Risk factors for syngeneic graft-versus-host disease after adult hematopoietic cell transplantation

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Syngeneic graft-versus-host disease (sGVHD) has been described after hematopoietic cell transplantation (HCT) but remains poorly defined. We retrospectively reviewed adult syngeneic HCTs at our center (1980-2002) for sGVHD to investigate incidence, morbidity, and risk factors with a primary focus on parity. Among 119 transplantations, there were 21 cases of biopsy-proven sGVHD. The cumulative incidence was 18%, with multiorgan involvement in 6 cases and 1 death. sGVHD was more frequent when the donor was parous (32%) than nulliparous (9%) or male (13%; $P = .03$) and when the recipient was parous (31%) than nulliparous (7%) or male (13%; $P = .02$). Other univariable risk factors included older age ($P < .01$), busulfan/melphalan/thiotepa conditioning ($P < .01$), interleukin-2 ($P = .02$), HLA-A26 ($P = .03$), and more recent transplantation year ($P < .01$). Overall, risk factors were similar to those described in GVHD. Although an independent effect of parity could not be completely separated from other factors, donor and recipient pregnancy history merits further investigation. (Blood. 2004; 104:1894-1897)

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Introduction

Cutaneous and gastrointestinal graft-versus-host disease (GVHD) are described after syngeneic hematopoietic cell transplantation (HCT), but the occurrence of syngeneic GVHD (sGVHD) remains controversial and seems to defy the concept that the host must appear "foreign" to the graft.[1-8] Although a number of mechanisms could potentially explain this observation, recent studies reveal that women harbor fetal cells decades after pregnancy, suggesting that HLA-incompatible fetal cells could be infused from parous donors.[9] We hypothesized that donor or recipient parity was a risk factor for sGVHD, and as an initial study retrospectively reviewed adult syngeneic HCTs over 22 years at our center for biopsy-proven sGVHD. In addition to a focused analysis on donor and recipient parity as potential risk factors, we also describe the incidence, morbidity, and other risk factors of this poorly defined syndrome.

Patients, materials, and methods

Study design and subjects

We retrospectively reviewed 126 consecutive first adult syngeneic bone marrow or peripheral blood HCTs at Fred Hutchinson Cancer Research Center (FHCRC) between January 1, 1980, and April 1, 2002. As sGVHD diagnosis required physician suspicion and biopsy, transplantations prior to the first sGVHD report (October 1979) were not considered.[1] There were 7 patients excluded (death before day 14 after transplantation, n = 5; insufficient data, n = 2), leaving 119 subjects. The FHCRC institutional review board approved the study.

Monozygosity testing

Siblings sharing birth date and sex underwent testing to determine whether they could be monozygotic (identical). Testing in earlier years involved matching human leukocyte antigens (HLAs), blood group, and red blood cell (RBC) antigens/isoenzymes. Although informative markers are identified in at least 80% of sibling pairs with this method, blood transfusion may lead to RBC antigen detection in a patient genetically negative for the antigen.[10] In 9 twin pairs (one with sGVHD), 1 to 3 RBC antigens in recipients were not present in their donors. Mismatches were attributed to blood transfusions. In 1991, DNA amplification of variable nucleotide tandem repeats (VNTRs) to confirm monozygosity became common. Informative polymorphisms can be identified in virtually all sibling pairs by testing 6 VNTR sequences.[10]

sGVHD definition

sGVHD was defined by characteristic histologic changes on a skin, intestinal, or liver biopsy with no evidence of infection. Skin biopsies earlier than day 20 were excluded. GVHD histologic findings included lymphocytic infiltration of the cutis/epidermis, apoptotic changes, vacuolar degeneration of epithelial basal cell layers, and eosinophilic coagulation necrosis (skin); apoptotic crypt endothelial cells and crypt cell dropout with/without lymphocytic aggregates (intestine); and apoptosis, dysmorphic small bile duct changes, ductopenia, and lobular acidophilic bodies (liver).

Candidate predictors

Potential risk factors included age, sex, cytomegalovirus (CMV) serostatus, pregnancy history, disease type, HLA (A26, DR2, DR3, DR4, DR7), cell

source, cell dose infused, immunosuppression/graft manipulation, immuno-therapy, conditioning regimen, donor buffy coat infusion, and transplantation year. Risk factors were abstracted from medical records and the FHCRC Transplant Database. Transfusion history outside our center could not be accurately determined. Some patients received methotrexate (n = 16) in an attempt to standardize syngeneic and allogeneic transplantations for future comparison studies. A stem cell infusion without conditioning was administered for aplastic anemia (n = 3) and paroxysmal nocturnal hemo-globinuria (n = 1). Inference of HLA-A26 from HLA-A10 (n = 2) was based on ethnicity.

Statistical analysis

Time to event analyses were performed to determine sGVHD incidence and risk factors. Cumulative incidence estimates treated death, relapse, or second transplantation before day 100 as competing events for sGVHD.[11] All survivors were censored 100 days after transplantation. Univariable comparisons used log-rank tests. Overall P values compared parous women with nulliparous women and men in univariable analysis. Analyses comparing parous with nulliparous women were performed in multivariable analyses. Hazard ratios (HRs) and associated 95% confidence intervals (CIs) were obtained using Cox proportional hazard regression models limited to 2 factors.

Results

Donor, recipient, and transplant characteristics are summarized in Table 1. Among 119 syngeneic hematopoietic cell transplant recipients, 21 developed biopsy-proven sGVHD, for a cumulative incidence of 18%. Median time to diagnosis was 35 days (range, 20-79 days). Disease course was often self-limited. Single organs (skin, n = 10; intestine, n = 5) were affected more frequently than multiple organs (n = 6). Treatment included no therapy (n = 8), supportive therapy (n = 2), corticosteroids (n = 10), and cyclospor-ine (CSP) and anti–thymocyte globulin (ATG; n = 1). Untreated cases were self-limited or diagnosed by autopsy or skin biopsy at discharge.

One death attributed to sGVHD occurred in a parous 47-year-old woman with acute myelogenous leukemia. Both twins were CMV seronegative and the donor was nulliparous. The patient underwent bone marrow transplantation with busulfan, cytoxan, and fractionated total body irradiation conditioning and did not receive methotrexate or immunotherapy. Severe rash, diarrhea, and hyperbilirubinemia developed. A skin biopsy was consistent with GVHD (day 23). Despite corticosteroids, CSP, and ATG, she died, with extensive GVHD findings on autopsy (day 63). Monozygosity testing of this twin pair involved HLA and blood group match. The twins may have been dizygotic (fraternal), as VNTR analysis was not performed. Genetic testing in other sGVHD cases was more extensive: HLA/VNTR (n = 15), HLA/VNTR/RBC antigen/isoenzyme panel (n = 3), HLA/RBC antigen/isoenzyme panel (n = 1), and HLA only (n = 1).

Discussion

In univariable analysis, factors significantly associated with sGVHD included older age, parous donors, parous recipients, parous donor/recipient combinations, HLA-A26, IL-2, busulfan/melphalan/thiotepa (BUMELT), and more recent transplantation year (Table 2). Recipient parity remained a significant independent risk factor in multivariable analysis after adjusting individually for acute lymphocytic leukemia (P = .03), BUMELT (P = .01), IL-2

Table 1. Donor, recipient, and transplant characteristics of 119 syngeneic HCTs

Factor	No. (%) or mean (range)
Age	42 (17-68)
Sex	
Female	49 (41)
Male	70 (59)
Parity in female donors, N = 45	
Nulliparous	11 (22)
Parous	34 (69)
Parity in female recipients, N = 49	
Nulliparous	14 (29)
Parous	35 (71)
Donor/recipient parity, N = 45	
Nulliparous/nulliparous	5 (11)
Nulliparous/parous	6 (13)
Parous/nulliparous	8 (18)
Parous/parous	26 (58)
CMV serostatus, donor/recipient, N = 76	
Negative/negative	28 (37)
Positive/negative	6 (8)
Negative/positive	8 (11)
Positive/positive	34 (45)
Disease type	
Hematologic malignancy	104 (87)
Other malignancy	10 (8)
Nonmalignant condition	5 (4)
HLA antigens	
A26, N = 117	6 (5)
DR2, N = 90	30 (33)
DR3, N = 90	23 (26)
DR4, N = 90	30 (33)
DR7, N = 90	15 (17)
Immunosuppression/graft manipulation, N = 118	
Methotrexate	16 (14)
CD34 selection	1 (1)
CD4 depletion, HIV infection	1 (1)
Conditioning regimen, N = 118	
TBI-containing	86 (73)
BUMELT	12 (10)
Other chemotherapy	16 (14)
None—simple infusion	4 (3)
Cell source	
Bone marrow	88 (74)
PBSC	31 (26)
Cell counts	
TNC/kg $\times 10^8$ in BM, N = 80	2.1 (0.0-4.8)
TMNC/kg $\times 10^8$ in PBSC, N = 21	7.6 (4.7-17.2)
CD34/kg $\times 10^6$ in PBSC, N = 28	8.1 (1.5-14.0)
Donor buffy coat infusion	32 (27)
Immunotherapy	
IL-2	9 (8)
Anti-idiotype myeloma vaccine	1 (1)
Alpha-interferon	1 (1)
Transplantation year	
1980 to 1987	58 (49)
1988 to 1995	43 (36)
1996 to 2002	18 (15)

Unless otherwise stated, percentages within a group were calculated using a denominator of 119 patients. TBI indicates total body irradiation; BUMELT, busulfan/melphalan/thiotepa; PBSC, peripheral blood stem cell; TNC, total nuclear cell count; TMNC, total mononuclear cell count, and IL-2, interleukin-2.

(P = .02), any immunotherapy (P = .02), donor buffy coat infu-sion (P = .03), HLA-A26 (P = .03), DR2 (P = .02), and year of transplantation (P = .02). Adjusting for age (P = .14) or recipient CMV status (P = .07) diminished the effect of recipient parity.

Table 2. Univariable analysis of factors associated with syngeneic GVHD

Factor	No.	GVHD incidence, No. (%)	HR	95% CI	Level-specific P value, > 2 levels	Overall P value, log-rank test
Age, y						
16 to 39	50	3 (6)	1.0	Reference	NA	
40 to 68	69	18 (26)	5.0	1.5-16.8	NA	< .01
Sex						
Male	70	9 (13)	1.0	Reference	NA	
Female	49	12 (24)	2.0	0.8-4.8	NA	.11
Donor parity, N = 115						
Nulliparous	11	1 (9)	1.0	Reference	Reference	
Parous	34	11 (32)	4.6	0.6-35.9	.07	
Male	70	9 (13)	1.7	0.2-13.1	.61	.03
Recipient parity						
Nulliparous	14	1 (7)	1.0	Reference	Reference	
Parous	35	11 (31)	6.0	0.8-46.2	.03	
Male	70	9 (13)	2.1	0.3-16.5	.44	.02
CMV serostatus						
Donor serostatus, N = 76						
Negative	37	8 (22)	1.0	Reference	NA	
Positive	41	11 (27)	1.4	0.5-3.4	NA	.51
Recipient serostatus, N = 105						
Negative	46	6 (13)	1.0	Reference	NA	
Positive	57	15 (26)	2.3	0.9-6.1	NA	.07
Acute lymphocytic leukemia						
No	112	18 (16)	1.0	Reference	NA	
Yes	7	3 (43)	2.7	0.8-9.2	NA	.10
HLA-A26, N = 117						
No	111	18 (16)	1.0	Reference	NA	
Yes	6	3 (50)	3.6	1.0-12.2	NA	.03
HLA-DR2, N = 90						
No	60	10 (17)	1.0	Reference	NA	
Yes	30	9 (30)	1.8	0.7-4.4	NA	.19
Cell source						
PBSC	31	8 (26)	1.0	Reference	NA	
Bone marrow	88	13 (15)	0.5	0.2-1.3	NA	.15
Donor buffy coat infusion						
No	87	18 (21)	1.0	Reference	NA	
Yes	32	3 (9)	0.5	0.1-1.5	NA	.19
Immunotherapy						
No	108	17 (16)	1.0	Reference	NA	
Yes	11	4 (36)	2.5	0.9-7.6	NA	.08
IL-2 therapy						
No	112	18 (16)	1.0	Reference	NA	
Yes	9	4 (44)	3.5	1.2-10.3	NA	.02
Conditioning regimen						
Non-BUMELT	107	16 (15)	1.0	Reference	NA	
BUMELT	12	5 (42)	3.6	1.3-9.8	NA	< .01
Transplantation year						
1980 to 1987	58	3 (5)	1.0	Reference	Reference	
1988 to 1995	43	12 (28)	5.2	1.5-18.6	< .01	
1996 to 2002	18	6 (33)	7.5	1.9-30.1	< .01	< .01

All P values are 2-sided. Variables listed include those with a P value ≤ .2 on univariable log-rank test. Level-specific P values were based on likelihood ratio tests from regression models. PBSC indicates peripheral blood stem cell; IL-2, interleukin-2; BUMELT, busulfan/melphalan/thiotepa; and NA, not applicable.

sGVHD appears to share many risk factors with autologous and allogeneic GVHD. Prior reports associated donor parity and GVHD, hypothesizing that allosensitization of maternal T cells to fetal antigens during pregnancy could prime cells to similar antigens in a transplant recipient.[12-14] Another intriguing hypothesis originates in the finding that fetal cells persist in parous women decades after childbirth. The recent finding that male DNA is frequently present in apheresis products from female donors suggests that donor fetal cells may be transferred during HCT.[9,15] In addition, host (recipient) tolerance to fetal cells from prior pregnancies may be altered with myeloablative therapy, potentially explain-

ing an association between sGVHD and recipient parity. Other sGVHD risk factors are consistent with those identified in autologous or allogeneic GVHD. Thymic aging may lead to increased autoreactivity in older adults. IL-2 and BUMELT are associated with autologous GVHD.[16-18] Finally, the association with more recent transplantation likely reflects increased physician awareness and lower threshold to biopsy tissue.

Limitations of our study include small numbers of nulliparous women and sGVHD cases, which made it difficult to separate a parity effect from all other factors. Another limitation is the need to analyze a variety of risk factors within a relatively small cohort.

Finally, it is also possible that a dizygotic twin pair may have been misclassified as monozygotic. However, in 48% of sGVHD cases, identity of HLA, blood group, and 4 unlinked genetic loci (VNTR) would theoretically result in a less than 1 in 500 chance of misclassification.[10]

Despite prior reports of GVHD-like syndromes, sGVHD remains controversial.[7,19] Our report of 21 sGVHD cases with associated morbidity and mortality suggests that the diagnosis be considered and treatment initiated. sGVHD is most likely multifactorial and shares many risk factors with autologous and allogeneic GVHD. Parity as a GVHD risk factor is essentially unexamined and merits further investigation.

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