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Severe Cytokine-Release Syndrome after T Cell–Replete Peripheral Blood Haploidentical Donor Transplantation Is Associated with Poor Survival and Anti–IL-6 Therapy Is Safe and Well Tolerated

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Use of high-dose post-transplantation cyclophosphamide for graft-versus-host disease prophylaxis has expanded the use of unmanipulated haploidentical hematopoietic cell transplantation. The immediate post-transplantation course in T cell–replete peripheral blood haploidentical hematopoietic cell transplantation (haplo-HCT) is often complicated by symptoms resembling cytokine-release syndrome (CRS), previously described in recipients of targeted cellular therapeutics. However, we know little about the incidence and impact of CRS on outcomes in these patients. To understand this syndrome in haplo-HCT patients, we reviewed data from 75 consecutive patients who received granulocyte colony–stimulating factor–mobilized T cell–replete peripheral blood haplo-HCT at a single center. Using CRS criteria described in recipients of chimeric antigen receptor T cell therapies, we found 65 of 75 (87%) met criteria for CRS, although most cases were only mild (grades 1 or 2). However, 9 patients (12%) experienced severe (grades 3 or 4) CRS. Median survival was 2.6 months (95% confidence interval [CI], .43 to 5.8) in patients with severe CRS, compared with 13.1 months (95% CI, 8.1 to not reached) in patients with mild CRS. Transplantation-related mortality was worse in the severe CRS cohort with a hazard ratio of 4.59 (95% CI, 1.43 to 14.67) compared with that in the mild CRS cohort. Severe CRS patients had a significant delay in median time for neutrophil engraftment. Serum IL-6 levels were measured in 10 haplo-HCT patients and were elevated in the early post-transplantation setting. Seven patients with CRS were treated with tocilizumab, resulting in a complete resolution of their CRS symptoms. Severe CRS represents a potential complication of peripheral blood haplo-HCT and is associated with worse outcomes. Anti–IL-6 receptor therapy is associated with rapid resolution of the CRS symptoms.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a cornerstone of therapy for hematologic malignancies, often constituting the only curative intent treatment available. HLA-matched sibling donors have historically offered the best clinical results. HLA-matched unrelated donors are traditionally considered second line, but availability is limited, especially for ethnic minorities [1,2]. In contrast, the majority of patients have readily available related haploidentical donors. Therefore, haploidentical HCT (haplo-HCT) offers a crucial alternative to traditional HLA-matched HCT. Several recent studies have shown that haplo-HCT patients have outcomes equivalent to those of HLA-matched unrelated donor transplantations [3,4]. Recent advances utilizing post-transplantation cyclophosphamide (PTCy) have allowed for selective depletion of post-transplantation alloreactive T cells while maintaining the graft-versus-leukemia effect and acceptable rates of graft-versus-host disease (GVHD) among recipients of haplo-HCT [3,5-9]. The most common source for haplo-HCT donor grafts is donor bone marrow, but peripheral blood constitutes an emerging option that many consider more convenient and less invasive for donors. Accompanying peripheral blood stem cells

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as a donor option are larger recipient T cell doses, which may bring added toxicities [3,5,10,11]. Previous studies comparing peripheral blood to bone marrow grafts in other settings have demonstrated improved engraftment but higher rates of chronic GVHD [10,11], but data in the haploidentical setting are lacking.

The syndrome of systemic inflammation—fevers, vascular leak, hypotension, and respiratory and renal insufficiency—in the context of elevated inflammatory markers and cytokine levels has previously been described as cytokine-release syndrome (CRS) [12-14]. CRS is characterized by high levels of inflammatory cytokines, including IL-6, interferon-γ, IL-2, and high peaks of C-reactive protein (CRP) that result from robust activation of the immune system. This syndrome was originally described after monoclonal antibody therapy and is now recognized as a common toxicity after chimeric antigen receptor (CAR) T cell cellular therapies [13-21]. A CRS grading system has been proposed by Lee et al., allowing the quantification of CRS symptoms, and has been employed in the CAR T cell literature [13]. Neurotoxicity is a common and highly morbid clinical feature of CRS that is supported by the literature [15,16,22,23]. This is captured in the Lee system under the catch-all term "organ toxicity," but it is not specifically broken out as a potential adverse effect. Given its central role in the pathophysiology of CRS, anti–IL-6 and anti–IL-6 receptor therapies such as tocilizumab have been used to disrupt the toxic effects associated with CRS [14,24]. Tocilizumab treatment of CRS after CAR T cell infusion has been shown to result in rapid defervescence and stabilization of blood pressure within 48 hours [14,21].

Multiple clinical series have reported an increased incidence of high-grade fever early after haplo-HCT [25-28]. Many of these patients lacked documented infection and recent evidence has implicated IL-6 in this post-transplantation systemic response [13,14,21,29]. Although these papers have described CRS symptoms among haplo-HCT patients in the post-transplantation period, they have not evaluated its impact on a patient's long-term clinical course and outcomes. With the increasing role of haplo-HCT, including patients with active disease in need of expedient HCT, understanding the unique complications of this transplantation approach and their effects on long-term outcomes is increasingly important [4,28]. Consequently, we performed a retrospective study to assess the incidence, severity, and impact of CRS on clinical outcomes in haplo-HCT patients. We also prospectively assessed IL-6 and other cytokine levels in 10 haplo-HCT recipients. Finally, we treated 7 haplo-HCT patients suffering from CRS with the IL-6–receptor antagonist tocilizumab and monitored their clinical response.

METHODS

Collection of Data

All patients who underwent granulocyte colony–stimulating factor–(G-CSF) mobilized T cell–replete peripheral blood haplo-HCT at Washington University in St. Louis between July 7, 2009 and April 28, 2015 were retrospectively identified using institutional databases. Patients with microbiologically confirmed infection before day +14 were excluded from the analysis. GVHD prophylaxis for all patients was with PTCy administered on days +3 and +4 and with mycophenolate mofetil starting on day +5. Detailed clinical data from post-transplantation days 0 to 14 were collected and used to grade CRS. These data included fever curves, vital signs, renal and hepatic function tests, CRP levels, the development of vasopressor dependence, oxygen requirement, and the need for mechanical ventilation. Pre- and post-transplantation ejection fractions from echocardiogram and or nuclear perfusion study results were also collected. Given the successful treatment of CRS with tocilizumab in other settings, 7 patients were treated clinically for severe CRS with this agent at a dose of 4 mg/kg of actual body weight and were included in our retrospective cohort.

To better characterize the cytokine milieu after haplo-HCT, 10 consecutive patients were recruited to undergo prospective cytokine analysis. After informed consent was obtained, serum cytokine levels were measured on day 0 (before transplantation) and on post-transplantation days +1 through +5 in these patients. This study was approved by the institutional review board and conducted in accordance with the declaration of Helsinki.

Analysis of Serum Cytokines

Peripheral blood was allowed to clot for 30 to 60 minutes before centrifugation at room temperature for 15 minutes at 2500 × g. The serum was removed, centrifuged at 10,000 × g for 10 minutes at 4°C and stored at –80°C. Frozen samples were thawed completely, vortexed, and centrifuged before use to remove particulates. Levels of G-CSF, granulocyte-macrophage–colony stimulating factor, IFNα2, IFNγ, IL-10, IL-12P70, IL-13, IL-15, IL-17A, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and TNFα in plasma were determined using a Milliplex Map Kit (EMD Millipore, Billerica, MA) according to the manufacturer's instructions. Briefly, fluorescent capture antibody-coated beads were mixed with standard or sample, incubated overnight at 4°C on an orbital shaker, washed, and then incubated with a biotinylated detection antibody. After washing, beads were incubated with a streptavidin phycoerythrin complex and the mean fluorescent intensities quantified on a Bio-Plex 200 system (Bio-Rad, Hercules, CA). All samples were measured in duplicate and data were analyzed using the Bio-Plex Manager software (Bio-Rad) and a 5-parameter logistic curve fit.

Definitions

CRS was graded according to the criteria shown in Table 1. This is based on the standards originally proposed by Lee et al. but was modified to highlight altered mental status and new-onset cardiomyopathy, which are common and potentially devastating sequelae of CRS [13]. Symptoms occurring before day +14 were included. CRS was distinguished from engraftment syndrome, which can also present as noninfectious fever and capillary leak, by onset before day +14 and the absence of skin rash [30]. Because CRS can also resemble sepsis syndrome, patients with a documented infection and clinical suspicion of sepsis did not receive a CRS grade and were excluded from the analysis. *Overall survival* (OS) was defined as the time from transplantation until death, and patients alive at the time of last follow-up were censored. Cause of death was determined by review of clinical notes, institutional records, and autopsy reports. *Neutrophil engraftment* was defined as the first of 3 consecutive days with an absolute neutrophil count > 500 cells/uL. *Platelet engraftment* was defined as the first of 3 consecutive days > 20,000 platelets/uL with no transfusion support in the previous 2 weeks. Patients who died with active disease were classified as cases of *disease-related mortality*. Patients who died before day +28 from any cause and patients who died after day +28 with no evidence of disease were classified as cases of *transplantation-related mortality* (TRM). Acute GVHD was graded according to the modified Keystone criteria [31]. Chronic GVHD was graded as limited or extensive according to published criteria [32]. To adjust for patients' underlying health status, the hematopoietic cell transplantation–specific comorbidity index (HCT-CI) was used [33].

Table 1
Grading of Cytokine Release Syndrome. Adapted from Lee et al. [11]

Grade 1	Symptoms are not life threatening and require symptomatic treatment alone. Includes fever, nausea, fatigue, malaise.
Grade 2	Symptoms require and respond to limited intervention: - Oxygen requirement <40%, ≤ 3 L nasal cannula or - Hypotension responsive to fluids or low dose of 1 vasopressor or - Grade 2 renal or hepatic toxicity
Grade 3	Symptoms require and respond to aggressive intervention: - Oxygen requirement ≥40%, >3 L nasal cannula or - Hypotension requiring high dose or multiple vasopressors or - Grade 3 renal toxicity or grade 4 transaminitis New-onset altered mental status without other explanation* New cardiomyopathy without wall motion abnormality
Grade 4	Life-threatening symptoms: - Requirement for ventilator support or - Grade 4 renal toxicity (excluding transaminitis)
Grade 5	Death

* Altered mental status of sufficient severity to warrant investigation by head imaging and or lumbar puncture.