Application of the Multiplicative-Additive Model in the Bone Marrow Transplantation Survival Data Including Competing Risks

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Abstract

Background: Cox proportional hazard model is a popular choice in modeling the survival data, but sometimes proportionality assumption is not satisfied. One of the tools for handling the non-proportional effects is the multiplicative-additive model named “Cox-Aalen model”. Recently these flexible regression models developed for competing risks setting. The aim of this paper is showing the application of the multiplicative-additive model in competing risks setting on real bone marrow transplantation (BMT) data when the proportionality assumption is violated.

Methods: The data was from a retrospective study on class III thalassemia patients who undergo hematopoietic stem cell transplantation (HSCT) in BMT ward of Shariatii Hospital, Tehran, Iran. The neutrophil engraftment time as the early outcome of HSCT on 37 patients who received mesenchymal stem cell infusion (MSC group) compared with 50 patients who did not. We fit the standard proportional models and flexible Cox-Aalen model in the sub distribution hazards.

Results: By day 30 after transplantation, the cumulative incidence of neutrophil recovery was 97% (95%CI: 89%-100%) and 76%(95%CI: 64%-88%) in MSC and control group, respectively. Based on the Cox-Aalen model for cumulative incidence function, the MSC infusion had a significant delay effect on neutrophil engraftment (P=.044). In patients who did not neutrophil recovery immediately after HSCT, those who received MSC had faster recovery.

Conclusion: Cox-Aalen model provides more accurate statistical description for time-varying covariate effects. There is a positive effect of MSCs on the neutrophil recovery, however further study on the advantages and disadvantages of MSCs are needed.

Keywords: Bone marrow transplantation, Competing risks, Multiplicative-additive model, Semi parametric Cox-Aalen model

Introduction

Beta thalassemia is the most common monogenic disorder worldwide. Currently, allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for these patients (1,2). Mesenchymal stem cells (MSCs) have been used in phase I and II studies on the autologous, allogeneic and haplo-identical or unrelated donor to improve the HSCT outcomes in hematologic...
malignancies. MSCs are non-hematopoietic cells with the capacity of self-renewal, which can differentiate into various cells lineages of mesenchymal origin (3-5). Generally, the studies have showed the feasibility and safety of MSCs co-infusion without immediate infusional or late MSC-associated toxicities. Safely engineered MSCs may provide targeted and effective cell therapy for graft versus host disease (GVHD). The engraftment capability of MSCs in terms of efficacy remains uncertain. Engraftment is an important milestone in transplant recovery. However, delayed neutrophil engraftment may cause early transplant related mortality primarily from infection(5). In order to evaluate the incidence of neutrophil engraftment in these patients considering the engraftment failures, we planned to use competing risks survival analysis. Recent developments in the field of survival data analysis have led to more powerful and proper data presentation. In the medical research the proportional Cox type models commonly used for regression modeling in the survival data, however it needs to hold the proportionality assumption (6-8). A very flexible alternative to the proportional hazard model is the additive hazard model proposed by Aalen (9,10). In the Aalen’s additive model, the unknown coefficients are allowed to be a function of time and the effect of a covariate may vary over time. It results in plots that are informative regards to the covariates effect on survival, but one limitation of application of Aalen’ additive model is that it is not available in commonly used computer packages (11). Another alternative is the additive-multiplicative model is so called Cox-Aalen model (12, 13). Recently the Cox-Aalen model extended for competing risks data (14, 15). In the competing risks data the occurrence of one event precludes the occurrence of another event. The Cox-Aalen model consists of two components including additive part (like an additive Aalen model) and multiplicative part (like a Cox regression model). This extended model used the Aalen’s model instead of simple baseline hazards on the proportional Cox model to handle non-proportional covariates in the model (11, 14, 15).

The aim of the study was to compare the incidence of neutrophil engraftment in patients with beta thalassemia major class III who undergo HSCT alone or in co-transplantation of donor bone marrow-derived MSCs. In these data, we illustrate the application of Cox-Aalen model in the case of proportionality assumption violation.

Materials and Methods

Patients, inclusion and exclusion criteria
In a retrospective study, data on class III thalassemia patients who underwent HSCT along with MSC between 2006 and 2012, in the Hematology-Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, Tehran University of Medical Sciences (TUMS) were collected and compared with a historical control of beta thalassemia patients class III who received HSCT alone between 1993 and 2012. Patients were included in the study if they had an HLA matched identical sibling donors and received busulfan and cyclophosphamide (BUCY) as the conditioning regimen and combination of CsA and methotrexateas GVHD prophylaxis. Written informed consent form was obtained before transplantation. The outcome of interest was the time to neutrophil engraftment, which is the time interval between the date of transplantation and the date of absolute neutrophil count (ANC) recovery. The ANC recovery defined as the first day of 3 consecutive days with ANC greater than .5×10^9/L. Occurrence of death without engraftment was defined as competing risk for neutrophil engraftment. Potential risk factors that were studied are listed in Table 1. The data were collected through data registry of research Center and missing data were completed from patient’s medical records by a physician.

Statistical analysis
For description of categorical variables, number and proportions were used and for continuous
variables, the median and range are presented. Patients, donors and transplant characteristics were compared between groups using chi square statistics for categorical variables and Mann-Whitney test for continuous variables. As the Kaplan Meier estimator overestimates the absolute risk in the presence of the competing risks, estimates of neutrophil recovery were calculated and tested between MSC and historical control group by the cumulative incidence function (CIF) in the univariate analysis (Fig. 1) (7). To examine the effect of MSC infusion on the incidence of ANC recovery in the multivariate analysis, different modeling approach on the competing risk setting were used. Along with MSC infusion covariate, all measured factors (Table 1) found to be significant ($P<.10$) in the univariate analyses were included in the multivariate analyses. For identifying variables significantly associated with the outcome in the multivariable model, factors that correlated with each other were not entered into the model simultaneously.

Table 1: Patients, donors and transplantation characteristics

| Group                | MSC                  | Historical Control | $P$  |
|----------------------|----------------------|--------------------|------|
| Number of patients   | 37                   | 50                 | ---  |
| Patients             |                      |                    |      |
| Median age, yr (range)| 12(3-15)             | 9(3-16)            | .003 |
| Sex                  |                      |                    |      |
| Male                 | 23(62)               | 25(50)             | .259 |
| Female               | 14(38)               | 25(50)             |      |
| Donors               |                      |                    |      |
| Median age, year (range)| 13(3-31)          | 12(2-38)           | .431 |
| Sex                  |                      |                    |      |
| Male                 | 18(51)               | 21(42)             | .538 |
| Female               | 19(49)               | 29(58)             |      |
| CMV serology antibody*|                     |                    | .735 |
| R+/D+                | 26(70)               | 33(66)             |      |
| R+/D-                | 4(11)                | 4(8)               |      |
| R-/D+                | 5(13)                | 7(14)              |      |
| R-/D-                | 1(3)                 | 4(8)               |      |
| R or D unknown       | 1(3)                 | 2(4)               |      |
| Stem cell source     |                      |                    | .499 |
| PBSBC                | 8(22)                | 14(28)             |      |
| BM                   | 29(78)               | 36(72)             |      |
| Median Total MNC infused×10⁶ /kg (range)** | 3.00(3-12) | 3.44(1.1-10.7) | .566 |
| Median MSC infused×10⁶ /kg (range)*** | 1.29(3-3.15) | --- | --- |

All patients had BUCY conditioning regimen and transplanted from HLA identical sibling donor. / MSC indicates mesenchymal stem cell; CMV, cytomegalovirus; R, recipient; D, donor; PBSBC, peripheral blood stem cell; BM, bone marrow; MNC, mononucleated cells; ( ), percent. / *Unknown recipient or donor serology test excluded in P-value calculation. / ** Data was missing for 7 patients for MNC infused cell in control group, respectively. / *** Data was available for 27 patients.
For modeling the CIF, an approach is modeling the hazard function associated with the CIF, which called sub distribution hazard (16). Cox-type proportional hazards model for sub distribution hazards proposed by Fine and Gray. First, we fit a Fine-Gray’s proportional sub distribution hazard model on the data. For testing the proportionality of the effects on the SH, we fit the model for each covariate by adding a time dependent covariate. The proportional hazard assumption was not hold for MSC infusion factor, so we used some alternative models. In the first alternative model, time dependent effect of MSC infusion was defined in a piecewise constant hazard regression manner, so the proportionality was satisfied in each time interval. The time was divided into two periods based on the number of events in each groups is approximately the same. The second alternative was to use the Cox-Aalen model for the CIF. This model extended the Fine and Gray model to handle the time-varying effect of covariates (14,15). To confirm the time-varying effect of the MSC infusion on the Cox-Aalen model, we fit a full nonparametric model of CIF (Fig. 2). The slop of the plot indicates that whether a particular covariate has a constant or time dependent effect. Deviation from a straight line gives an evidence of a time varying effect for covariate. When the covariate has no effect on the hazard, the cumulative regression function will have roughly zero slop during periods. Positive (or negative) slopes are associated with increases (or decreases) in the hazard function along with increasing the covariate value. Finally, we compare the coefficients and their standard error across the models.

All analyses were done with R 2.15.1, including the survival, spline, cmprsk and timereg libraries for R (17). More details of applied regression models are given in appendix.

![Graph](image)

**Fig. 2:** Cumulative regression function estimation and 95% confidence bands for ANC recovery from the full nonparametric regression model of the cumulative incidence function, (a) MSC group and (b) stem cell sources.

**Results**

**Patients, donors and transplant characteristics**

Eighty-seven patients with median age of 10 years (range: 3-16 years) who received hematopoietic stem cell transplantation with or without mesenchymal stem cell (MSC) infusion (MSC group, n=37 (42%), Control group, n=50 (58%)) included in the study. Patients, donors and transplant characteristics are given in Table 1. Comparison between the groups showed significant differences in the patient ages. The
The patient’s median age in MSCs group was more than the control group (12 yr versus 9 yr) ($P=.001$) but it did not affect the neutrophil engraftment ($P=.168$). The follow-up time was complete for all patients. Thirty-seven (100%) and 47 (94%) patients achieved neutrophil engraftment at the end of the follow-up time, both in the MSC and control group, respectively. Three patients in the control group died before engraftment. The reason of death was intracerebral hemorrhage (ICH) in one and cardiac toxicity in two patients.

**Neutrophil engraftment**

The median time to ANC recovery was 16 days for both groups (range: 5-40 days in MSC group versus range: 10-73 days in control group). By day 30 after transplantation, the cumulative incidence of neutrophil recovery was 97% (95% CI: 89%-100%) and 76% (95% CI: 64%-88%) in MSC and control group, respectively ($P=.147$). The cumulative incidence probability of ANC engraftment is shown in Fig. 1.

In the univariate analysis, peripheral blood stem cell (PBSC) source ($P<.001$) along with higher number of infused mono-nucleated cells (MNC) ($P<.001$) were associated with more incidence of ANC recovery. There was a strong association between number of MNC infusion and stem cell source, but due to missing data on MNC variable, only the stem cell source was entered in the multivariate analyses. Cumulative incidence of neutrophil engraftment was not related to the patient’s age and sex ($P=.168$ and .272, respectively) and also donor’s age and sex ($P=.196$ and .764, respectively).

For assessing the effect of MSC infusion on the ANC engraftment rate, which was adjusted for stem cell source, we considered the Fine and Gray model. The model showed that the bone marrow (BM) stem cell source had a lower incidence rate of engraftment (HR=.357, $P=.001$), but the MSC infusion had no effect (HR=1.105, $P=.688$) (Table 2). We checked the proportionality assumption and the analyses showed a non-proportional effect of MSC infusion on the SH ($P<.001$ and $P=.056$ for the proportionality of MSC infusion and stem cell source effect, respectively). To accommodate the time-varying effect of MSC infusion on the SH, we fit a piecewise constant sub distribution hazard model. With the cut point of 16 days, the analysis showed that the MSC infusion had a positive delay effect on ANC recovery and patients who received MSC had significantly faster recovery after 16 days of the transplantation (HR=2.68, $P=.025$) (Table 2).

**Table 2:** Estimated hazard ratio and 95% confidence interval for different models of sub distribution hazard of ANC engraftment

| Models/Factors                        | SHR  | 95% CI   | P-value |
|--------------------------------------|------|----------|---------|
| Fine-Gray model †                    |      |          |         |
| MSCs infusion (yes)                  | 1.11 | .68-1.80 | .688    |
| Stem cell source (BM)                | .36  | .19-.66  | .001    |
| Cox-Aalen model                      |      |          |         |
| MSCs infusion (yes)                  |      | Time varying effect | .044 |
| Stem cell source (BM)                | .35  | .19-.64  | <.001   |
| Piecewise constant proportional model|      |          |         |
| MSCs infusion (yes) <=16 days        | .85  | .48-1.53 | .600    |
| >16 days                             | 2.68 | 1.13-6.32| .025    |
| Stem cell source (BM)                | .57  | .31-1.06 | .074    |

SHR indicates subdistribution hazard ratio; MSC, mesenchymal stem cell; BM, bone marrow.

† The P-values for the test of proportionality are .008 and .088 for MSC infusion and stem cell source covariates, respectively which indicate the non proportionality of MSC infusion effect.
Without selection of an arbitrary cut time point, we fit a Cox-Aalen model. Figure 2 shows the estimated cumulative regression functions and their 95% confidence bands. The plot (a) indicate that the MSC infusion had a time varying effect and a significant delay effect on ANC engraftment ($P=.008$ for time varying effect and $P=.044$ for the significant effect of MSCs). In addition, the plot (b) are evident that the stem cell source had a constant significant effect over time ($P=.088$ for time varying effect and $P<.001$ for the significant effect of stem cell source) (Table 2).

**Discussion**

The allogeneic hematopoietic stem cell transplantation have used the bone marrow derived MSCs in patients with hematologic and non-hematologic malignancies (3,4). As the MSCs can support hematopoiesis after transplantation, in this study we assessed the neutrophil engraftment time as the early outcome of HSCT on class III thalassemia patients who received MSCs and compared their results with patients who did not. There are limited published studies about the clinical outcomes of MSCs in the HSCT. Koc et al. performed a trial on the 28 patients with breast cancer to determine feasibility, safety, and hematopoietic effects of culture-expanded autologous MSCs after a high-dose chemotherapy and autologous HSCT. Autologous MSCs were infused without any toxicity and hematopoietic recovery was rapid (18). In a multicenter clinical trial, culture-expanded allogeneic MSCs derived from BM of HLA-identical sibling donors were infused in 46 patients undergoing myeloablative HSCT for various hematological malignancies (19). There were no infusion-related toxicities. The median times of both groups to engraftment of neutrophils $\geq 500/\mu$L were 14 days and in comparison with historical controls, no acceleration of hematopoietic engraftment was observed. These studies had some limitations such as few numbers of patients and different underlying diseases, and preparative regimen or GvHD prophylaxis, which may affect the results. Our study was conducted on homogeneous patients concerning the clinical class of thalassemia patients, conditioning regimen and GvHD prophylaxis.

In our study, three patients died early after transplantation in the control group but none in the MSC group. The median days of neutrophil engraftment were 16 days in both MSC and control groups which is comparable with other studies on thalassemia patients(20,21). Furthermore, this result was similar to the result mentioned by the Lazarus et al., which indicates no difference between groups in term of median days of neutrophil engraftment. However, Koc et al. reported rapid hematopoietic recovery in their patients, but their study was not a randomized clinical trial (18).

In addition, our results showed that by day 30, the cumulative incidence of neutrophil engraftment was higher in MSC group versus control group, which indicate higher rate of engraftment for patients with delayed ANC recovery in MSC group. This result may highlight the usefulness of MSCs only for patients with high risk of delayed engraftment. However, benefits of MSCs about other HSCT complications such as GvHD should be considered.

In line to previous studies on HSCT, our study showed that PBSCT was related to faster engraftment independent of MSC factor (22,23). One explanation could be the higher number of cell dose transplanted in PBSC transplantations.

In our data, there were problems with the proportionality regards to MSC groups, indicating that the fit of proportional model are not satisfactory. Without checking the proportionality assumption, the rate of ANC engraftment was not differing between two MSC groups adjusted for stem cell source. One possible strategy to deal with non-proportionality would be piecewise constant proportional hazard regression. It has been demonstrated that by selecting an arbitrary cut point choosing from the data, the P value from this procedure may be suspected, unless very small P values (6). Therefore, we used the multiplicative-additive models as another flexible strategy for handling the time varying effects (12-
In these models instead of having simple baseline intensity, Aalen’s additive model applied as its covariate dependent baseline, which can handle the time varying effect. Applying the Cox-Aalen model to our data confirmed the results from the piecewise constant model, but the estimations from Cox-Aalen model have relatively smaller standard errors and so narrower confidence intervals rather than piecewise constant time varying effect and sub distribution hazard model (Table 2).

Some limitations of our study were retrospective information of control group and relatively small sample size. Considering the limitations of our study, we suggest further studies to evaluate the advantages and disadvantages of MSCs for short and long-term outcomes of transplantation in patients with nonmalignant disease. In addition, in our data as the time-varying behavior of the MSC infusion on the ANC engraftment was significant; the knowledge of this structure in the data was preferred. Using this knowledge, showed a significant effect of the MSC infusion on the incidence of the engraftment which is in concordance with the MSCs potential effect. It was not seen from the applied proportional models. These results indicate that as the Cox-Aalen models can be fitted easily, it would be more appropriate approach for the similar situations as our data.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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