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Timeline, Epidemiology, and Risk Factors for Bacterial, Fungal, and Viral Infections in Children and Adolescents after Allogeneic Hematopoietic Stem Cell Transplantation

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Advances made in the field of hematopoietic stem cell transplantations (HSCT) over the past 20 years may have had an impact on the distribution of posttransplantation infections. We sought to retrospectively analyze the epidemiology and risk factors for bacterial, fungal, and viral infections in children after allogeneic HSCT in a cohort of 759 children who underwent allogeneic HSCT in a single institution between 1990 and 2009. The association between infections and risk factors of interest at 0 to 30 days, 31 to 100 days, and 101 days to 2 years posttransplantation was evaluated using logistic regression. Difference among the subtypes within each category was studied. There were 243 matched-related donors, 239 matched-unrelated donors (MUDs), and 176 haploidentical donor transplantations. Era of transplantation (0-30 days), peripheral blood stem cell product, acute graft-versus-host disease (aGVHD; 31-100 days), and chronic GVHD (cGVHD; 101-730 days) were associated with higher risk for bacterial infections at the respective time periods. Patients with aGVHD (31-100 days), cGVHD, and older age (101-730 days) were at higher risk for fungal infections. Cytomegalovirus (CMV) donor/recipient (D/R) serostatus (0-100 days), era of transplantation, MUD HSCT (31-100 days), and cGVHD (101-730 days), influenced viral infections. Gram-positive outnumbered gram-negative bacterial infections; aspergillosis and candidemia were equally prevalent in all time periods. Haploidentical donor HSCT was not associated with an increased risk of infections. There seems to be a continuum in the timeline of infections posttransplantation, with bacterial, fungal, and viral infections prevalent in all time periods, particularly late after the transplantation, the risk affected by GVHD, CMV, D/R status, product type, older age, and use of unrelated donors.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment modality for patients with certain hematologic malignancies and for many congenital and acquired disorders of the hematopoietic system. Infections contribute significantly to the morbidity and mortality from this procedure. The manner in which the transplantation is performed has changed substantially over the past 2 decades. This may have had an impact on the type of pathogens and timeline of infections posttransplantation.

Data predominantly from the adult literature show that gram-positive (GP) and gram-negative (GN) bacteria [1], *Candida* and *Aspergillus spp* [2], are major pathogens in the pre-engraftment phase. With neutrophil recovery, bacterial infections decrease, and infections by cytomegalovirus (CMV) [3] and fungi predominate, particularly in association with graft-versus-host disease (GVHD) [4]. The risk of invasive fungal infections in the late recovery phase increases with age, CMV infection, GVHD, and in recipients of matched-unrelated donor (MUD) transplantation [5,6]. With the use of pre-emptive therapy, disease due to CMV [3], and herpes simplex virus (HSV) [7] has shifted to the late recovery phase. Haploidentical donor transplantations have been reported to influence the risk of fungal [8] and adenoviral (ADV) [9] infections in this phase. Total-body irradiation (TBI) has been associated with an increased risk of symptomatic parainfluenza virus (PIV) [10] and ADV infection [11].

Thus, there is a complex interplay of factors including patient demographics, neutropenia, GVHD, transplantation modality, TBI, product type, CMV donor/recipient (D/R) status, and era of transplantation, contributing to infection risk. The effect of these factors on risk of bacterial, fungal, and viral infection, at 0 to 30 days, 31 to 100 days, and 101 days to 2 years posttransplantation, after adjusting for confounding variables, has not been well studied both in children and adults. This is the largest retrospective study providing a timeline of infections in young HSCT recipients.

PATIENTS AND METHODS

This retrospective cohort consisted of 786 patients who underwent a first allogeneic HSCT over a 20-year period (January 1990 through December 2009) at St. Jude Children's Research Hospital (SJCRH). Patients who were older than 21 years of age at transplantation (19 patients) and those who underwent a cord blood transplantation (8 patients) were excluded. A total of 759 patients were included in this analysis. The study was approved by the SJCRH Institutional Review Board. Patients were followed for 10 years after transplantation or until age 18 years whichever was later. Follow-up was frequent in the first 2 years after transplantation and yearly thereafter. The mean duration of follow-up for surviving patients was 7.96 years (range, 0.21-19.91 years).

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Microbiologic Methods

Microbiologic records were reviewed to identify patients with documented bacterial, fungal, and viral infections as diagnosed by culture (bacteria, fungi, viruses), direct fluorescent antibody testing, and PCR (viruses). Infection was defined as isolation or detection of an organism that was associated with symptoms or disease and included fungal and viral pathogens detected on pre-emptive screening. Colonization detected in surveillance cultures and positive blood cultures due to contamination were not considered as infections. Infections with the same organism occurring more than 14 days after the last negative culture or diagnostic test were recorded as 2 separate infectious episodes. Only the first episode of bacteremia and the first episode of GP, GN, yeast, mold, and specific viral infection in a given patient during the specified time period was included in the analyses. The day of onset of infection was defined as the day when the first positive diagnostic sample was collected. Infection as the primary cause of death was used for analyses. In patients who relapsed from their underlying disease, or those with GVHD who died from infection, infection was not noted as the primary cause of death.

Peripheral blood samples were collected once weekly starting in February 2000 to prospectively screen for CMV and starting in February and July 2002 to prospectively screen for Epstein-Barr virus (EBV) and ADV by quantitative real-time PCR, as previously described [12], using an ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA). Before 2000, CMV and ADV were detected by viral blood culture and EBV using endpoint PCR with detection by Southern blot. Weekly screening on whole blood for galactomannan was performed from August 2003. Pre-emptive treatment with ganciclovir, rituximab, and cidofovir was given based on the results of surveillance testing. Invasive fungal infection was defined according to accepted criteria [13]. Patients who met the definition of possible invasive fungal infections were not included.

Infection Prophylaxis

All patients received prophylaxis against *Pneumocystis jirovecii* for up to 1 year posttransplantation. Between 1993 and 2000, patients who were seropositive for CMV or had a seropositive donor received ganciclovir until day +120. After 2000, patients at risk for CMV or HSV reactivation received acyclovir prophylaxis until 1 year posttransplantation. Patients received antifungal prophylaxis with amphotericin B or lipid-based amphotericin formulations between 1990 and 2003. Subsequently, prophylaxis was with echinocandins until engraftment and with voriconazole thereafter. Antibacterial prophylaxis with fluoroquinolones was not given. Patients with chronic GVHD (cGVHD) received PenVK, cotrimoxazole, acyclovir, and voriconazole prophylaxis.

Era of Transplantation

The population was divided into 4 eras. There were 111, 218, 219, and 211 patients who underwent HSCT in the eras 1990 to 1994, 1995 to 1999, 2000 to 2004, and 2005 to 2009, respectively. The years 2000 onward represented a boundary for introduction of peripheral blood stem cell (PBSC) products, viral monitoring by quantitative real-time PCR, testing for galactomannan, and use of haploidentical donors.

Transplantation Methods

Transplantation-related variables were abstracted from a prospectively collected database that included patient demographics, underlying diagnosis, remission status, donor and product type, CMV D/R status, conditioning regimen, GVHD prophylaxis, and grading if present. The conditioning and GVHD prophylaxis have been previously described [14,15]. A regimen based on TBI and cyclophosphamide was used for 326 of the 427 patients (76%) with acute leukemia. Cyclosporine with methotrexate or mycophenolate mofetil was predominantly used for GVHD prophylaxis. For haploidentical donor transplantations, 49 patients (28%) received TBI-based conditioning; the remaining 127 patients (72%) received fludarabine, thiotepa, and melphalan-based conditioning. Ex vivo T cell depletion of the graft was performed by anti-T cell Abs and complement in 17 patients (10%) or immune-magnetic selection using the Miltenyi CliniMACS system (Miltenyi Biotec GmbH, Teterow, Germany) in 159 patients (90%), respectively. Patients received GVHD prophylaxis with either a calcineurin inhibitor or mycophenolate mofetil. The day of engraftment was defined as the first of 3 consecutive days of achieving an absolute neutrophil count >500 cells/μL. Assessment of acute GVHD (aGVHD) was based on consensus criteria [16]. The aGVHD grade 3 and 4 were classified as severe. Immunization was according to standard recommendations [17] starting 1 year posttransplantation.

Statistical Analysis

The association among infections (bacterial, fungal, and viral) and the risk factors of interest was first evaluated using univariate logistic regression. The risk factors that were evaluated included age at transplantation, era of transplantation, donor type (haplo vs non-haplo identical), product type (PBSC vs marrow), T cell depletion, aGVHD (≥grade 3 vs others), cGVHD, TBI, myeloablative vs reduced-intensity conditioning (RIC), CMV D/R status (+/+ vs +/− vs −/+ vs −/−), and MUD vs matched-related donor (MRD) transplantation. To identify the exact sources of differences among eras and CMV status, Bonferroni correction was used to adjust for multiple comparisons, and P values were noted to be significant at level $\alpha = 0.05/6 = 0.008$. The analyses for each type of infection were conducted independently. All the factors that were significant at level $\alpha = 0.15$ in the univariate analyses were included in the multiple logistic regression models. The final model reports the results of all factors that remained significant at the 5% level.

The association between cumulative incidence of infectious deaths and the risk factors described above were assessed using Fine and Gray's approach [18] within the framework of the Cox proportional hazards model. Deaths due to any other cause were treated as the competing events, and research participants alive at the last follow-up were considered as censored events.

The cumulative incidence of bacteremia within 30 days post-transplantation was defined as the time from the date of transplantation to the first episode of bacteremia within 30 days, with death within 30 days treated as a competing event. Patients who survived 30 days posttransplantation without bacteremia were treated as censored. Cumulative incidence of bacteremia within 30 days among different eras was estimated as described by Kalbfleisch and Prentice [19] and compared using Gray's test [20].

Chi-square tests were used to test whether there was a significant difference among the proportions of different subtypes within each category of interest (ie, GP vs GN; yeast vs mold; Candidemia vs *Aspergillus*; HSV vs CMV vs EBV vs ADV; PIV vs influenza vs respiratory syncytial virus (RSV) within each time period. SAS version 9.2 (SAS Institute, Cary, NC) and StatXact (Cytel Corporation, Cambridge, MA) Windows version 8 were used for statistical analyses.

RESULTS

Patient Characteristics

A total of 759 patients underwent an allogeneic HSCT between1990 to 2009 at SJCRH. Demographics and patient characteristics are presented in Table 1.

Infections in the Posttransplantation Period

Infections were documented in 621 patients (82%). GP infections were more prevalent than GN infections in all 3 time periods posttransplantation (Table 2, Figure 1). There were 225 patients (30%) with bacteremia including 133 patients with GP and 92 patients with GN bacteremia. The median onset of bacteremia was 87 days posttransplantation. *Staphylococcus epidermidis* 70 cases (31%) and viridians streptococci 16 cases (7%) were the most common GP organisms and *Klebsiella spp* 18 cases (8%), *Escherichia coli* 17 cases (8%), and *Pseudomonas spp* 14 cases (6%) were the most common GN organisms causing bacteremia. There were 110 patients (14%) with *C. difficile* infection, and 13 patients (2%) with *Enterococcus spp* bacteremia in the entire cohort including 6 patients with *E. faecalis* bacteremia, which were vancomycin-susceptible, and 7 patients with *E. faecium* bacteremia, which were vancomycin-resistant.

Yeasts and molds were equally prevalent in all 3 time periods posttransplantation (Table 2, Figure 1). Candidemia was detected in 26 patients (3%). *Candida albicans* and non-*albicans Candida spp* were equally prevalent. *C. parapsilosis* and *C. glabrata* were the most common non-*albicans Candida spp* isolated. In 49 patients (6%) with proven aspergillosis, *Aspergillus flavus* and *A. fumigatus* were the most frequent isolates, in 17 and 10 patients, respectively. A probable diagnosis of invasive fungal infection was made in 40 patients (5%). Candidemia and aspergillosis were equally prevalent in all 3 time periods posttransplantation (Figure 1).

The distribution of HSV, CMV, EBV, and ADV at 0 to 30 days posttransplantation was significant (Figure 1A). HSV infections were more common than ADV and EBV infections ($P < .0001$). Infections by EBV were also less common than