirubin, indicating a higher instantaneous risk of death with higher levels of bilirubin. Our log-transformed model on the other hand goes the other way, and groups with higher levels of bilirubin have lower risk…

**Table 4:** Mean hazard ratio estimates for groups stratified by serum bilirubin compared to a group with 1 mg/dL of serum bilirubin.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline serum bilirubin mg/dL (n)** | | | | | |
|  | <=1.9 (249) | (1.9-3.8](76) | (3.8-7.6] (50) | (7.6-15.2](27) | >15.2 (16) | Total (418) |
| **Mean hazard ratio with linear model(mean (SD))** | .998(.020) | 1.091(.030) | 1.255(.067) | 1.67(.194) | 2.519(.505) | 1.147(.343) |
| **Mean hazard ratio with log-transformed model(mean(SD))** | 1.012(.034) | .923(.015) | .872(.014) | .825(.014) | .789(.011) | .958(.077) |

**6.** **Figure 3:** Comparison of fitted models for estimated hazard ratio as predicted by serum bilirubin (mg/dL). Analyses were performed as described in problems 3-5. 

The log-transformed model is a straight line with a slope of 0, while the other models, though largely linear have a slight curve and definitely have non-zero slopes.

**7.** a. From our descriptive statistics, we know that age and sex were not particularly well distributed across groups defined by bilirubin levels, so if either factor influences bilirubin levels independent of disease, they could cause confounding, since it is well known that age and sex are both associated with mortality.

**B.** If variability in age or sex lead to consistently higher or lower measurements of bilirubin for people of a certain age or sex, than we would have increased variability within groups, that might limit our ability to detect across-group differences. Again, because age and sex were not very evenly distributed across groups stratified by bilirubin, there could be an association.

**c.** **Methods:** A Cox proportional hazards regression with a Breslow method for ties was run on 418 subjects, with 161 observed deaths and robust SE. Age and sex were adjusted for in our model. Wald-based estimates were used to generate a 95% CI and two-sided P-value.

**Inference:** We estimate a mean difference of instantaneous risk of death of 16% for groups differing by 1 mg/dL of bilirubin where the group with higher bilirubin would be at higher risk, adjusting for effects by sex and age. This finding is statistically significant with p<.001. A 95% CI predicts it would not be unusual if the true difference in mortality between groups differing by 1 mg/dL in bilirubin were between 12.5% and 19.9%. There was also a statistically significant effect of age on mortality, with an estimated increase in risk of 3.8% for groups 1 year older (p<.001, CI: 1.93%-5.79%). There was no statistically significant effect on mortality due to sex (p=.085).

**8.** If we assume the treatment had a significantly positive effect on survival time, I believe adjusting for it would have added precision to our estimates. As it was, we probably had more variability in our data because we did not analyze those on placebo and those on d-penicillamine separately. Also, because there was a higher proportion of subjects on treatment in the three groups with lowest serum bilirubin levels, improved survival time in those groups over the groups with higher levels may have been due in part to treatment, rather than initially low bilirubin levels. This would have been confounding our inference.