FINAL PROJECT ASSIGNMENT:

Methotrexate and PBC

Group 6

BIOST 517

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Instructor: Emerson

Summary

The goal of this analysis was to asses if primary biliary cirrhosis (PBC) patients being treated with methotrexate in a randomized clinical trial would experience lung toxicity. Lung toxicity was assessed by comparing the difference between diffusing capacity of the lung for carbon monoxide (DLCO) measurements at baseline and DLCO measurements at the end of the treatment period between the placebo and methotrexate groups. The observed mean of these differences was 1.51 (ml/min/mmHg) for the placebo group and 1.59 (ml/min/mmHg) for the methotrexate group, indicating a difference of only 0.082 (ml/min/mmHg) between the two groups. This difference was not statistically significant (P=0.88) and the 95% confidence interval indicated that the observed data would not be unusual if the true value for the treatment group was between 1.12 (ml/min/mmHg) higher and 0.95 (ml/min/mmHg) lower than the placebo group. Thus, these data do not provide evidence that methotrexate impairs lung function in PBC patients.

Comment: A very nice report. Your summary was lacking some important information, but the rest of your paper had it very nicely laid out.

Comment: assess?

Comment: tell us much more about the RCT. How many patients treated for how long? How often monitored?

Background

Primary biliary cirrhosis is a rare liver disease that arises as a result of scarring of normal liver tissue. The name of the disease derives from the observation that obstructed intrahepatic bile ducts inhibit the normal bile excretory function of the liver, with the subsequent damage to normal liver cells eventually resulting in liver failure. In addition to adversely affecting normal liver function, PBC can affect other organs. PBC affects women more than men (~90% of patients are female), and, in total, affects approximately 200 out of every million people. Onset of the disease is typically in midlife (usually between age 35 and 60), with a typical disease course of five to ten years after diagnosis. Currently there is no known cure for PBC, however changes to diet (decrease in alcohol intake), and use of medications (e.g. Ursodiol and Cholestyramine) have been shown to assuage side effects of the disease (e.g. cholestasis and itching). A successful liver transplant is generally associated with recovery, however the lack of supply and risk of rejection of donor livers make other forms of treatment preferable[1,2].

The lack of current treatments for PBC has motivated the current study, in which the effects of methotrexate on prolonging the survival or progression free survival of patients diagnosed with PBC were investigated in a randomized, double blind, placebo controlled clinical trial. Methotrexate treatment has previously been used to treat other diseases, and toxic side effects resulting from methotrexate treatment have been identified. One such side effect is the impairment of normal lung function. In order to assess whether patients treated with methotrexate have impaired lung function, data were collected on lung diffusion capacity by measuring DLCO. DLCO is an estimate of the ability of lungs to transfer gases between alveoli and the bloodstream, with lower measurements corresponding to worse lung function.

Questions of Interest

The question posed for this analysis is: “Does treatment with the study drug lead to impaired lung function as measured by DLCO?” Although the study design (randomized controlled trial) allows us to look for the causality, the fact that patients in both the treatment and placebo arms are PBC patients should not be missed. The

appropriate question is thus "Does treatment with the study drug lead to impaired lung function as measured by DLCO in PBC patients?" and this is the question that we tried to address.

Source of the Data

Data were obtained from 12 geographically diverse clinical centers in the U.S.A. A total of 535 patients were screened for possible entry into the study, with 265 patients meeting the enrollment criteria. These patients were randomized in a double blind fashion to receive UDCA plus methotrexate or UDCA plus placebo. The randomization was stratified by the histological stage of liver disease as determined by pathologists. The resultant study consisted of 132 patients given methotrexate (62 and 70 with stage 1 or 2, and stage 3 or 4 disease, respectively), and 133 patients given placebo (64 and 69 with stage 1 or 2 and stage 3 or 4 disease, respectively). Patient visits were arranged according to the following schedule: biweekly visits for the first 4 weeks, monthly visits for the first 6 months, and 3 month intervals for the remainder of the study. The following data were provided based on these clinical visits: DLCO measurements at the time of randomization (baseline), at the time the patient discontinued use of methotrexate or placebo (final), and the minimum measurement observed at any follow up visit; characteristic information such as age, sex, height, and weight; the stage of the disease; the treatment arm for each patient; and outcome data describing the times until progression or last follow up, discontinuation of the study drug or last follow up, and death or last follow up. The DLCO measurements will be used to address the question regarding effects of methotrexate on lung function. The remaining variables will allow us to address any confounding issues, identify cases of missing data that might impact our analysis, as well as identify any errors in measurements. There are 20 patients missing both their final and minimum DLCO measurements; a single patient is missing their baseline DLCO measurement; these patients cannot be used to study the effects of methotrexate on lung function.

Comment: How long were the patients eventually followed?

Comment: actually only measured annually

Comment: actually this patient could be used—you don't need a baseline measurement in an RCT. I do note that you have more precision ignoring this patient and using the baseline.

Statistical Methods

The purpose of this analysis was to determine if methotrexate impaired lung function, as measured by DLCO values, in PBC patients over time. As such, the value of interest is the change from the patient's baseline DLCO measurement to the last measurement taken while on the study drug. Thus, the difference between a patient's baseline DLCO measurement to that patient's final DLCO measurement while taking a study drug was calculated. This difference will be positive if DLCO measurements decreased over time while on the study drug, and thus if methotrexate adversely affects lung function (leading to lower DLCO measurements) patients in the treatment group would have larger, positive differences than patients in the placebo group. The difference between baseline and final DLCO values was used, rather than the difference between baseline and the minimum observed value, to assess the toxicity over time. Using minimum values would test for a large drop in DLCO values at any point while on methotrexate; such a drop is of less concern if DLCO values subsequently recover to near baseline values, but is of concern if they do not. Thus, final measurements were used as the best way to assess the effect of methotrexate on lung function over the duration of the treatment.

Comment: Excellent clarification of your measurement scale

Comment: Also excellent observation

In order to test if the treatment group and placebo group had different average differences, a two-sided t-test was done on the mean difference for each group. The t-test computes the probability that the observed difference between the two groups could have occurred due to chance if the actual difference in means were zero. The computed 95% confidence intervals give a range of possible true values for the difference for which the observed data would not be an extreme outcome (occurring less than 5% of the time).

Missing data were assumed to be missing at random, as there is no scientific reason to expect that patients with missing DLCO values would be more or less likely to suffer toxicity as a result of methotrexate.

Comment: Unless their lung toxicity made them stop. (I know of no such case, but it could happen)

Data were analyzed using Stata version 9.2 running under Windows XP.

Results

The sample population consists of 265 patients diagnosed with Primary Biliary Cirrhosis divided into a treatment and a placebo group as described in Table 1. The placebo and treatment groups are similar with respect to the ages, weights and heights of the participants. Only twenty male patients are represented in the sample population, making up just under 8% of the participants (they are divided equally between the treatment and placebo groups). The raw data as collected contained two notable errors. The variable stopdrug, representing the number of days from randomization until treatment with either the study medication or placebo was discontinued, exceeded the value of obsdeath, the number of days until death or last follow-up, in 34 observations. This was corrected by setting stopdrug to the minimum of stopdrug or obsdeath. The variable dlcoMin, the lowest value of DLCO recorded while on the study medication or placebo, was greater than the value of dlcoFnl, the final measurement made while on the study medication in 19 observations. As such, this was corrected by replacing dlcoMin with dlcoFnl, if dlcoFnl was less than dlcoMin. The corrections and revised measurements were confirmed with the study team as the correct values to use for analysis.

Comment: Severity of their PBC was probably also of interest. I would have included some of those variables

Comment: Perfect description for a report to a collaborator. Hopefully they would all be fixed before going for publication.

DLCO measurements were available at baseline for 264 of the 265 participants. Table 2 shows that these baseline measurements were similar across treatment groups. DLCO final measurements (the last measurement while on the study drug) were available on 245 of the 265 patients. These final measurements were generally lower than the baseline measurement for all participants (20.0 vs. 18.5). These measurements were also similar across the two treatment groups. Data were also available on the minimum DLCO measured while on the study drug. These measurements were also similar across treatment groups. Note that for some participants, the minimum DLCO measurement was the final DLCO measured while on the study drug.

Comment: It is very much of interest to know how long patients were followed on each arm. This is true to ensure we aren't missing patients with toxicity, as well as just to give an idea of how much experience we have with MTX in PBC

Figure 1 shows the distribution of differences between baseline and final DLCO measurements for each patient by time on study drug and treatment group. This graph shows that the average difference of baseline to final is slightly positive for all participants, as predicted by lower DLCO measurements over time. However, there is not a noticeable difference between the two treatment groups, nor does there appear to be a general trend of the difference to increase or decrease with longer time on the study drug.

Comment: OK, this probably suffices for my last comment. A picture is worth a thousand words here.

Looking at the differences over time is important to assess the possibility that one reason patients might have stopped taking the study drug early was due to lung toxicity. However, the graph shows that patients who stopped early were not the ones with the highest differences, and patients who stopped early were approximately equally distributed between the placebo and treatment groups. Thus, there is no obvious evidence that there was a subgroup of patients on methotrexate who stopped taking it early due to lung toxicity.

Comment: Excellent point

Table 3 shows point estimates and 95% confidence intervals for the difference of baseline DLCO value to final DLCO value by treatment group. Both groups had slightly positive differences, indicating that DLCO values are decreasing over time. However, the difference in the difference between treatment groups was small (0.082 higher for the treatment group) and not statistically significant (two-sided P=0.88). This means that these data do not provide evidence that methotrexate impairs lung function, as measured by a decreased DLCO value. The 95% confidence interval for the difference suggest that the observed data would not be unusual if the true value for the treatment group difference was between 1.12 (ml/min/mmHg) higher and 0.95 (ml/min/mmHg) lower than the placebo group.

In addition to the primary analysis, limited exploration into possible associations of DLCO measurements with age, height, weight, platelet count and disease stage was performed. In general, DLCO measures tended to decrease with increasing age but there was no significant difference between the treatment and placebo groups. DLCO measures against height showed a general increase with increasing height, but no differentiation between the placebo and treatment groups. Similarly, DLCO values with weight showed a general increasing with increasing weight, although this relationship is likely confounded by height. Although there were only 20 males in the data set, the males had noticeably higher DLCO values, indicating that DLCO is likely associated with sex. However, this effect was similar across the two treatment groups. Finally, more specific to the PBC participants, DLCO measures against platelet count did not show any association, nor did DLCO and disease stage. In summary, exploratory analysis did not identify age, height, weight, sex, platelet count or disease stage, as potential confounders in our analysis of DLCO measurements, nor did they appear to be predictors of the change in DLCO values while on the study medication.

Discussion

In this randomized controlled trial, we didn't detect any statistically significant difference in the change in DLCO over time between the methotrexate and placebo arms. Based on the statistical method that we used, we can therefore state that this trial did not provide evidence that the use of methotrexate (defined by the study protocol) caused a change in the DLCO measurements over time.

Pulmonary toxicity of methotrexate is generally seen with both high and low dose treatment and may present with acute or chronic symptoms. Either symptomatic or asymptomatic radiographic lung damage may be due to inflammation, infection, or methotrexate related neoplasia[3]. Our results are not compatible with other studies that

estimate the prevalence of pulmonary toxicity is between 2 and 8 percent of patients receiving methotrexate, and even some reports suggest an incidence as high as 33 percent[4-7]. One possible explanation is that we looked at the DLCO ratio of baseline to final rather than the actual lung toxicity.

In fact, DLCO doesn't seem to be a good measure to access the pulmonary function. It has been shown that healthy individuals will have variable results based on the laboratory and also in different times in the same laboratory. This variability is even more prominent among people with any kind of lung disease. This fact is important in our analysis because it is known that methotrexate may cause some pulmonary problems which can't be detected by the DLCO, so it is possible that patients in the treatment arm may have a higher pulmonary dysfunction comparing to the placebo arms (which can be detected by other means than DLCO) and it may affect the variability of the DLCO measures in our population. Another important issue is the fact that methotrexate causes myelodepression, which results in anemia and pancytopenia (decrease in all types of blood cells). On the other hand anemia can cause a decreased DLCO. We didn't look at this factor as it hasn't been provided in the dataset. Although we didn't observe any decrease in the treatment group, adjusting for the anemia status would increase the quality of this analysis.

Comment: Yes. Can you relate this back to the width of the CI? And is the CI so wide that our study is essentially inconclusive, or should we feel pretty comfortable?

For future studies it seems that looking at the pulmonary toxicity by different clinical laboratory tests or alternative histopathological definitions will help in detection of more pulmonary toxic side effects. The Multiparameter scoring systems (devised by Searles and McKendry) which is a diagnostic criterion for methotrexate lung toxicity may be the best way in this regard. This scoring system considers DLCO only as a minor criterion which is another confirmation for the need of using other measurements[8,9].

References

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		N	Mean(SD)	Min	25th %	50th %	75th %	Max
All	age (years)	265	51(8.7)	25	46	51	57	70
N=265	weight (kg)	264	71.7(15.4)	42.7	61	68.8	81.7	149.1
♀=245	height (cm)	264	163.7(8.0)	142.8	157.4	162.6	169.1	191.9
MTX	age (years)	132	50(8.7)	31	44.5	50	56	70
N=132	weight (kg)	132	70.1(14.4)	42.7	59.4	68.3	77.1	114.2
♀=122	height (cm)	132	163.7(8.0)	142.8	157.5	162.2	170.3	181.6
Placebo	age (years)	133	52(8.6)	25	47	53	59	67
N=133	weight (kg)	132	73.3(16.2)	46	62.7	70.3	84.4	149.1
♀=123	height (cm)	132	163.7(8.1)	149.5	157.4	162.7	168.4	191.9

Table 1: Descriptive statistics for the entire sample and treatment groups.

		N	Mean(SD)	Min	25th %	50th %	75th %	Max
All N=265	Baseline	264	20.0(5.0)	8.9	16.4	19.3	22.8	41
	Final	245	18.5(4.9)	8.5	15.4	18.5	20.9	39.4
	Minimal	245	16.8(4.3)	7	14.3	16.4	18.8	34.6
MTX N=132	Baseline	131	20.3(5.1)	8.9	16.4	20	23.5	37.7
	Final	120	18.7(4.8)	8.6	15.4	18.8	21.4	35.8
	Minimal	120	16.9(4.4)	7	14.4	17.2	19.2	34.6
Placebo N=133	Baseline	133	19.7(5.0)	11.5	16.3	18.4	22.2	41
	Final	125	18.4(5.0)	8.5	15.7	18.2	20.8	39.4
	Minimal	125	16.7(4.2)	8.5	14.3	16.2	18.6	32.7

Table 2: Diffusing capacity of the lung for carbon monoxide (DLCO) outcomes for the entire sample and by treatment group. Measurements aside from N are in ml/min/mmHg.

	Estimate (95% CI)
MTX	1.59 (0.85 – 2.34)
Placebo	1.51 (0.78 – 2.24)
Difference	0.082 (-0.95 – 1.12); *P*=0.88)

Table 3: Point estimates and 95% confidence intervals for the difference of baseline DLCO to final DLCO value (in ml/min/mmHg); P value for difference is two-sided.

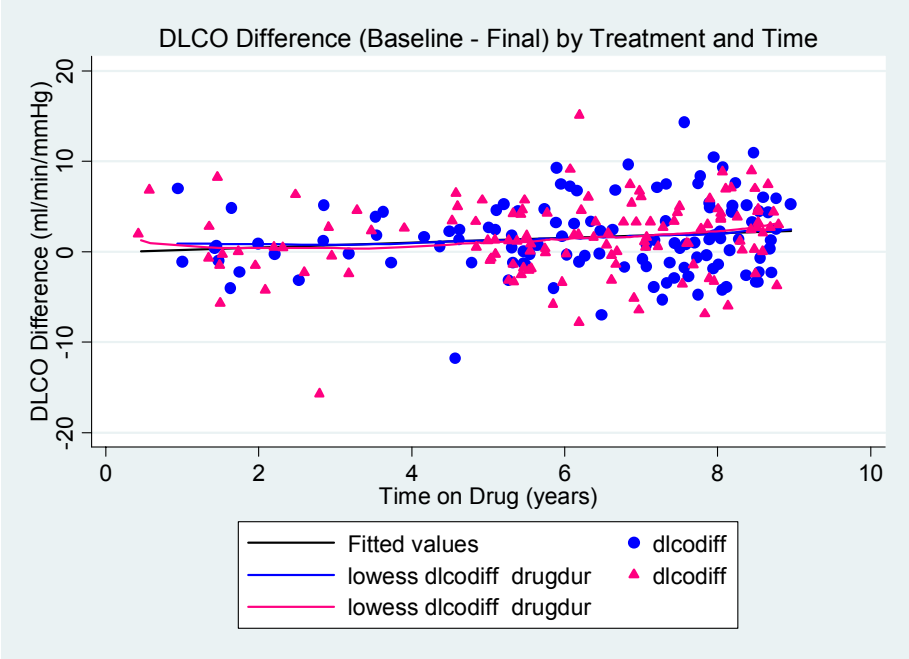

Figure 1: Difference in DLCO values between baseline and final measurement taken while on study drug for placebo group (pink triangles) and treatment (MTX) group (blue circles). Superimposed are lowess smooths by treatment group and the least squares estimate line for the entire sample is superimposed in black.