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Bone marrow toxicity

Marrow damage and hematopoietic recovery following allogeneic bone marrow transplantation for acute leukemias: Effect of radiation dose and conditioning regimen

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ABSTRACT

Background and purpose: Total body irradiation (TBI) is a common component of hematopoietic cell transplantation (HCT) conditioning regimens. Preclinical studies suggest prolonged bone marrow (BM) injury after TBI could contribute to impaired engraftment and poor hematopoietic function.

Materials and methods: We studied the longitudinal changes in the marrow environment in patients receiving allogeneic HCT with myeloablative (MA, $n = 42$) and reduced intensity (RIC, $n = 56$) doses of TBI from 2003–2013, including BM cellularity, histologic features of injury and repair, hematologic and immunologic recovery.

Results: Following MA conditioning, a 30% decrease in the marrow cellularity persisted at 1 year post-transplant ($p = 0.03$). RIC HCT marrow cellularity transiently decreased but returned to baseline by 6 months even though the RIC group received mostly umbilical cord blood (UCB) grafts (82%, vs. 17% in the MA cohort, $p < 0.01$). There was no evidence of persistent marrow vascular damage or inflammation. Recipients of more intensive conditioning did not show more persistent cytopenias with the exception of a tendency for minimal thrombocytopenia. Immune recovery was similar between MA and RIC.

Conclusions: These findings suggest that TBI associated with MA conditioning leads to prolonged reductions in marrow cellularity, but does not show additional histological evidence of long-term injury, which is further supported by similar peripheral counts and immunologic recovery.

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Total body irradiation (TBI) is commonly used in the treatment of advanced or high-risk hematologic malignancies as a component of the preparative regimen prior to allogeneic hematopoietic cell transplantation (HCT). Historically, myeloablative (MA) transplant regimens have included high doses of cytotoxic chemotherapy and/or TBI with the goal of eradicating residual disease and achieving immunosuppression to allow for donor stem cell engraftment. Increasing the intensity of the conditioning regimen has been shown to reduce the risk of disease relapse albeit with a corresponding increase in the severity of the acute treatment-related toxicities [1].

Over the past two decades, reduced intensity conditioning (RIC) regimens have emerged as lower toxicity alternatives, especially in older patients or those with significant medical comorbidities. Reduced treatment-related mortality has followed HCT using these regimens [2–4]. In contrast, the development of novel targeted approaches concentrating the dose of radiation to the marrow (total marrow irradiation, or TMI) explored by our group and others is designed to maximize tumor killing while sparing more radiosensitive tissues that typically limit radiation dose [5–7]. Such novel approaches may extend the curative potential of allogeneic HCT to patients who may otherwise be at substantial relapse risk (e.g., high-risk myelodysplastic syndromes or acute leukemias with minimal residual disease prior to HCT). As the spectrum of treatment options expands from RIC to novel, dose-escalated, marrow-targeted therapeutic radiation protocols and both are fur-

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ther integrated with chemotherapy, it becomes critical to understand the long term impact of different treatments on hematopoietic competence and marrow environment health.

Pre-clinical studies reveal prolonged bone marrow injury after TBI [8]. Specifically, the bone marrow demonstrates an increase in the number of adipocytes and a corresponding decrease in hematopoietic precursors following exposure to chemotherapy or ionizing radiation [9–11]. Following HCT, expanded marrow adipocyte populations have also been demonstrated to negatively influence post-transplant hematopoietic engraftment in a murine model [12]. Conditioning-induced structural and functional marrow damage beyond the necessary immunosuppression for successful engraftment is not well understood in clinical HCT. While numerous studies have examined treatment responses in a variety of diseases utilizing either MA or RIC regimens [13–16], less is known regarding the bone marrow microenvironment, and the relative effect of the pre-transplant irradiation dose on hematopoietic engraftment and recovery [9–11]. Furthermore, it is unclear whether post-transplantation peripheral blood cell counts accurately reflect the extent of marrow damage. Several prior studies have shown that structural changes to the marrow environment may lead to hematopoietic dysfunction [17,18] as well as decreased bone mineralization and higher fracture risk [19–21].

We examined the features of marrow in response to TBI-containing MA and RIC regimens in a cohort of patients undergoing HCT for treatment of advanced or high-risk leukemia. We additionally explored the hematopoietic and immunological recovery to determine if there is a demonstrable effect of the conditioning regimen on the bone marrow histological structure and function over time. We correlated the degree of structural marrow change with hematopoietic function as demonstrated by peripheral blood cell production to better characterize marrow response and recovery following two commonly-utilized treatment regimens. This will inform clinical decision-making in the selection of appropriate conditioning protocols, including the optimization of novel TMI regimens.

Methods

Patients

Medical records from 98 patients (47 male, 51 female) receiving allogeneic HCT using either TBI-containing MA or RIC regimens at the University of Minnesota between January 2003 and June 2013 were reviewed. Patients were limited to age 45–55 at HCT to avoid confounding age-related differences in marrow cellularity [22,23].

Preparative regimens

MA conditioning included cyclophosphamide 60 mg/kg on day-6 and -5 pre-HCT followed by 1320 cGy TBI delivered in 8 twice-daily fractions. Those receiving umbilical cord blood (UCB) grafts had the same MA conditioning, but with the addition of fludarabine 25 mg/m^2/day $\times$ 3 days. Patients receiving RIC regimens received cyclophosphamide 50 mg/kg on day-6 in addition to fludarabine 40 mg/m^2 on day-6 to -2 pre-transplant followed by TBI delivered in a single 200 cGy fraction on day-1. Total body radiotherapy for both MA and RIC groups was delivered via an opposed lateral technique [24]. Custom aluminum compensators for head/neck, lung and legs to account for the transverse tissue deficit were used to provide a uniform dose homogeneity within 10% of the prescribed dose.

Anti-thymocyte globulin was added to patients receiving UCB grafts without prior exposure to combination chemotherapy within 3 months prior to transplantation. Due to the retrospective nature of the study, there was heterogeneity in the patient populations receiving MA and RIC in addition to differences in the treatment protocols. We therefore analyzed the groups separately. The characteristics of MA and RIC patients including diagnosis, disease risk, prior autologous transplants, performance status and CMV serostatus were extracted from the medical records and reported.

Transplant procedures and engraftment

The graft and graft versus host disease (GVHD) prophylaxis protocols were extracted from the medical records for each group of patients. Factors impacting engraftment in each group were evaluated.

Bone marrow analysis

Iliac crest bone marrow specimens for each patient were examined at baseline prior to pre-transplant conditioning and at 21 days, 6 months and 1 year post-transplant according to the normal monitoring protocol for our institution. The average bone marrow cellularity for each specimen was determined from original clinical bone marrow biopsy reports. Data were censored for specimens that demonstrated evidence of recurrent leukemia or if the patient had undergone treatment for relapsed disease prior to specimen collection. The white blood count (WBC), absolute neutrophil count (ANC), hemoglobin and platelet count were also obtained for each patient on the same dates as the bone marrow collection. A subset of patients randomly selected from those having excellent quality archived bone marrow core biopsy slides available for review (i.e. large specimen, minimal artifacts that would interfere with interpretation). (10 MA, 10 RIC) underwent more comprehensive microscopic evaluation of the primary bone marrow biopsy samples. The goal was to elucidate changes in bone marrow environment not routinely characterized in detail during clinical evaluation of marrow sections that might be relevant to long term changes in the hematopoietic microenvironment. In addition to standard examination of the hematopoietic component, marrow samples were evaluated for vascular changes (edema, congestion, sinusoidal dilation, vasculitis), evidence of inflammation and necrosis, hemosiderosis, and fibrohistiocytic proliferation. For each characteristic, samples were scored as not present (0), mild (1), moderate (2), marked (3) or severe (4). All samples were evaluated by a single investigator (LS) and then independently verified by a second board certified hematopathologist (SY).

Immune reconstitution

Samples were prospectively analyzed for the absolute numbers of lymphocytes, NK cell (CD3−CD56+), T cells (CD3+ lymphocytes), CD4+ T cells (CD3+CD4+ cells), CD8+ T cells (CD3+CD8+) and B cells (CD19+ lymphocytes) at 3, 6 and 12 months according to an institutional immune monitoring protocol. Comparison of analytes by conditioning at individual time-points was carried out by the non-parametric General-Wilcoxon test. Comparison of the overall average level of each cell type was evaluated at each time point among only those patients without non-relapse mortality prior to 1 year. Values for each cell type were transformed by the natural logarithm to yield a normal distribution assumption in the repeated measures analysis. Linear mixed models was employed to evaluate the repeated measures of each cell type using time from transplant as a continuous variable.

Definitions and statistical analysis

Neutrophil engraftment, defined as 3 consecutive days with ANC $\geqslant 0.5 \times 10^9$/L, was determined for each patient. In order to