Group 3
BIOST 517 project

Treatment with methotrexate and survival in primary biliary cirrhosis

Summary

The present study explores whether treatment with a study drug, methotrexate (MTX), affects survival or progression-free survival relative to placebo for patients with PBC using a double blind, randomized, placebo controlled, clinical trial. The study included 265 patients with primary biliary cirrhosis (PBC) from a larger clinical trial of PBC treatments. From Kaplan-Meier estimates of survival and progression-free survival, advanced stage of liver disease was observed to lead to worse survival after six years and five years, respectively, though stratification by stage revealed comparable patterns of survival and progression-free survival. Through the use of proportional hazard regression analysis for survival we were able to determine that the risk of death with treatment of methotrexate was 1.23 times higher than those in the placebo group. A 95% confidence interval of 0.462 to 3.296 suggests that these results are typical if the true risk of death with treatment was .46 that of the placebo group to 3.30 times greater with the use of methotrexate. Based on a two-sided *p*-value of .675 we fail to reject the null hypothesis of no association between risk of death and treatment. Through the use of proportional hazard regression analysis for progression-free survival we estimated that at any given time the risk of progression with methotrexate treatment tends to be 1.26 times that of the placebo group. A 95% CI suggests these results are typical if the true risk of progression in the treatment group is 0.75 to 2.12 times that of the placebo group. Based on a two-sided *p*-value of 0.381, we fail to reject the null hypothesis of no association between progression and treatment.

Comment: multicenter

Comment: Also a good idea to note how many deaths and/or progressions over what length of observation time (do a KM of the censoring time).

Comment: estimate

Comment: perhaps remark that this is a VERY wide CI

Comment: All of this a bit wordy for an abstract, but fine for the Results section.

Background

Primary biliary cirrhosis (PBC) severely damages the functionality of the liver. PBC causes scarring and blockage of the intrahepatic bile ducts outside the liver and impedes the liver's ability to excrete bile into the gastrointestinal tract. Bile assists with digestion, specifically of fats, and with a bile deficiency the body experiences an accumulation of bilirubin in tissues causing jaundice. In early stage PBC, the bile ducts of the liver are blocked. After a period of time, ongoing blockage during early stage PBC causes damage to the liver cells. This cellular damage limits the ability of the liver to carry out important functions including the synthesis of proteins, the metabolism of fats and glucose, and the detoxification of chemicals. Specifically, the advanced stage of PBC leads to cirrhosis of the liver and is characterized by the following malfunctions in the body that may impact survival:

1. Excretory malfunctions that include increased bilirubin, increased estrogen and accumulation of copper in the liver and other organs in the body.
2. The breakdown of liver cells such that certain enzymes, which are typically only found in the liver, are released into other parts of the body. Detecting these enzymes may signify liver damage.
3. Malfunction in protein formation such that albumin levels in the blood decrease which causes swelling in the body. Also, proteins necessary for blood coagulation and the production of platelets are not sufficiently produced.
4. Scarring of the liver impacts the flow of fluids from the liver and through the body.
5. The damaged liver impedes effective fat metabolism as shown by changes in cholesterol and triglyceride levels in the blood.

Comment: liver function

It has been hypothesized that an auto-immune component may play a key causal role that results in disease progression whereby the body's own immune system attacks and damages the liver. Most often, PBC is initially diagnosed between the ages of 35 and 60 and tends to more frequently affect women than men. For some patients, the disease lacks symptoms, but for those patients who demonstrate symptoms of liver disease, death tends to occur within 5 to 10 years.

Overall goal and specific aims of current study

The present study explores the impact of a study drug, methotrexate (MTX), on survival and progression-free survival for patients with PBC using a double blind, randomized, placebo controlled, clinical trial. Progression-free survival indicates that no advancements of the disease were observed. Our analysis is meant to answer the question of whether treatment with methotrexate imparts differential survival or progression-free survival relative to placebo. We hypothesize that it will.

Comment: define

Data source

The study included 265 patients with primary biliary cirrhosis. Participants came from multiple areas of the United States (including multiple data collection sites) and were identified by clinical investigators as having sufficiently advanced PBC to be included in the study. Data on participant age, gender, height and weight were gathered at the time of their inclusion in the study as well as on several other potential covariates related to their condition. These include their history of PBC, as measured by time from diagnosis to study inclusion; hepatocellular damage, as measured by alkaline phosphatase and ALT levels in the blood (enzymes released by damaged liver cells); liver inflammation and cirrhosis, as measured by presence or absence of splenomegaly, platelet count in the blood, and pathologic staging of PBC (1-4); and lack of liver function, as measured by bilirubin, albumin, and cholesterol levels in the blood and the time required for blood clots to form. Participants were then stratified by histologic stage of liver disease, randomized to treatment condition, methotrexate or placebo, and monitored for the study period. Methotrexate and placebo were administered in 2.5 mg tablet form with alterations throughout the study period in dosage if side effects and/or bone marrow toxicity occurred. Measurements throughout this period included the time participants took their particular study drug and the time until progression of PBC and/or death or, barring the occurrence of either, the time to last follow-up.

Comment: ???

Statistical methods

For this analysis, we used the statistical analysis software Stata (Version 9.2). Stata is an extremely flexible, interactive, and widely used statistical package with excellent implementation of biostatistical methods and is, therefore, well suited to our analysis. Analysis was performed by stratifying data according to the histologic stage of liver disease. Based on the randomized, double blinded design of the study, we did not expect to observe a difference between subjects in the two treatment groups. But in order to assess the comparability of treatment groups in each stratum and types of patients used in the trial, stratified descriptive statistics of participant characteristics by treatment groups, including demographic characteristics (sex, age, weight and height) and baseline measurements (history of disease, hepatocellular damage, liver inflammation, etc.), are provided. For participants with missing values, the values of other participants were carefully examined (whether they were within the range of values in the corresponding group) to ensure that the values are missing at random.

Comment: usually last sentence

Comment: omit

Comment: we were sort of hoping for a difference in survival…

Comment: we can never ensure they were missing at random. We can sometimes detect situations that make us suspect they were not missing at random.

Since the observed time to death or progression is a right-censored variable, the usual sample mean, median, standard deviation and variance are not appropriate to analyze the treatment effect. Instead Kaplan-Meier survival curves were used to estimate the distributions of time to death or progression (defined by death, liver transplantation, bleeding varices, hepatic encephalopathy or development of ascites). A 95% confidence interval (CI) and KM estimates of survival probabilities at a few relevant fixed time points for treatment groups in each stratum were calculated. Cox proportional hazard regression analysis was used to compare treatment effect by reporting the hazard ratio, corresponding 95% CI and two-sided *p*-values for both survival and progression-free survival.

We provide both confidence intervals and two-sided *p*-values since each plays an important role in comparing the effect of treatment. Confidence interval (CI) estimates the values of the population parameter for which the observed data are fairly typical (here, we define typical as the central 95% of the sampling distribution). It gives the set of all true parameter values that reasonably result in the observed sample parameter value. *P*-value is associated with hypothesis tests. It is the probability of obtaining a result at least as "impressive" as that obtained, assuming the truth of the null hypothesis that the finding was the result of chance alone. Generally, one rejects the null hypothesis if the observed *p*-value is smaller than or equal to the *a priori* significance level. In this analysis, we set the level to be 0.05, which is mostly commonly used for 2-sided tests. The results are, therefore, only 5% likely to be as extraordinary as observed, given that the null hypothesis is true.

Results

In order to ensure treatment and placebo group equivalence we examined the demographic and other potential covariate measures from baseline for our subjects and found no noticeable difference between the two groups (see Table 1). The population in this study consisted of a large majority of females (over 90% of participants). The ages of subjects ranged from 25 to 70 years old. Ages seemed to be evenly distributed throughout the four groups, though the placebo group at advanced stage PBC had a slightly higher average age than the other three groups. All other distributions of these variables seemed to be similar throughout the four groups. The missing values among these measures seemed to occur in equal numbers across the two treatment groups and four strata. This ensured that the randomization of the study participants was effective in creating equivalent treatment groups and our comparisons between these two groups would be statistically valid.

Comment: assess

Comment: wording is a little too strong

[Insert Table 1 here]

The Kaplan-Meier survival curves for both survival and progress free survival showed only slight differences between the four stratified groups (see Figure). This seems to indicate that there was not a significant difference in the outcomes between the four strata.

[Insert Figure here]

From Kaplan-Meier estimates of survival, advanced stage of liver disease was observed to lead to worse survival after six years (see Table 2). But when we compared within stage stratum we observed that both treatment groups follow a comparable pattern. From Kaplan-Meier estimates of progression-free survival, advanced stage liver disease was observed to lead

Comment: Giving estimates for the most important time point in the text is probably a good idea, even though it is also in the table.

to worse progression-free survival after 5 years. Again, when we compared within stage stratum we observed that both treatment groups follow a comparable pattern.

[Insert Table 2 here]

Through proportional hazard regression analysis we were able to determine that the risk of death for those receiving treatment with methotrexate was 1.23 times higher then for those in the placebo group. A 95% confidence interval of 0.462 to 3.296 suggested that these results are typical if the true risk of death with treatment was .46 that of the placebo group to 3.30 times greater with the use of methotrexate. A two-sided *p*-value of .675 suggested that we could not reject the null hypothesis that there is no difference in survival between the two treatment groups.

Comment: This CI is huge! Comment on this lack of precision.

Through proportional hazard regression analysis for progression-free survival we were able to estimate that at any given time the risk of progression for participants in the methotrexate treatment tends to be 1.26 times that of the placebo group. A 95% CI suggested that these results are typical if the true risk of progression for participants in the treatment group is 0.75 to 2.12 times that of those in the placebo group. Based on an observed two-sided *p*-value of 0.381, we failed to reject the null hypothesis of no association between progression and treatment.

Discussion

The present study failed to demonstrate that the study drug, methotrexate, improved survival or progression-free survival for patients with primary biliary cirrhosis. Furthermore, stratification by stage of disease also did not demonstrate an impact of the study drug on improved survival or progression-free survival for patients with PBC. The present study demonstrated a number of limitations. Suggestions for improvement in future analyses are listed below.

Comment: So was this just a terrible study?

Comment: What were you looking for here?

Future investigation of the effectiveness of methotrexate on survival and progression-free survival may be improved if changes in the methodology for data collection and analyses are carried out. First, increasing the sample size of patients with PBC in the present study and tracking them over a longer period of time may strengthen future investigation of the effectiveness of methotrexate (Carithers 2003). Also, the present study included a small number of male participants, less than 8% in both the placebo and treatment groups. Although 90% of patients with PBC are women (Worman 1999), including more men in the future investigation of the effectiveness of MTX, improves the overall generalizability of the study's findings to both men and women with PBC. The present study's findings are not necessarily generalizable to men with PBC.

Comment: Not if you want to generalize to the true disease population—it is 90% women

For many individuals, PBC is asymptomatic. However, for those individuals who develop symptoms, death tends to occur within five to ten years. As a result, following more individuals who are in varying stages of PBC progression for a longer period of time than ten years may offer greater insight into the effectiveness of MTX (Carithers 2003). Perhaps, patients should be tracked for at least 15 years to more thoroughly investigate long term survival and progression-free survival of PBC for patients on methotrexate.

Lastly, PBC is most often first diagnosed between the ages of 35 and 60 and for those who develop symptoms, as stated above, deaths tend to occur within 5 to 10 years. As a result, including more individuals in the 50 to 60 year old range may improve analysis of the effectiveness of methotrexate. Half of the participants in the present study were under age 53;

Comment: Only if it works in the more advanced disease rather than in the early stages. The question is: Will the early stage people in this study go on to advanced disease or not?

younger participants may not be symptomatic. Asymptomatic patients demonstrate longer survival periods (Pares and Rodes, 2003).

Also, further analyses by stratification and investigation of additional outcomes of MTX may be helpful in improving the design of the present study. First, stratifying on some of the baseline potential covariates, such as age and gender, may provide additional insight into the effectiveness of methotrexate on survival and progression-free survival of patients with PBC. Age and gender have been shown to relate to the diagnosis of individuals with PBC and may relate to both the survival and progression-free survival of individuals with PBC.

Comment: Why would such stratification help? Are these important prognostic variables?

In addition, to investigate the long term effects of methotrexate use, more data are needed on covariates such as alkaline phosphatase, ALT, presence of splenomegaly, platelets, stage of liver disease, bilirubin, albumin, time of clot and cholesterol at time intervals beyond baseline. Also, additional measures such as quality of life indicators, specifically, self-rated health and physical capabilities should be assessed to further examine the effectiveness of methotrexate on patients with PBC. Lastly, because methotrexate has not been shown to produce strong positive outcomes for patients with PBC, additional drugs should also be developed and investigated that may demonstrate improved health outcomes and lengthened survival and progression-free survival for patients with primary biliary cirrhosis.

Comment: How would you have used these? As surrogates?

References

Cariters, RL, Jr. Primary biliary cirrhosis: specific treatment. Clin Liver Dis. 2003 Nov;7(4):923-39.

Pares, A. and Rodes, J. Natural history of primary biliary cirrhosis. Clin Liver Dis. 2003 Nov;7(4):779-94.

Worman, HJ. What is Primary Biliary Cirrhosis (PBC)? Columbia University Medical Center, 1999. http://www.cumc.columbia.edu. Accessed November 30, 2006.

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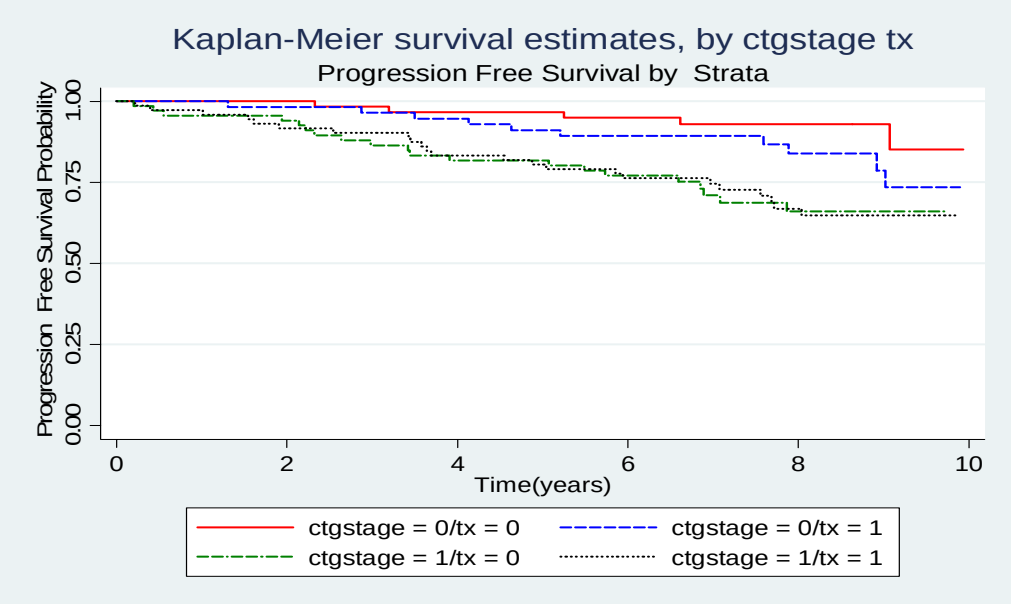
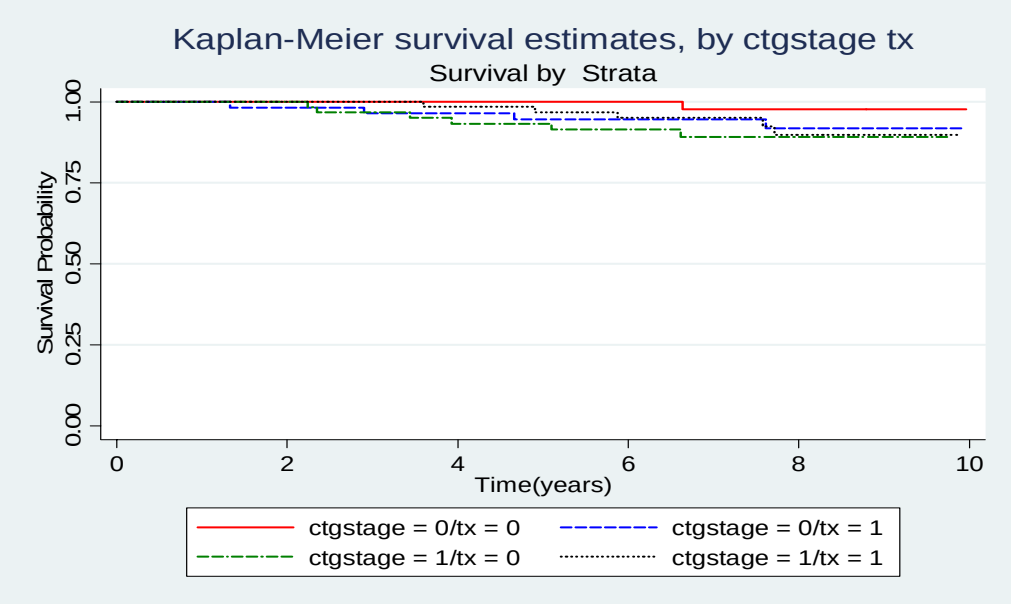
Table 1
Summaries of baseline measurements by histologic stage of liver disease and treatment group

	Stratum 1 (placebo and early stage)					Stratum 2 (methotrexate and early stage)				
variable	**n**	**Mean**	**sd**	**min**	**max**	**n**	**Mean**	**sd**	**min**	**max**
age	64	50.2	9.3	25.0	67.0	60	50.4	8.0	31.0	70.0
% male	64	3.1%				60	8.3%			
weight (kg)	63	75.7	17.1	47.3	149.1	60	69.5	14.8	44.4	114.2
height (cm)	63	164.0	8.0	149.7	191.9	60	164.7	7.7	148.2	179.0
days between diagnosis & randomization	64	1146.6	1110.6	198.0	5038.0	60	1158.1	1158.0	202.0	5652.0
alkaline phosphatase (U/1)	64	213.1	192.5	63.0	1127.0	60	238.6	134.1	58.0	572.0
ALT (U/1)	63	44.7	44.1	9.0	314.0	60	52.4	42.2	9.0	175.0
splenomegaly (1= absent, 2= present, 3= unknown)	64	1.1				59	1.0			
platelets (1000 cells/ cu mm)	64	269.3	81.4	94.0	561.0	60	264.5	94.9	119.0	619.0
stage of liver disease	64	1.6	0.5	1.0	2.0	60	1.8	0.4	1.0	2.0
bilirubin (mg/dl)	64	0.6	0.3	0.1	1.8	60	0.7	0.5	0.1	2.8
time of clot (sec)	64	11.3	1.0	8.9	13.5	57	11.4	1.2	8.5	14.6
albumin (g/dl)	64	4.0	0.3	3.2	4.6	60	4.0	0.4	3.3	5.9
serum cholesterol (mg/dl)	64	240.8	63.9	128.0	560.0	59	240.7	62.1	137.0	440.0
time on drug	64	2266.3	729.4	247.0	3212.0	60	2188.7	870.7	46.0	3273.0
DLCO	64	21.0	4.9	12.7	40.0	60	21.1	5.1	10.2	31.7
	Stratum 3 (placebo and advanced stage)					**Stratum 4 (methotrexate and advanced stage)**				
variable	**n**	**Mean**	**sd**	**min**	**max**	**n**	**Mean**	**sd**	**min**	**max**
age	69	54.0	7.7	33.0	66.0	72	50.4	9.2	31.0	69.0
% male	69	11.6%				72	6.9%			
weight (kg)	69	71.2	15.0	46.0	108.0	72	70.6	14.2	42.7	106.6
height (cm)	69	163.4	8.2	149.5	185.1	72	162.9	8.0	142.8	181.6
days between diagnosis & randomization	69	1517.7	1249.3	185.0	5269.0	72	1382.2	1342.1	202.0	6552.0
alkaline phosphatase (U/1)	69	274.4	179.0	73.0	996.0	72	246.4	155.5	50.0	930.0
ALT (U/1)	69	55.2	38.9	13.0	252.0	71	55.9	41.0	10.0	199.0
splenomegaly (1= absent, 2= present, 3= unknown)	69	1.1				72	1.1			
platelets (1000 cells/ cu mm)	69	202.6	71.6	77.0	402.0	72	226.1	79.5	86.0	546.0
stage of liver disease	69	3.3	0.5	3.0	4.0	72	3.2	0.4	3.0	4.0
bilirubin (mg/dl)	69	0.8	0.4	0.1	2.3	72	0.7	0.4	0.1	2.1
time of clot (sec)	69	11.4	1.2	8.6	13.9	71	11.1	1.1	9.0	13.3
albumin (g/dl)	69	4.0	0.4	3.1	4.9	72	4.0	0.3	3.0	4.7
serum cholesterol (mg/dl)	66	230.9	53.5	141.0	378.0	71	237.9	55.1	140.0	472.0

Comment: Usually we would call early stage one stratum and late stage another stratum. Placebo and MTX would be the "arms" of the trial.

Figure. Survival and progression-free survival by histologic stage of liver disease and treatment group





NOTE: tx=0 corresponds to placebo, tx=1 corresponds to methotrexate, ctgstage = 0 corresponds to early PBC stage, and ctgstage = 1 corresponds to advanced PBC stage

Table 2
Kaplan-Meier survival estimates by histologic stage of liver disease and treatment group

Survival			Progression-Free Survival		
Time	**Survivor Function**	**95% CI**	**Time**	**Survivor Function**	**95% CI**
Early Stage and Placebo			**Early Stage and Placebo**		
5	NA	NA	**5**	0.9669	0.8742 0.9916
6	NA	NA	**6**	0.9494	0.8510 0.9834
7	0.9778	0.8525 0.9968	**7**	0.9283	0.8188 0.9727
8	0.9778	0.8525 0.9968	**8**	0.9283	0.8188 0.9727
9	0.9778	0.8525 0.9968	**9**	0.9283	0.8188 0.9727
Early Stage and Methotrexate			**Early Stage and Methotrexate**		
5	NA	NA	**5**	0.9110	0.7993 0.9620
6	NA	NA	**6**	0.8931	0.7775 0.9505
7	0.946	0.8419 0.9823	**7**	0.8931	0.7775 0.9505
8	0.9182	0.7919 0.9693	**8**	0.8389	0.6985 0.9176
9	0.9182	0.7919 0.9693	**9**	0.7865	0.6039 0.8919
Advanced Stage and Placebo			**Advanced Stage and Placebo**		
5	NA	NA	**5**	0.8176	0.7011 0.8921
6	NA	NA	**6**	0.7710	0.6491 0.8552
7	0.8913	0.7719 0.9501	**7**	0.7095	0.5766 0.8075
8	0.8913	0.7719 0.9501	**8**	0.6599	0.5176 0.7692
9	0.8913	0.7719 0.9501	**9**	0.6599	0.5176 0.7692
Advanced Stage and Methotrexate			**Advanced Stage and Methotrexate**		
5	NA	NA	**5**	0.8051	0.6932 0.8796
6	NA	NA	**6**	0.7625	0.6459 0.8451
7	0.9499	0.8525 0.9836	**7**	0.7451	0.6258 0.8314
8	0.8985	0.7681 0.9575	**8**	0.6688	0.5384 0.7699
9	0.8985	0.7681 0.9575	**9**	0.6485	0.5160 0.7532
Cox Proportional Hazard Ratio:			**Cox Proportional Hazard Ratio:**		
1.234 (two-sided p-value= .675)			**1.261 (two-sided p-value= .381)**		

Comment: This layout makes it hard to compare the treatment arms. Also, NA's probably should be 1.00. It is also possible to get CI for this.

In any case, you probably could abbreviate this table for a paper, but in the absence of knowing exactly what years to focus on, it might not be so bad to give a collaborator this report at first.

Comment: Why not CI for these?