Group 15
Biostat 517
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Tables: 4
Graphs: 2

Summary:

Our aim in this study is to look at medical treatment in prolonging the time to disease progression of primary biliary cirrhosis with the use of methotrexate. This was a double-blinded placebo controlled trial that enrolled 535 patients from twelve different institutions. Of the 535 patients, 265 were eligible for randomization after screening. The results showed no difference in time to disease progression between the methotrexate treatment group and placebo. The five-year difference in progression-free survival from the Kaplan-Meier curves was 0.038 (95% CI -0.044 to 0.120) greater for the methotrexate group than placebo. The Cox proportional regression analysis estimated the risk of progression for the treatment group to be 1.261 (95% CI 0.751 to 2.120) times that of the placebo group. The populations were further stratified by stage of disease. The Cox proportional regression for the early stage estimated the risk of progression for the treatment group to be 2.114 (95% CI 0.721 to 6.194) times that of the placebo. Late stage analysis estimated the risk for the treatment group to be .993 (95% CI 0.547 to 1.804) times that of placebo. Several methodological and scientific concerns with the design and clinical basis of this study were addressed.

Comment: screened

Comment: What was length of follow-up and number of events? This tells us about our statistical information.

Comment: Stated too strongly. Perhaps "no statistically significant differences". We have a pretty wide CI here

Comment: NO!!! The 5 yr PFS was better for the placebo group.

Also, you should tell us what it was for each group.

Comment: I would say instead "further analyzed within subgroups defined by stage"

Comment: "addressed" or "discussed"?

Background:

Primary biliary cirrhosis (PBC) is an inflammatory process of the intra and extra hepatic bile ducts that leads to eventual cirrhosis of the liver. The cause of PBC is unknown, however it is generally believed to be autoimmune in nature due to the presence of autoantibodies. The overall prevalence of disease is approximately 5 in 100,000. Most patients with this disease are women who make up about 90% of this disease population. Age groups that are most affected are those in their 40's to 60's.

Comment: may lead to

The pathophysiology of PBC can be followed by clinical and histological data. Abnormal laboratory values consistent with mechanical obstruction of the biliary system and liver failure are consistent with progressive PBC. As disease progresses bilirubin tends to increase, protein synthesis such as albumin and clotting factors decrease, platelet count decreases as a result of sequestration from an enlarged spleen, and other metabolic disturbances such as hypocalcemia becomes clinically severe enough to require treatment. Histologically, PBC is divided into four stages based on the level and amount of damage to the biliary and hepatic parenchyma. The stage of disease is usually a good indicator of PBC severity because it reflects the mechanical obstruction of the bile ducts as well as damage to the liver itself.

The final outcome of PBC is fulminant liver failure. There have been many attempts at medical treatment for symptomatic relief such as bile salt binders; however there has not been any evidence that this prolongs the interval to definitive treatment. The mainstay treatment of PBC is orthotopic liver transplantation. The success rate of transplantation is very good with an overall survival of 80% in 2 years.

Comment: What would have been these patients' survival if they had not been transplanted? (Someone once showed me some observational data suggesting that liver transplantation in less advanced disease did not seem to do as well as we would hope. I have never seen this in print, however.)

The interval of progression free survival is essential to these patients because of the scarce supply of available organs for transplantation. Since PBC is assumed to be a disease caused by an inflammatory process, some have postulated that medical therapy based on decreasing this inflammatory process may slow the disease progression. Methotrexate is an antimetabolite that reduces cell turnover that also has anti-

inflammatory properties. In fact, it is a well-known medical treatment for other inflammatory disease such as ulcerative colitis. Thus, methotrexate is an attractive drug to study because it may prolong the disease free progression of PBC and better bridge the patient for liver transplantation.

Question of Interest

Our aim is to determine whether or not methotrexate affects the progression-free survival of patients with primary biliary cirrhosis relative to placebo. Specifically, is there a clinically significant difference in the time to disease progression between our treatment group and our placebo? Our secondary aim is to determine whether or not the stage of disease affects disease progression among those who receive our study drug.

Comment: How are you going to assess this?

Comment: Are you interested in gaining precision? addressing confounding? assessing effect modification? assessing association between stage and progression? (Your sentence reads like the latter to me, and I would argue that that is of no interest.)

Source of the Data

Twelve clinical centers enrolled 535 patients for a randomized double-blinded, placebo controlled clinical trial. All patients underwent review of their clinical records. These patients were also screened to satisfy inclusion criteria for PBC. Some patients did not meet inclusion criteria because of the severity of their disease. Those who satisfied these screening criteria continued to the clinical trial. Overall, 265 individuals were eligible for this study. The patients were randomized to placebo and treatment by stage of disease. Overall, there were 126 patients that were in the early stage of PBC (stages 1 and 2) and 139 in the late stage (stages 3 and 4). In the early stage group, 62 of the 126 were randomized to receive the treatment while 70 in the late stage were randomized to receive the treatment.

Comment: screened

Patients entered into this study had multiple clinically relevant variables measured. These included, four demographic variables (age, sex, weight, height); duration of disease before randomization; estimation of hepatocellular damage (alkaline phosphatase and ALT); liver inflammation and cirrhosis (splenomegaly, platelet count, and stage); and lack of liver function (bilirubin, PT, cholesterol, and albumin). There were no missing observations for our variables of interest.

Statistical Methods

We began our analysis by descriptively evaluating all of the clinical variables of interest to ensure that there was no informative censoring of the data. To initially test our primary hypothesis of a difference in the time to disease progression, Kaplan-Meier analysis at the five-year interval was performed due to the censoring of the data. Specifically, some patients left the study before progression could be observed. To estimate the risk of progression at any point in the study period, Cox proportional hazard regression analysis was performed allowing for robust standard error estimates. All statistical output was derived using the Stata version 9 software package. We performed the same Cox proportional hazard model among early and late disease stages.

Comment: No travelog, here. Just tell us the methods used.

Comment: There is nothing in the data that can tell you whether there was or was not informative censoring. There are things that can make us worry about it, however.

Comment: Cox regression can only estimate the relative risk of progression between two groups, and it then takes some weighted average of the risk.

As mentioned above, censoring for progression of disease may have occurred for a variety of reasons (ie, general withdrawal from study, death, recipient of a transplant, etc). For Kaplan-Meier to be appropriate, we must be able to assume censoring occurred at random and did not provide any information in regards to a patient's progression.

Further, when a patient is censored, Kaplan-Meier requires his or her progression of disease to be similar to those patients still present in the study. The Kaplan-Meier estimates are calculated by considering all individuals at risk for progression at a given time interval, and all patients who had disease progression during that same interval.

More basic techniques could have been performed, such as a test of proportions at year five, if censoring were absent. A five-year cut-off point for disease progression was used because of its clinical significance as a standard time period among studies investigating the treatment and progression of disease.

Comment: Good discussion for a collaborator, but it would be omitted in a published manuscript.

To calculate the difference in progression-free survival at year five, the absolute difference in the proportion of methotrexate patients progression-free and the proportion of placebo patients progression-free is estimated from the Kaplan-Meier curves. A standard error for the difference is calculated from the standard errors of the two groups. A 95% confidence interval and a respective p-value are then calculated. A p-value greater than approximately 0.05, or a 95% confidence interval containing 0 for the difference, would provide evidence that we cannot reject the hypothesis there is no difference in the five year progression-free survival.

Comment: I'll bet you used Greenwood's formula. You would have to note this when performing this analysis, because we do have other methods.

The hazard ratio is the relative risk of disease progression at any given time. To estimate a difference in hazard ratios for the methotrexate and placebo groups, we used the Cox proportional hazard models, allowing the risk ratio to vary over time. A p-value greater than approximately 0.05, or a 95% confidence interval containing 1.0 for the hazard ratio, would provide evidence that we cannot reject the hypothesis there is no difference in the risk of disease progression between either the treatment or placebo group.

Results

At randomization the clinical variables measured between the treatment and placebo groups were examined descriptively (Table 1). There were no obvious differences between the two treatment groups, however some generalizations about the overall study population could be made. The patients were primarily female (92.5%), the range of ages was from 25 to 70 years, and measurements reflective of hepatic function were within normal range.

At 5 years, the Kaplan-Meier analysis did not show a statistically significant difference in progression free survival for treatment and placebo groups (Figure 1). The survival for the placebo group was 0.038 greater than methotrexate, with a respective 95% CI -0.044 to 0.120 and a two sided p-value of 0.368. The Cox proportional hazard regression analysis between the treatment and placebo group was performed and we estimate that at any given time the risk of progression for the methotrexate group tends to be 1.261 times (95% CI 0.751 to 2.120) that of the placebo group with a p-value 0.381. These results suggest no statistical significance between the progression free survival between the methotrexate and placebo group. (Table 2 and 3)

Comment: It would be good to describe the length of follow-up (use KM estimates of censoring distribution) and the length of time patients took drug (this is a post-randomization variable, and could give info about adverse effects, generalizability of results, etc.)

Comment: Tell us the number of events observed. This is informative about the precision you will have. We would also be interested in number of deaths, even if we lacked power to assess differences

Comment: probably should use a few more words here to highlight that the direction was wrong and to explain that -0.044 would mean that MTX would be better and 0.120 would mean Placebo would be better.

Subset analysis among treatment and placebo groups were divided into early stage disease (stage 1 and 2) and late stage disease (stage 3 and 4) (Figure 2). For early stage disease, Cox proportional hazard regression analysis between the treatment and placebo group was performed. We estimate that at any given time the risk of progression for the methotrexate group tends to be 2.114 times (95% CI 0.721 to 6.194) that of the placebo group with a p-value 0.172. These results suggest that in the early stage, there is no

statistical significance in the risk of progression between the methotrexate and placebo groups. The same analysis was performed on the late stage group. Cox proportional hazard regression analysis between the treatment and placebo group shows that at any given time the risk of progression in the methotrexate group tends to be 99% (95% CI 0.547 to 1.804) that of the placebo group with a p-value 0.982. These results suggest that in the late stage, there is also no statistical difference in the risk of progression between the methotrexate and placebo groups.

Comment: Do you make anything of the different estimates in the early and late stage groups? I note that you are looking for effect modification here, rather than what your Question of Interest seemed to suggest.

Discussion

Discovering medical treatments that prolong the progression of primary biliary cirrhosis is important because the cure to this disease is dependent on the availability of liver organs for transplantation. Because the supply is limited and the demand is high, by successfully bridging these patients until transplantation (organ availability) we can expect the survival of these patients with this disease to improve. This study examined the potential of using methotrexate, an antimetabolite with anti-inflammatory properties, to prolong the progression of disease in patients with PBC.

Our study design was a randomized double blinded, placebo controlled clinical trial that enrolled 535 patients from twelve clinical centers in the United States. We examined disease progression as our outcome of interest because of the clinical implications. We were also interested in overall survival; however we felt that this study was underpowered to sufficiently examine this outcome.

Overall, the difference in disease progression between the methotrexate and placebo group was not statistically significant. Specifically, the point estimate of 5 years and the relative risk of progression at any given point in time between the methotrexate and placebo groups were not statistically different. This suggests that there would be no benefit to the overall population of PBC patients in terms of disease progression if they were treated with methotrexate versus not being with this drug. We examined this further to determine whether or not the possibility of a successful outcome by our medical treatment was masked by the stage of disease. We stratified the data by early staged and late staged individuals. Early staged PBC patients were determined by their histologic grade as being 1 or 2, while late staged PBC patients were defined as being in either grade 3 or 4. Our analysis within strata of disease stage also did not show any statistical difference, by Cox proportional hazard regression. We did note a trend in the hazard ratios of the different strata. The early stage disease population has a hazard ratio of 2.114, while the late stage had a hazard ratio of 0.993. While it is tempting to state that there is an association of faster disease progression in the early stage treatment group compared to placebo, the wide confidence intervals (95% CI 0.721 to 6.194) do not give us the precision to suggest this.

Comment: Would a 4% improvement in survival at 5 years have been clinically important? Or was our study underpowered?

This study attempted to address an important medical treatment to a complicated disease process. One of the main weaknesses of our study is the lack of scientific knowledge concerning both the actual cause and pathophysiology of this disease. With this being poorly understood, perhaps the mechanism at which methotrexate works does not offer the appropriate counter mechanisms in preventing the disease progression of PBC. From a study design perspective, one of our many concerns was the low number of individuals in this study who actually had an observed progression event. Although we had a 10-year study, the clinical progression of disease in individual patients actually

varies from 5 to 20 years. In addition, this may have been exacerbated by our inclusion and exclusion criteria where patients with advanced disease were selected out of this study. There would have been more observed events with these individuals included in our study. This would have improved our ability to detect an association, if one truly existed, between treatment and disease progression. When we further stratified among stages this compromised the numbers of individuals in our analysis and decreased the number of observed progressions.

The other issue we had been that drug compliance was not followed throughout the study. For example, 10% of the study population had at least a 2-year gap between last drug dose and event to censoring or progression to event. These were evenly split between the placebo and treatment groups. Thus, any inference on the effect of MTX versus placebo would require that the patient has taken the drug at least once and not be restricted by a particular scheduled drug regimen.

In summary, we made a valid attempt at prolonging progression free survival of PBC using methotrexate. However, we did not find such evidence. In retrospect we could improve this study by increasing the number of patient recruited, increasing patient follow up time, and allowing for patients with late stage disease to participate in the study. Only then, when the power of the study is improved can we make a more definitive statement concerning treatment with MTX.

Figures and Tables

Table 1

Descriptive Statistics at Randomization by Treatment Groups									
Variable	**N**	**Missing**	**Mean**	**Std Dev**	**Min**	**25th %ile**	**50th %ile**	**75th %ile**	**Max**
Placebo Group									
Age	133		52.2	8.6	25	47	53	59	67
Female	133		0.94						
Weight (kg)	132	1	73.35	16.17	46.00	62.70	70.25	84.35	149.10
Height (cm)	132	1	163.69	8.07	149.50	157.40	162.71	168.35	191.90
Disease Duration (days)	133		1339.1	1194.7	185	376	867	1979	5269
Stage	133		2.5						
Splenomegaly	133		0.11						
Bilirubin	133		0.72	0.39	0.10	0.50	0.70	0.90	2.30
Albumin	133		4.00	0.34	3.10	3.80	4.00	4.20	4.90
Alkaline phosphate	133		244.93	187.44	63	120	200	277	1127
ALT	132	1	50.2	41.6	9	26.5	38	61	314
Prothrombin time	133		11.37	1.083	8.60	10.90	11.50	12.10	13.90
Cholesterol	130	3	235.77	58.81	128	199	224.5	267	560
Platelets	133		234.7	83.2	77	166	235	291	561
Methotrexate Group									
Age	132		50.4	8.7	31	44.5	50	56	70
Female	132		0.92						
Weight (kg)	132		70.12	14.42	42.70	59.40	68.30	77.10	114.20
Height (cm)	132		163.71	7.92	142.80	157.50	162.15	170.29	181.60
Disease Duration (days)	132		1280.3	1262.1	202	340	983	1646	6552
Stage	132		2.6						
Splenomegaly	131	1	0.08						
Bilirubin	132		0.66	0.44	0.10	0.40	0.60	0.80	2.80
Albumin	132		4.00	0.35	3.00	3.80	3.95	4.20	5.90
Alkaline phosphate	132		242.9	145.8	50	148	208	304.5	930
ALT	131	1	54.3	41.4	9	27	42	67	199
Prothrombin time	128	4	11.25	1.13	8.50	10.60	11.35	12.00	14.60
Cholesterol	130	2	239.2	58.2	137	204	233	266	472
Platelets	132		243.5	88.6	86	179	231.5	299	619

Comment: Far better to have Plc and MTX groups side by side so we can compare. We would then sacrifice having all the descriptive statistics, but the means, SDs are most important from the standpoint of possible confounding, and the min, max are of interest from the Materials and Methods standpoint.

Figure 1

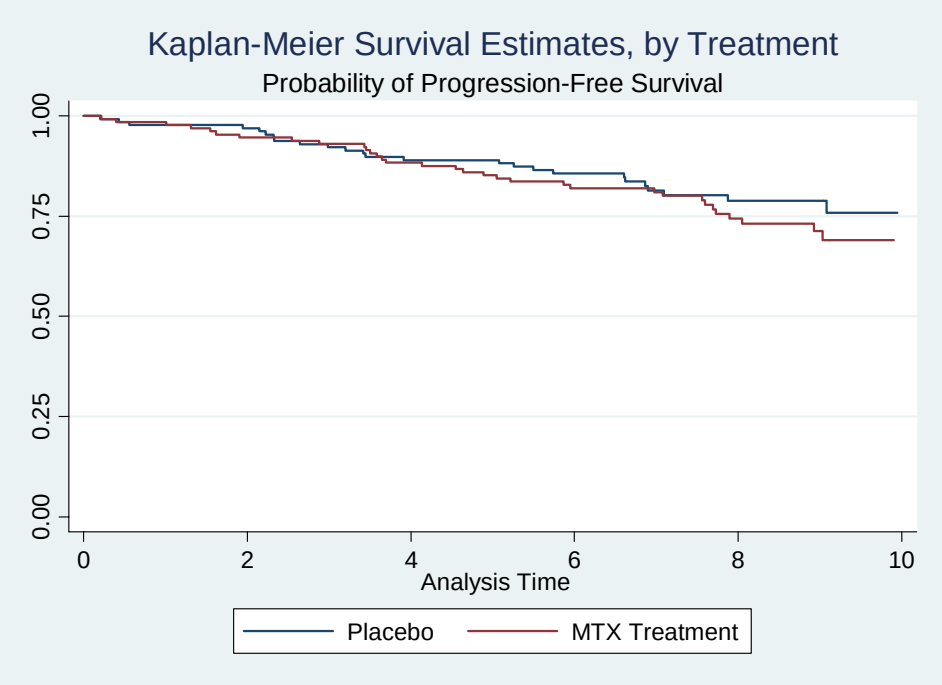
Kaplan-Meier Survival Estimates, by Treatment
Probability of Progression-Free Survival
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
Analysis Time
Placebo
MTX Treatment

Table 2 Difference in 5-year survival

Time	Treatment	Beginning Total	Fail	Survivor Function	Std. Error	95% Conf. Int.	
						Lower Bound	Upper Bound
Year 5	Placebo	110	14	0.8897	0.0278	0.8209	0.9332
Year 5	MTX	109	19	0.852	0.0313	0.7779	0.903

Table 3 Cox proportional hazard progression between Placebo and MTX

Hazard Ratio	Robust Std. Err.	P-value	95% Confidence Interval	
			Lower Bound	Upper Bound
1.261	0.334	0.381	0.751	2.120

Comment: Probably ought to stress that this HR is MTX : Plc rather than Plc : MTX

Figure 2

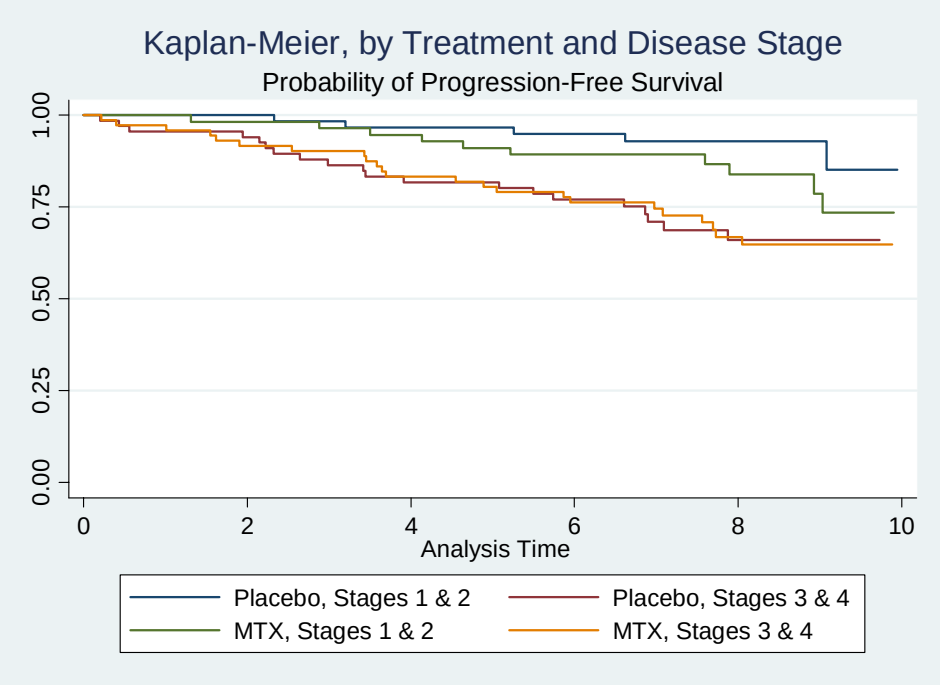
Kaplan-Meier, by Treatment and Disease Stage
Probability of Progression-Free Survival
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
Analysis Time
Placebo, Stages 1 & 2
Placebo, Stages 3 & 4
MTX, Stages 1 & 2
MTX, Stages 3 & 4

Table 4 Cox proportional hazard of progression of disease for early and late stage disease

Stage 1 & 2:			95% Confidence Interval	
Hazard Ratio	Robust Std. Err.	P-value	Lower Bound	Upper Bound
2.114	1.159	0.172	0.721	6.194

Stage 3 & 4:			95% Confidence Interval	
Hazard Ratio	Robust Std. Err.	P-value	Lower Bound	Upper Bound
0.993	0.302	0.982	0.547	1.804