Biost 517: Applied Biostatistics I
Emerson, Fall 2005

Homework #6
November 28, 2005

Written problems: To be handed in at the beginning of class on Monday, November 21, 2005.

> *On this (as all homeworks) unedited Stata 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.)*

The written problems all refer to the DFMO data set as stored on the class web pages. My guess is that you will find this problem easiest to do using the "wide" format for the data, but it does not make too much of a difference either way.

In this homework, you will perform several alternative analyses to assess whether DFMO has an effect on spermidine levels in the colon mucosa. In this homework (as opposed to homework #5), you should perform the two sample comparisons. In all problems, provide as complete statistical inference as possible (i.e., provide point estimates, confidence intervals, and p values where possible, along with a statement of your scientific/statististical conclusions).

Note: I wrote each answer to "stand on its own". That is, in a report of a clinical trial, I would want to give some idea of the range of measurements in order to allow readers to assess possibly toxic actions of the drug. (I note that after years of analyzing data from clinical trials of DFMO, I still do not have an idea about a dangerously low or high level of spermidine in the colonic mucosa. Nevertheless, it is still a good idea to provide information on the absolute levels and perhaps the change.)

In the answers that follow, I urge you to consider the following points. In making these points, I am considering the precision with which we were able to reject the null hypothesis by looking at how low the P value was. This is not totally fair, because few of the analyses were testing the same things. But it is illustrative to consider the general properties of the various choices for analysis.

- *We generally had the greatest precision to distinguish the means (rather than geometric means or medians) of the measurements made at 12 months (rather than considering the change from baseline). There was a slight loss of precision with the geometric means (the data was not really all that skewed, and it is in the presence of positively skewed data that the geometric mean will tend to be more precisely estimated). Sample medians tend to be imprecisely measured, and the mean will do better unless there is a similarly high propensity to outliers ("heavy tails") in both groups being compared.*

- *The Wilcoxon test would tend to have decent power in this setting, but the lack of a correspondence to a scientifically meaningful estimate of treatment effect is a major drawback to me.*

- *In a clinical trial, looking at the difference between mean measurements made at the end of the clinical trial and looking at the difference between mean change in measurements*

(final minus initial) are estimating the same treatment effect, because the dose groups are equivalent at baseline due to randomization. Hence it is of great interest to compare the two approaches to analysis.

- *Note that when looking at the change in spermidine levels, we actually had a bit less precision than when just looking at the 12 month measurement. This is often quite surprising to many nonstatisticians, but it can be mathematically proven. You can actually lose precision in a clinical trial by analyzing the difference between the follow-up and baseline measurements.The exact behavior depends on the correlation between measurements made at baseline and follow-up. In fact, if the correlation between the initial and final measurements within dose groups is less than 0.5 in a randomized clinical trial, you do better to completely ignore the baseline measurements rather than analyze the difference. In this study, the estimated correlation between initial and final measurements was 0.39 in the placebo group and -0.16 in the high dose group, and the loss of precision we observed was consistent with that. I note that the best way to adjust for baseline values is to model them as a covariate using multiple regression—something we will address in Biost 518.*

- *Note that we did have less precision when we dichotomized the change in spermidine levels (decrease vs not), as compared to the analysis based on the means. This is not too surprising—we are losing some information when we dichotomize the data. However, it is sometimes the case that we are more interested scientifically in the proportion of patients having a decrease than in the average decrease (a large decrease in one person might not be clinically relevant.*

1. Perform an analysis to assess whether the mean spermidine level was different between the dose 0.4 group and the placebo group after 12 months of treatment.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, spermidine levels at 12 months were observed to range between 1.01 and 5.91 μ mol/mg protein, and averaged 3.26 μ mol/mg protein. The 95% CI for the true mean spermidine level in a population receiving placebo is 2.75 to 3.77 μ mol/mg protein. In the group randomized to receive 0.4 g / m^2 / day of DFMO, spermidine levels after 12 months on study ranged from 2.46 to 3.42 μ mol/mg protein and averaged 1.95 μ mol/mg protein. The 95% CI for the true mean spermidine level in a population receiving the highest dose of DFMO is 1.58 to 2.32 μ mol/mg protein. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with an average spermidine level that is 1.31 μ mol/mg protein lower than what it would be in the absence of treatment with DFMO (95% CI 0.69 to 1.92 μ mol/mg protein lower). These results are highly statistically significant (two-sided P= 0.0001), and thus these data are not consistent with results that might be observed by random chance in the absence of a treatment effect.

2. Perform an analysis to assess whether the geometric mean spermidine level was different between the dose 0.4 group and the placebo group after 12 months of treatment.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, spermidine

levels at 12 months were observed to range between 1.01 and 5.91 μ mol/mg protein, with a geometric mean of 3.01 μ mol/mg protein. The 95% CI for the true geometric mean spermidine level in a population receiving placebo is 2.56 to 3.53 μ mol/mg protein. In the group randomized to receive 0.4 g / m^2 / day of DFMO, spermidine levels after 12 months on study ranged from 2.46 to 3.42 μ mol/mg protein, with a geometric mean of 1.71 μ mol/mg protein. The 95% CI for the true geometric mean spermidine level in a population receiving the highest dose of DFMO is 1.25 to 2.34 μ mol/mg protein. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with a 43.1% lower geometric mean spermidine level relative to what it would be in the absence of treatment with DFMO (95% CI 19.2% to 59.6 % lower). These results are statistically significant (two-sided P= 0.0022), and thus these data are not consistent with results that might be observed by random chance in the absence of a treatment effect.

3. Perform an analysis to assess whether the median spermidine level was different between the dose 0.4 group and the placebo group after 12 months of treatment. (Use bootstrapped estimates of the standard errors for each group, along with the methods for combining estimates that are approximately normally distributed.)

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, spermidine levels at 12 months were observed to range between 1.01 and 5.91 μ mol/mg protein, with a median of 2.82 μ mol/mg protein. The 95% CI for the true median spermidine level in a population receiving placebo is 2.24 to 3.39 μ mol/mg protein. In the group randomized to receive 0.4 g / m^2 / day of DFMO, spermidine levels after 12 months on study ranged from 2.46 to 3.42 μ mol/mg protein with a median of 1.93 μ mol/mg protein. The 95% CI for the true median spermidine level in a population receiving the highest dose of DFMO is 1.60 to 2.26 μ mol/mg protein. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with a median spermidine level that is 0.89 μ mol/mg protein lower than what it would be in the absence of treatment with DFMO (95% CI 0.23 to 1.55 μ mol/mg protein lower). These results are statistically significant (two-sided P= 0.0085), and thus these data are not consistent with results that might be observed by random chance in the absence of a treatment effect.

4. Perform an analysis to assess whether the probability was 0.5 that a randomly chosen subject from the dose 0.4 group had a lower spermidine level at 12 months than a randomly chosen subject from the placebo group.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, spermidine levels at 12 months were observed to range between 1.01 and 5.91 μ mol/mg protein, and in the group randomized to receive 0.4 g / m^2 / day of DFMO, spermidine levels after 12 months on study ranged from 2.46 to 3.42 μ mol/mg protein. Based on the Wilcoxon rank sum test, we reject the null hypothesis that a spermidine measurement in the high dose group was equally likely to be higher or lower than a measurement made in the placebo group (two-sided P= 0.0002).

(Note that the Wilcoxon test is not easily related to any particular point estimate of treatment effect. In fact, because the Wilcoxon test considers the "strong null" (exact equality of

distributions), I cannot precisely state the confidence with which I might conclude that the probability is greater than 0.5 that a high dose subject would have a lower spermidine level than a placebo patient.)

5. Perform an analysis to assess whether the mean change in spermidine levels was different between the dose 0.4 group and the placebo group after 12 months of treatment.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, the change in spermidine levels over 12 months were observed to range between a decrease of 3.20 and an increase of 2.53 μ mol/mg protein, with an average decrease of 0.04 μ mol/mg protein. The 95% CI for the true mean change in spermidine levels in a population receiving placebo is from a decrease of 0.64 to an increase of 0.55 μ mol/mg protein. In the group randomized to receive 0.4 g / m^2 / day of DFMO, the change in spermidine levels after 12 months on study ranged from a decrease of 5.48 and an increase of 1.46 μ mol/mg protein, with an average decrease of 1.76 μ mol/mg protein. The 95% CI for the true mean change in spermidine levels in a population receiving the highest dose of DFMO is from a decrease of 0.74 to a decrease of 2.77 μ mol/mg protein. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with an average decrease in spermidine level that is 1.71 μ mol/mg protein more than any change in the absence of treatment with DFMO (95% CI 0.56 to 2.87 μ mol/mg protein lower). These results are statistically significant (two-sided P= 0.0048), and thus these data are not consistent with results that might be observed by random chance in the absence of a treatment effect.

6. Perform an analysis to assess whether the change in geometric mean spermidine level over 12 months of treatment was different between the dose 0.4 group and the placebo group.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, the spermidine levels at 12 months were observed to range between 56% lower to 89% higher than levels at the time of randomization, with the geometric mean at 12 months estimated to be 0.17% higher than that at randomization. The 95% CI for the true proportionate change in geometric mean levels at 12 months in the placebo group is from 16.2% lower to 19.7% higher than the geometric mean at randomization. In the group randomized to receive 0.4 g / m^2 / day of DFMO, the spermidine levels at 12 months were observed to range from complete suppression (i.e., spermidine levels below the limit of detectability) to levels that were 2.88 times the measurements obtained at randomization, with the geometric mean at 12 months estimated to be 46.8% lower than that at randomization. The 95% CI for the true proportionate change in geometric mean levels at 12 months in the highest dose group is from 4.0% lower to 65.8% lower than the geometric mean at the time of randomization. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with an proportionate decrease in geometric mean spermidine levels that is 46.9% lower than any proportionate decrease in geometric means in a population treated with placebo (95% CI 15.0% lower to 66.8% lower). These results are statistically significant (two-sided P= 0.0104), and thus these data are not consistent with results that might be observed by random chance in the absence of a treatment effect.

7. Perform an analysis to assess whether the median change in spermidine levels was different between the dose 0.4 group and the placebo group after 12 months of treatment.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, the change in spermidine levels over 12 months were observed to range between a decrease of 3.20 and an increase of 2.53 μ mol/mg protein, with a median increase of 0.34 μ mol/mg protein. The 95% CI for the true median change in spermidine levels in a population receiving placebo is from a decrease of 0.66 to an increase of 1.34 μ mol/mg protein. In the group randomized to receive 0.4 g / m^2 / day of DFMO, the change in spermidine levels after 12 months on study ranged from a decrease of 5.48 and an increase of 1.46 μ mol/mg protein, with a median decrease of 1.08 μ mol/mg protein. The 95% CI for the true median change in spermidine levels in a population receiving the highest dose of DFMO is from a decrease of 2.55 to an increase of 0.40 μ mol/mg protein. Based on these data, we thus estimate that prescription of a dose of 0.4 g / m^2 / day of DFMO is associated with a median decrease in spermidine level that is 1.41 μ mol/mg protein more than the median change in the absence of treatment with DFMO (95% CI 3.20 μ mol/mg protein lower to 0.37 μ mol/mg protein higher). These results are not statistically significant (two-sided P= 0.12), and thus we are not able to state that these observations are different from what might reasonably be observed when the median change in spermidine levels did not differ between the high dose and placebo groups.

8. Perform an analysis to assess whether the probability was 0.5 that a randomly chosen subject from the dose 0.4 group had a greater change in spermidine level at 12 months than a randomly chosen subject from the placebo group.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the 32 subjects in the group randomized to receive placebo. In the placebo group, the change in spermidine levels over 12 months were observed to range between a decrease of 3.20 and an increase of 2.53 μ mol/mg protein, and in the group randomized to receive 0.4 g / m^2 / day of DFMO, the change in spermidine levels after 12 months on study ranged from a decrease of 5.48 and an increase of 1.46 μ mol/mg protein. Based on the Wilcoxon rank sum test, we reject the null hypothesis that the change in spermidine measurement in the high dose group was equally likely to be higher or lower than the change in measurement made in the placebo group (two-sided P= 0.0095).

(Note again that the Wilcoxon test is not easily related to any particular point estimate of treatment effect. In fact, because the Wilcoxon test considers the "strong null" (exact equality of distributions), I cannot precisely state the confidence with which I might conclude that the probability is greater than 0.5 that a high dose subject would have a greater decrease in spermidine level than a placebo patient.)

9. Perform an analysis to assess whether the proportion of subjects having a decrease in spermidine levels after 12 months of treatment differed between the dose 0.4 group and the placebo group.

Ans: After 12 months of treatment, colon mucosal spermidine levels were available on 20 of the 28 subjects in the group randomized to receive 0.4 g / m^2 / day of DFMO and 28 of the

32 subjects in the group randomized to receive placebo. In the placebo group, the change in spermidine levels over 12 months were observed to range between a decrease of 3.20 and an increase of 2.53 μ mol/mg protein, with 13 out of 28 (46.4%) exhibiting a decrease in spermidine levels. The 95% CI for the true proportion expected to have decreased spermidine levels in a population receiving placebo is from 26.7% to 66.1%. In the group randomized to receive 0.4 g / m^2 / day of DFMO, the change in spermidine levels after 12 months on study ranged from a decrease of 5.48 and an increase of 1.46 μ mol/mg protein, with 16 out of 20 (80.0%) exhibiting a decrease in spermidine levels. The 95% CI for the true proportion expected to have decreased spermidine levels in a population receiving the highest dose is from 60.8% to 99.2%. Based on these data, we thus estimate that the absolute difference in the proportion of individuals expected to experience a decrease in spermidine levels on the highest dose compared to placebo is 33.5% (95% CI 8.1% to 59.0%), with a higher proportion of participants having a decrease when taking DFMO. These results are statistically significant (two-sided P= 0.019), and thus we are able to state that these observations are different from what might reasonably be observed when similar proportions of participants would have decreases in spermidine levels in both the high dose and placebo groups.

(Note that difference in proportions was statistically significant, even though the two confidence intervals for the dose groups overlapped.)