Exploratory analysis of identifying factors used for informing patient prognosis with bone marrow transplant

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1. Introduction

Acute leukemia is a relatively rare form of cancer. There are two main types of acute leukemia, acute lymphocytic leukemia (ALL) and acute myelocytic leukemia (AML), which affect approximately 6,000 and 20,000 Americans each year, respectively. Both types are very deadly. ALL has a 68.8% 5-year relative survival rate. AML has a 28.7% 5-year relative survival rate (1). Bone marrow transplant is a common treatment for acute leukemia. However, recovery after transplantation depends on a variety of factors, including patient and donor demographics, disease stage, and the occurrence of acute graft-versus-host disease (aGVHD). The effects of these factors on survival following transplantation is not well understood.

A multicenter study was conducted to identify factors that may predict patient survival following allogeneic marrow transplantation. Between March 1, 1984 and June 20, 1989, 137 patients were enrolled at four hospitals in Columbus, Ohio; Philadelphia, Pennsylvania; Melbourne, Australia; and Sydney, Australia. All patients in the study participated in a radiation-free conditioning regime of oral Busulfan and intravenous cyclophosphamide.

We are primarily interested in understanding how baseline factors like patient and donor age, patient and donor sex, and French-American-British classification impact relapse and survival. We are especially interested in understanding the roles of aGVHD, exploring characteristics of patients developing aGVHD, the effect of aGVHD on negative outcomes and the methotrexate use for aGVHD. We also focused on the effect of normal platelet functions on leukemia relapse and death. If we could understand the risk factors of relapse and death after transplantation better, we can prevent negative outcomes effectively by controlling these factors and paying attention to high-risk populations.

2. Methods

We conducted this study as an exploratory investigation to evaluate whether patient and donor characteristics as well as unfolding clinical events are predictive of leukemia return or death in remission among patients receiving allogeneic marrow transplantation.

In our study, we released the assumption of distribution of the time to event for baseline function and mainly fitted Cox proportional hazards regression models (Cox PH models) to evaluate risk factors of a negative outcome (death or relapse). In this way, the baseline hazard functions were unspecified and hazard ratios with confidence intervals were estimated. In addition, we would not stratify patients into different hospitals in the models, as this was a small sample size group, which would reduce the power of the study and increase the margin of error. We used a significance level of 0.10 to compensate for the relatively small sample size in the study.

2.1 Characteristics of the estimated distribution of disease-free survival time

The outcome of interest is time to disease-free survival, of which the initiating event is bone marrow transplantation and terminating event is leukemia relapse or death in remission. Data for patients who did not relapse or die by the end of study or who were lost to follow up were considered as right-censored data. In order to investigate the main characteristics of the estimated distribution of disease-free survival time,

we used the Kaplan-Meier method to provide a curve of survival estimates with 90% confidence intervals for all patients enrolled in the study and estimated the median time until relapse or death with its 90% confidence interval.

2.2 Comparisons of baseline characteristics by FAB classification and disease group

In order to understand how patients differ by FAB classification and disease group, we created tables comparing the groups. Baseline characteristics were summarized with frequency distributions for categorical variables and mean and standard deviation for continuous variables.

2.3 Baseline factors associated with disease-free survival among all patients

We were interested in the association between the following measured baseline characteristics and the risk of a negative outcome (relapse or death): patient age and gender, donor age and gender, patient and donor cytomegalovirus (CMV) immune status, the wait time from diagnosis to transplantation, disease group, French-American-British (FAB) classification based on standard morphological criteria, and prophylactic use of methotrexate to prevent aGVHD.

In order to determine whether any of the measured baseline factors were associated with differences in disease-free survival, we used Cox proportional hazards regression. The Cox PH model allows us to easily adjust for the various baseline factors and borrows information across subgroups. We used a descriptive model building strategy, because we were going through the data to describe potentially interesting effects and did not have a defined model before conducting the trial. We tested each of the baseline factors in separate Cox models and also fit a complete model with all of the factors. Since prior studies had found that patient CMV status may be a confounder, we included that in each model. We included each factor with a p-value of less than 0.1 in a combined model. We then added the remaining covariates into the model one-by-one and conducted a log-rank test, keeping only the factors that were significant.

2.4 Effect of aGVHD on disease-free survival and relapse

We built explanatory models to examine the hypotheses that aGVHD occurrence after transplantation is associated with improved disease-free survival and decreased risk of relapse. Because the predictor of interest, aGVHD can occur at random times during the recovery process, we treated it as a time varying variable.

First, we investigated the effect of aGVHD on disease-free survival among all enrolled patients. The event of interest was leukemia relapse or death. Cox PH models accounting for a time-varying variable were fitted to estimate hazard ratios with 90% confidence intervals unadjusted and adjusted for potential confounders. Potential confounding factors were identified a priori (2-5), including donor's gender, patient's CMV immune status, disease group, FAB classification and prophylactic use of methotrexate.

Second, we examined the association of aGVHD with the risk of relapse. The outcome of interest was time until relapse, of which the initiating event was bone marrow transplant and the terminating event is leukemia relapse. Similarly, we fitted Cox PH models with a time-varying variable to estimate the hazard ratios of relapse with 90% confidence intervals unadjusted and adjusted for potential confounders. Based on the scientific background, we included donor's gender, patient's CMV immune status, disease group, FAB classification and prophylactic use of methotrexate as confounding variables in the adjusted model.

2.5 Baseline factors associated with disease-free survival among patients developing aGVHD

This was a subgroup analysis and we would develop descriptive models. First, only patients who develop

aGVHD would be included in this analysis. The survival object in this question was the same as that in question 3, which took time until relapse or death (in days) and the disease-free survival indicator. And then Cox PH models were applied in this question. First, we adjusted one variable at a time to build an adjusted model, to test the null hypothesis that any of the measured baseline factors was not associated with differences in disease-free survival, and then found those with p-value smaller than significance level 0.1. In addition, we also built a full model including all those baseline variables and selected those with p-value smaller than significance level 0.1. We included each factor with a p-value of less than 0.1 in a combined model. We then added the remaining covariates into the model one-by-one and conducted a log-rank test, keeping only the factors that were significant.

2.6 Effect of prophylactic Use of Methotrexate on aGVHD occurrence

In order to explore the risk of developing aGVHD for patients either administered methotrexate or not, we first plotted a Kaplan-Meier survival curve for each subgroup. The curves for two groups seemed to have a Weibull distribution.

Explanatory models were developed to evaluate the hypotheses that prophylactic use of methotrexate is associated with decreased risk of developing aGVHD. The predictor of interest was prophylactic use of methotrexate and the outcome of interest was time from transplantation until occurrence of aGVHD. We first fitted an unadjusted Cox PH model and an adjusted Cox model adjusting for potential confounders to estimate the hazard ratios with 90% confidence intervals.

Then, we identified the measured confounding factors a prior based on the scientific background. It appeared that donor age increased the risk of aGVHD, but a fatal outcome of this complication was influenced by recipient age (6). Female to male transplants were associated with a higher risk of aGVHD (6). And for those populations, they were more likely to have prophylactic use of methotrexate to prevent aGVHD. Besides, patients with advanced ALL had better overall survival (OS) when they developed aGVHD with or without grades I and II aGVHD (7). And those patients were more likely to have prophylactic use of methotrexate to prevent aGVHD. Therefore, in this question, we decided to consider patient age and gender, donor age and gender and disease group as relevant confounding factors.

Furthermore, to estimate the survival function of time from transplant until onset of aGVHD separately for patients either administered methotrexate or not, we assumed that the baseline survival function is a Weibull distribution. And then we fitted an unadjusted and an adjusted Accelerated Failure Time Models (AFT) Weibull models adjusting for potential confounders mentioned to estimate the ratio of mean survival time with 90% confidence intervals. We also provided an estimate of survival function of survival time separately for patients administered methotrexate or not.

2.7 Effect of recovery of normal platelet levels on disease-free survival and relapse

To evaluate the hypotheses that recovery of normal platelet levels (RNPL) is associated with improved disease-free survival and decreased risk of relapse, explanatory models were built. The predictor of interest, recovery of normal platelet levels was treated as a time-varying variable because it can vary over time.

To assess the effect of RNPL on disease-free survival among all enrolled patients, Cox PH models accounting for a time-varying variable were fitted. The event of interest was leukemia relapse or death. Hazard ratios with 90% confidence intervals were estimated with and without adjustment for potential confounders. According to prior literature (8,9), we considered patient's age, patient's CMV immune status, disease group and FAB classification as potential confounding variables.

Then, we examined the effect of RNPL on the risk of relapse. The event of interest was leukemia relapse.

Cox PH models with a time-varying variable were fitted to estimate hazard ratios of relapse with 90% confidence intervals unadjusted and adjusted for potential confounders. Potential confounding factors were identified a priori, including patient's age, patient's CMV immune status, disease group and FAB classification.

3. Results and Discussions

3.1 Characteristics of the estimated distribution of disease-free survival time

All of the 137 enrolled patients were included into this study. Among these 137 patients, 83 patients experienced relapse or death. The survival probability decreased with time (see Figure 1), with a faster decrease rate from the enrollment to the approximate 100 days, than after 100 days. The median disease-free survival time is 481 (90% CI: 383, 677) days since the time of transplantation.

3.2 Comparisons of baseline characteristics by FAB classification and disease group

Patients were split into two groups by FAB classification:

- Group 1: FAB grade 4 or 5 and AML
- Group 2: All other patients

Table 2A provides a comparison between these two groups on a variety of baseline measurements. The two groups were similar across most baseline characteristics, including patient age, patient sex, CMV status, donor age and donor sex.

There were differences between the groups in methotrexate use and wait time. Roughly half as many patients in group 1 prophylactically used methotrexate to prevent aGVHD than in group 2: 17.8% in group 1 versus 34.8% in group 2. Patients in group 2 waited an average of 50% longer from diagnosis to transplantation: average of 206.3 days in group 1 versus 308.7 in group 2. However, there was a wide variation in wait time among both groups. Group 1 had a standard deviation of 163.9 days and group 2 had a standard deviation of 426.9 days.

Patients were split into three groups by disease group:

- ALL: acute lymphoblastic leukemia
- AML Low Risk: acute myelocytic leukemia low risk
- AML High Risk: acute myelocytic leukemia high risk

Table 2B provides a comparison between these two groups on a variety of baseline measurements. The three groups were similar across most baseline characteristics, including donor age, donor sex and donor CMV status.

There were differences between the groups in age, wait time, and methotrexate use. Patients in the ALL group were the youngest, with an average age of 24.4 years vs 29.4 in the AML Low Risk and 30.4 in the AML High Risk Groups. Patients in the ALL group also waited longer from diagnosis to transplantation, an average of 477.2 days vs 138.1days in the AML Low Risk and 268.9 in the AML High Risk. Patients in the ALL group were most likely to prophylactically use methotrexate to prevent aGVHD, 44.7% vs 22.2% in the AML Low Risk Group and 24.4% in the AML High Risk Group.

3.3 Baseline factors associated with disease-free survival among all patients

We found that the only baseline factors associated with differences in disease-free survival were disease group and FAB grade. We estimate that the hazard for individuals in disease group 2 (AML low risk) is 0.4047 (95% CI: 0.2388-0.6857) times that of the individuals in disease group 1 (ALL), adjusting for FAB and patient CMV status. We estimate that the hazard for individuals in disease group 3 (AML high risk) is 0.9486 (95% CI: 0.5567-1.6164) times that of the individuals in disease group 1 (ALL), adjusting for FAB and patient CMV status. We estimate that the hazard for individuals in FAB grades 4 or 5 is 2.1578 (95% CI: 1.3808-3.3720) times that of the individuals with all other FAB grades adjusting for disease group and patient CMV status. Details can be found in Table 3A and 3B.

This analysis has some limitations. It's possible that we are missing some important baseline factors. We considered ten baseline factors. However, there are many more that potentially could be considered, including patient BMI, smoking history, alcohol consumption, etc. These could have important effects that we are missing. Additionally, we did not consider interactions or higher order terms in our modeling. With ten factors, that would be 45 potential first order interactions and any combination thereof, making the model space extremely large.

3.4 Effect of aGVHD on disease-free survival and relapse

To assess the effect of occurrence of aGVHD after transplantation on disease-free survival, we fitted an unadjusted Cox PH model and an adjusted Cox PH model adjusting for donor's gender, patient's CMV immune status, disease group, FAB classification and prophylactic use of methotrexate based on previous studies. The unadjusted and adjusted HRs were 1.14 (90% CI: 0.72-1.79) and 1.23 (90% CI: 0.77-1.97), respectively. Among patients with the same donor's gender, CMV immune status, disease group, FAB classification and prophylactic use of methotrexate, the hazard of leukemia relapse or death for individuals who experienced aGVHD is 23% higher than those who didn't experience aGVHD. However, this association is not statistically significant at level 0.1(see Table 4A).

To investigate the association between occurrence of aGVHD after transplantation and the risk of relapse, we fitted an unadjusted Cox PH model and an adjusted Cox PH model adjusting for the same confounders. The unadjusted and adjusted HRs were 0.64 (90% CI: 0.29-1.40) and 0.68 (90% CI: 0.31-1.50), respectively. Among patients with the same donor's gender, CMV immune status, disease group, FAB classification and prophylactic use of methotrexate, the hazard of leukemia relapse for individuals who experienced aGVHD is 32% lower than those who didn't experience aGVHD. However, this association is not statistically significant at level 0.1(see Table 4B).

Based on our statistical analysis, we cannot reject the null hypothesis that aGVHD doesn't associate disease free survival and the null hypothesis that aGVHD is not associated with the risk of leukemia relapse after transplantation at level 0.1. Therefore, there is no evidence that aGVHD is a prognostic event.

The analysis is subject to some limitations. Though we take multiple potential confounding variables into account, it is still possible that the effect is confounded by unmeasured factors, such as socioeconomic factors. Furthermore, we didn't consider interaction between primary predictor and other covariates in our modeling.

3.5 Baseline factors associated with disease-free survival among patients developing aGVHD

There were only 26 patients included in this question. When we adjusted one variable at a time to build an adjusted Cox PH model, only the donor's age and disease group 3 which was labeled AML high risk were associated with differences in disease-free survival (see Table 5A) at significance level 0.1. In addition, if

we fit a full Cox PH model including all those baseline variables, donor's age, the wait time from diagnosis to transplantation, disease group 3 which was labeled AML high risk and prophylactic use of methotrexate to prevent aGVHD were all associated at significance level =0.1 (see Table 5B). And then we included these four baseline measurements with a p-value of less than 0.1 in a combined model, all of them got p-value smaller than 0.1. We then added the remaining covariates into the model one-by-one and conducted a log-rank test, none of them were significant.

Therefore, among the patients who develop aGVHD, donor's age, the wait time from diagnosis to transplantation, disease group and prophylactic use of methotrexate to prevent aGVHD were associated with differences in disease-free survival. However, the small sample size may reduce the power of our study analysis. Furthermore, we failed to consider interaction between baseline variables in the models because of the limitation of small sample size.

3.6 Effect of prophylactic Use of Methotrexate on aGVHD occurrence

The plot of Kaplan-Meier survival estimate for the risk of developing aGVHD for patients either administered methotrexate or not was presented below (see Figure 6). From the plot, patients with prophylactic use of methotrexate were more likely to survive longer than those without prophylactic use of methotrexate, indicating that prophylactic use of methotrexate was associated with decreased risk of developing aGVHD.

First, we fit an unadjusted and an adjusted Cox PH model. From the unadjusted Cox PH model, the hazard of developing aGVHD in patients with prophylactic use of methotrexate was 0.74 times those without that, with 90% confidence interval of (0.34, 1.60). From the adjusted Cox model, the hazard of developing aGVHD in patients with prophylactic use of methotrexate was 0.54 times those without, with 90% confidence interval of (0.24, 1.19), adjusting for patient age and gender, donor age and gender and disease group (see Table 6A). However, we got p-values of each model larger than 0.1, and we could not reject the null hypothesis that the prophylactic use of methotrexate was not associated with decreased risk of developing aGVHD.

Then, we fit an unadjusted and an adjusted AFT Weibull model. From the unadjusted model, we got the survival function of time form transplant until onset of aGVHD separately for patients administered methotrexate and without were $S(t|mtx=1) = S_0(e^{-0.59}t)$ and $S(t|mtx=1) = S_0(t)$ separately, where $S_0(t)$ was the baseline survival function $S_0=exp(-(58100t)^{0.33})$. And the mean survival time of individuals with prophylactic use of methotrexate is 1.8 times (90% CI: 0.17, 18.8) the mean survival time of individuals without usage.

Then, for the adjusted AFT Weibull models, we got the survival function of time form transplant until onset of aGVHD separately for patients administered methotrexate and without were

$$S(t|mtx=1) = S_0(e^{1.69-0.57*age-1.1*donor\ age+2.23*male+1.06*donor\ male+2.53**disease\ group2+3.67**disease\ group3}t)$$

and

$$S(t|mtx=0) = S_0(e^{-0.57*age-1.1*donor\ age+2.23*male+1.06*donor\ male+2.53**disease\ group2+3.67**disease\ group3}t)$$

separately, where $S_0(t)$ was the baseline survival function $S_0=exp(-(1620000t)^{0.35})$.

And the mean survival time of individuals with prophylactic use of methotrexate is 5.41 times (90% CI:

0.55, 53.8) the mean survival time of individuals without usage, adjusting for donor age, donor cytomegalovirus (CMV) immune status and disease group (see Table 6B).

Therefore, we had no evidence to reject the null hypothesis that prophylactic use of methotrexate was not associated with a decreased risk of developing aGVHD when both un-adjusting and adjusting relevant confounding variables. The results of the AFT Weibull models agreed qualitatively with the results from Cox PH models and they both indicated a nonsignificant association at level 0.1.

In this analysis, we fitted Cox PH and AFT models using two different modeling strategies - semi-parametric and parametric models. Semi-parametric model allows us to release the assumption of baseline survival function, but it cannot give estimates of survival functions. Though the parametric model depends on the assumption about a distribution of baseline survival function, it allows us to estimate the survival functions. It's a trade-off to choose an appropriate model.

3.7 Effect of recovery of normal platelet levels on disease-free survival and relapse

To evaluate the effect of recovery of normal platelet levels (RNPL) after transplantation on disease-free survival, we fitted Cox PH models with and without adjustment for patient's age, patient's CMV immune status, disease group, and FAB classification. The unadjusted and adjusted HRs were 0.12 (90% CI: 0.08-0.16) and 0.13 (90% CI: 0.09-0.19), respectively (see Table 7A). Among patients with the same age, CMV immune status, disease group, and FAB classification, the hazard of leukemia relapse or death for individuals who experienced RNPL is 87% lower than individuals who didn't experience RNPL, and this association is statistically significant at level 0.1.

To investigate the association between occurrence of RNPL after transplantation and the risk of relapse, we fitted an unadjusted Cox PH model and an adjusted Cox PH model adjusting for the same confounders. The unadjusted and adjusted HRs were 0.14 (90% CI: 0.09-0.23) and 0.18 (90% CI: 0.11-0.30), respectively (see Table 7B). Among patients with the same age, CMV immune status, disease group, and FAB classification, the hazard of leukemia relapse for individuals who experienced RNPL is 82% lower than individuals who didn't experience RNPL, however, and this association is statistically significant at level 0.1.

Therefore, we can conclude that RNPL is significantly associated with improved disease-free survival and the decreased risk of relapse. However, similar to part 3.4, unmeasured confounding factors and interaction could be concerns of the results.

4. Tables and Figures

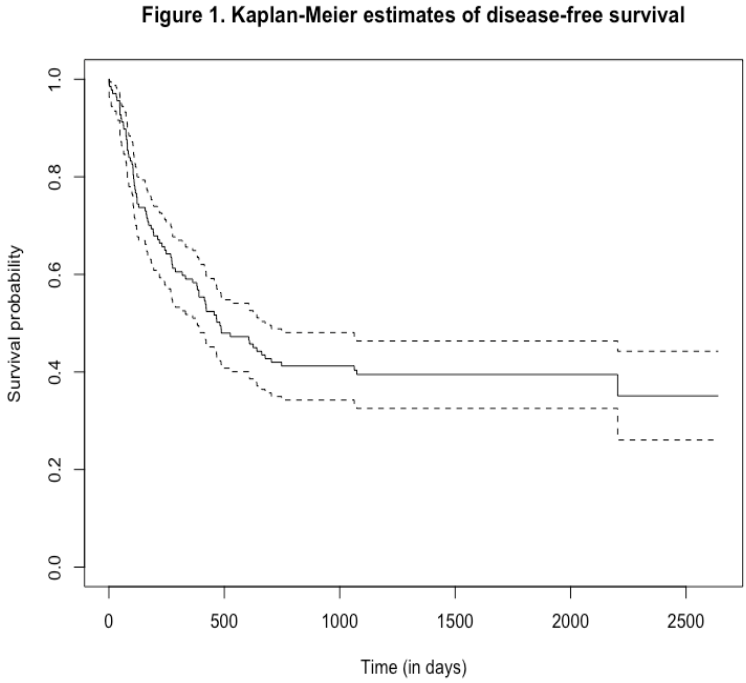

Table 2A. Baseline Characteristics by FAB Classification

Variable	Description	FAB Grade 4 or 5 and AML (N=45)	Otherwise (N=92)	Overall (N=137)
Patient Age	Mean (SD)	27.9 (9.8)	28.6 (9.5)	28.4 (9.6)
Sex	Female	21 (46.7%)	36 (39.1%)	57 (41.6%)
	Male	24 (53.3%)	56 (60.9%)	80 (58.4%)
CMV	Negative	21 (46.7%)	48 (52.2%)	69 (50.4%)
	Positive	24 (53.3%)	44 (47.8%)	68 (49.6%)
Wait Time	Mean (SD)	206.3 (163.9)	308.7 (426.9)	275.1 (364.7)
Methotrexate	No	37 (82.2%)	60 (65.2%)	97 (70.8%)
	Yes	8 (17.8%)	32 (34.8%)	40 (29.2%)
Disease Group	AML High Risk	27 (60.0%)	18 (19.6%)	45 (32.8%)
	AML Low Risk	18 (40.0%)	36 (39.1%)	54 (39.4%)

Donor Age	Mean (SD)	27.0 (11.1)	29.0 (9.7)	28.3 (10.2)
Donor Sex	Female	15 (33.3%)	34 (37.0%)	49 (35.8%)
	Male	30 (66.7%)	58 (63.0%)	88 (64.2%)
Donor CMV	Negative	31 (68.9%)	48 (52.2%)	79 (57.7%)
	Positive	14 (31.1%)	44 (47.8%)	58 (42.3%)

Table 2B. Baseline Characteristics by Disease Groups

Variable	Description	ALL (N=38)	AML Low Risk (N=54)	AML High Risk (N=45)	Overall (N=137)
Patient Age	Mean (SD)	24.4 (7.3)	29.4 (8.8)	30.4 (11.2)	28.4 (9.6)
Sex	Female	12 (31.6%)	24 (44.4%)	21 (46.7%)	57 (41.6%)
	Male	26 (68.4%)	30 (55.6%)	24 (53.3%)	80 (58.4%)
CMV	Negative	23 (60.5%)	28 (51.9%)	18 (40.0%)	69 (50.4%)
	Positive	15 (39.5%)	26 (48.1%)	27 (60.0%)	68 (49.6%)
Wait Time	Mean (SD)	477.2 (598.9)	138.1 (74.5)	268.9 (210.7)	275.1 (364.7)
Methotrexate	No	21 (55.3%)	42 (77.8%)	34 (75.6%)	97 (70.8%)
	Yes	17 (44.7%)	12 (22.2%)	11 (24.4%)	40 (29.2%)
FAB	Otherwise	38 (100.0%)	36 (66.7%)	18 (40.0%)	92 (67.2%)
Donor Age	Mean (SD)	26.8 (8.9)	28.1 (9.2)	29.9 (12.1)	28.3 (10.2)
Donor Sex	Female	12 (31.6%)	20 (37.0%)	17 (37.8%)	49 (35.8%)
	Male	26 (68.4%)	34 (63.0%)	28 (62.2%)	88 (64.2%)
Donor CMV	Negative	21 (55.3%)	32 (59.3%)	26 (57.8%)	79 (57.7%)
	Positive	17 (44.7%)	22 (40.7%)	19 (42.2%)	58 (42.3%)

Table 3A. Estimates and p-values for each single-variable adjusted model

Variable	Hazard ratio	p-value
Patient age	1.01	0.43
Patient gender	0.79	0.28
Donor age	1.01	0.29
Donor gender	0.98	0.94

Patient CMV	1.17	0.48
Donor CMV	1.00	0.99
Wait time	1.00	0.79
Disease group 2	0.56	0.04*
Disease group 3	1.44	0.18
FAB	1.87	0.01*
Use of methotrexate	1.47	0.11

* p-value<0.1

Table 3B. Estimates and p-values for the all-variables adjusted model

Variable	Hazard ratio	p-value
Patient age	1.01	0.50
Patient gender	0.90	0.65
Donor age	1.00	0.91
Donor gender	1.03	0.89
Patient CMV	0.94	0.81
Donor CMV	0.95	0.85
Wait time	1.00	0.38
Disease group 2	0.35	0.00*
Disease group 3	0.83	0.61
FAB	2.23	0.00*
Use of methotrexate	1.34	0.25

* p-value<0.1

Table 4A. Estimates of the hazard ratios and p-values of the adjusted Cox PH model for the association between aGVHD and disease-free survival

	Hazard ratio	90% Confidence Interval	p-value
aGVHD	1.23	0.77-1.97	0.46
Donor's gender	0.97	0.69-1.37	0.88
Patient's CMV	0.96	0.68-1.36	0.84

Disease group2	0.52	0.32-0.85	0.03*
Disease group3	1.03	0.62-1.72	0.92
FAB	1.94	1.30-2.91	0.01*
Use of methotrexate	1.35	0.93-1.97	0.19

* p-value<0.1

Table 4B. Estimates of the hazard ratios and p-values of the adjusted Cox PH model for the association between aGVHD and risk of relapse

	Hazard ratio	90% Confidence Interval	p-value
aGVHD	0.68	0.31-1.50	0.425
Donor's gender	1.63	0.94-2.82	0.14
Patient's CMV	1.26	0.75-2.11	0.47
Disease group2	0.19	0.08-0.45	<0.01*
Disease group3	0.66	0.30-1.47	0.40
FAB	4.09	2.08-8.05	<0.01*
Use of methotrexate	1.25	0.73-2.16	0.49

* p-value<0.1

Table 5A. Estimates and p-values for each single-variable adjusted model

Variable	Hazard ratio	p-value
Patient age	1.02	0.31
Patient gender	1.32	0.59
Donor age	1.07	0.063 *
Donor gender	0.84	0.73
Patient CMV	0.79	0.63
Donor CMV	1.88	0.25

Wait time	1.00	0.38
Disease group 2	1.45	0.56
Disease group 3	1.35	0.04 *
FAB	1.52	0.42
Use of methotrexate	1.72	0.35

* p-value<0.1

Table 5B. Estimates and p-values for the all-variables adjusted model

Variable	Hazard ratio	p-value
Patient age	0.98	0.73
Patient gender	0.86	0.83
Donor age	1.12	0.075 *
Donor gender	0.79	0.77
Patient CMV	0.47	0.34
Donor CMV	1.96	0.35
Wait time	1.00	0.097 *
Disease group 2	3.37	0.32
Disease group 3	11.67	0.10 *
FAB	1.51	0.67
Use of methotrexate	9.10	0.019 *

* p-value<0.1

Figure 6. Kaplan-Meier survival estimate for the risk of developing aGVHD for patients either administered methotrexate or not

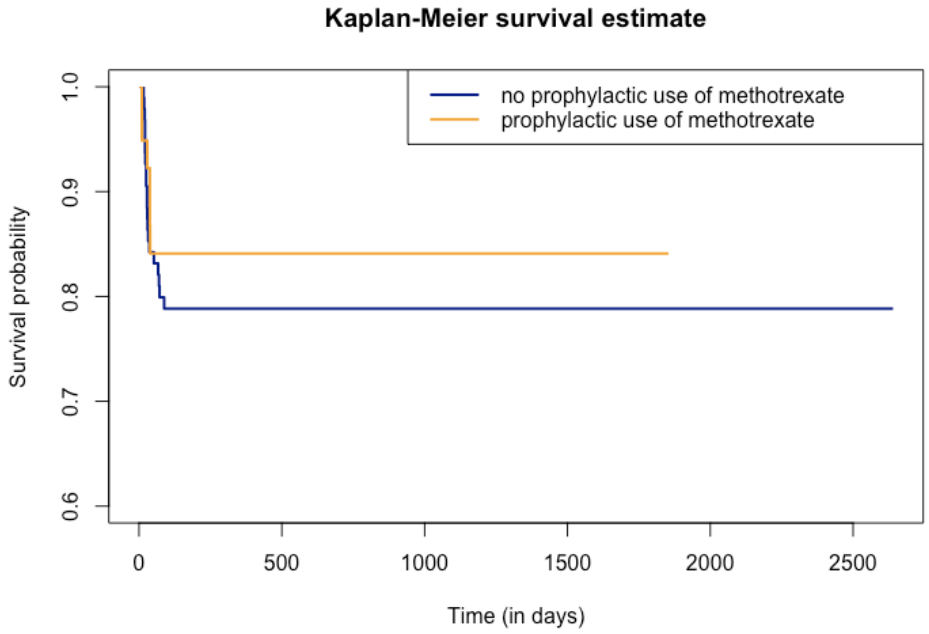

Table 6A. Estimates of hazard ratio and p-values for the adjusted Cox model

Variable	Hazard ratio	p-value
Use of methotrexate	0.54	0.20
Age	1.05	0.16
Donor age	1.03	0.34
Male	0.89	0.77
Donor male	0.68	0.34
Disease group 2	0.55	0.24
Disease group 3	0.29	0.031 *

* p-value<0.1

Table 6B. Estimates of ratios of mean survival times for the adjusted AFT model

Variable	Ratio of mean survival time
Use of methotrexate	5.41
Age	3.15
Donor age	3.39
Male	1.83
Donor male	2.90
Disease group 2	3.40
Disease group 3	10.71

Table 7A. Estimates of the hazard ratios and p-values of the adjusted Cox PH model testing the association between RNPL and disease-free survival

	Hazard ratio	90% Confidence Interval	p-value
RNPL	0.12	0.09-0.19	<0.01*
Patient's age	1.02	1.01-1.04	0.01*
Patient's CMV	0.81	0.60-1.09	0.24
Disease group2	0.49	0.32-0.74	<0.01*
Disease group3	0.71	0.46-1.11	0.20
FAB	1.98	1.38-2.84	<0.01*

* p-value<0.1

Table 7B: Estimates of the hazard ratios and p-values of the adjusted Cox PH model testing the association between RNPL and risk of relapse

	Hazard ratio	90% Confidence Interval	p-value
RNPL	0.18	0.11-0.30	<0.01*
Patient's age	1.02	1.00-1.04	0.14
Patient's CMV	1.12	0.75-1.68	0.63

Disease group2	0.28	0.15-0.54	<0.01*
Disease group3	0.59	0.32-1.11	0.17
FAB	3.18	1.88-5.38	<0.01*

* p-value<0.1

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