# Impact of Donor and Recipient Parity and Gravidity on Risk of Graft-versus-Host Disease in the Liver

# after Allogeneic Hematopoietic Cell Transplant

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**Introduction:**

Chronic graft vs. host disease (cGVHD) is a major cause of non-relapse mortality and morbidity in patients following an allogeneic hematopoietic cell transplant (HCT), a common therapy for hematological malignancies. cGVHD is caused by immunological activation of donor immune cells against recipient tissues and generally affects tissues including the skin, eyes, gut, liver, lungs and salivary glands. Although there are known factors that mediate risk of cGVHD overall, such as Human Leukocyte Antigen (HLA) matching, stem cell source, donor and recipient age and gender, T-cell dose and presence of acute GVHD, risk factors for specific organ involvement have been less well elucidated. The pathophysiology of cGVHD is not well understood, but involves reactions between donor T-lymphocytes and recipient antigens.[1]

One hypothesis is that donor and recipient microchimerism (the trafficking of cells between the fetus and the mother, subsequent engraftment, and persistence of low levels of cells) function allo-reactively and contribute to the development of cGVHD. This hypothesis is supported by studies that show an increase in cGVHD when female donors are parous or previously gravid[2,3,4]. Recipient parity has also been shown to increase risk of cGVHD[5]. Interestingly, donor and recipient parity also appear to increase risk of cGVHD in the syngeneic (identical twin) transplant setting, again implying a role for microchimeric cell populations because identical donor and recipient cells should not be allo-reactive to each other[6].

The liver is of specific interest to the fetal microchimerism hypothesis for several reasons. First, during fetal development, fetal blood stem cells originate in the liver before they travel to the thymus to undergo immune education to become mature lymphocytes. Second, microchimeric cells are frequently detected in the liver[7]. Third, the body is particularly tolerant to liver transplants even when they are not HLA-matched. All of these observations suggested that liver microchimerism in particular could be important in modulating immune cell interactions. To our knowledge there is no prior study specifically examining any risk factors for liver cGVHD. If an association between microchimerism and liver cGVHD exists, it could contribute to the development of hypotheses for the pathophysiologic role of microchimerism in cGVHD and other transplant settings. We therefore sought to assess the association between parity and gravidity of HCT donors and/or recipients with the development of cGVHD with liver involvement. We hypothesized that donor parity/gravidity would increase the risk of non-liver cGVHD in transplant recipients, and that this risk would be higher when examining liver cGVHD alone. We also hypothesized that the risk of non-liver cGVHD would be higher when the recipient was parous/previously gravid, and that this association would be reversed when liver cGVHD was examined specifically.

**Methods:**

Study Design, Setting and Subjects:

We conducted a case-control study based at Fred Hutchinson Cancer Research Center (FHCRC) /Seattle Cancer Care Alliance (SCCA). Study subjects included all patients (N=2,305) who received an allogeneic HCT at FHCRC/SCCA between 1991 and 2011, excluding cord blood transplants and patients who died or underwent a second transplant before day 425 post-transplant. Of note is the composition of the study cohort, which included transplant patients with both myeloablative and reduced-intensity conditioning regimens as well as transplants with HLA-matched related, mismatched related, and matched unrelated donors.

The primary outcome assessed in this study was a positive determination of cGVHD with liver involvement. There were 153 patients who qualified as a case based on either of following two criteria: (1) A clinical diagnosis of liver cGVHD in addition to a positive diagnosis of liver cGVHD via liver biopsy. (2) A positive clinical diagnosis of cGVHD in at least one organ other than the liver, as well as either a minimum two-fold increase of normal upper limit of alkaline phosphatase values with concomitant two-fold increase of normal upper limit of bilirubin levels on at least one laboratory test between day 60 and 425 post-transplant. Alternatively a patient could have a three-fold increase in either serum alkaline phosphatase or total serum bilirubin values on at least one such test. Recipients fulfilling those criteria were excluded if they had any

complicating diagnoses including viral hepatitis, drug-induced liver injury, sinusoidal obstruction syndrome, nonalcoholic steato-hepatitis, extra-hepatic obstruction, late-acute liver GVHD or another preexisting liver complications. The other case group, cGVHD group, was comprised of 1102 patients with a clinical diagnosis of cGVHD in at least one organ other than the liver, with no clinical diagnosis of liver GVHD, and no elevated liver enzymes (using our case criteria) between day 60-425 post-transplant. The non-cGVHD control group was composed of 1,050 patients who had no clinical diagnosis of any type of cGVHD and no elevated liver enzymes between day 60 and 425 post-transplant, or at any point thereafter.

The primary exposures that were evaluated were parity and gravidity, respectively, of transplant donors and recipients. Parity, or number of live/stillborn deliveries after 20 weeks, was categorized as nulliparous (no deliveries) or parous (one or more deliveries). Gravidity, or number of pregnancies, was defined as nulligravid (no pregnancies) or previously gravid (one or more pregnancies). Twins were considered one pregnancy and one delivery.

Data collection

Data were collected from three sources: 1) the Gateway transplant database, operated by the FHCRC, containing basic patient and transplant data as well as pre- and post-transplant laboratory data, 2) manual review of recipient and donor medical charts in the Optical Web Library (OWL) managed by FHCRC, and 3) the National Marrow Donor Program (NMDP), which maintains basic patient data for unrelated donors in the program. Pre-transplant parity and gravidity was determined by review of intake forms and medical charts in OWL if not present in the Gateway transplant database. Data for gravidity for some unrelated donors were provided by the NMDP. All other data on donor and recipient confounders, effect modifiers, exposures and outcomes were obtained from the Gateway transplant database. If data from the Gateway transplant database were missing or implausible, recipient charts were manually reviewed to find or verify data.

Data Analysis

We used Stata 11 for statistical analyses. Polytomous logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the separate associations of donor and recipient parity (and gravidity) with liver and non-liver cGVHD compared to non-cGVHD controls. Known and potential confounders assessed in the analysis were donor age and recipient age (continuous), donor type (matched related, mismatched related, unrelated), cell source (bone marrow, peripheral blood, both), acute GVHD grade (0-I, II-IV), ABO compatibility (matched, major incompatibility, minor incompatibility), type of cancer (CML, other), donor/recipient CMV serostatus (Yes/Yes, Yes/No, No/Yes, No/No), GVHD prophylaxis (calcineurin inhibitor + methotrexate +/- other, other), conditioning regimen (myeloablative, reduced intensity). Donor age (<45 years, ≥45 years) was also examined as an effect modifier because of a possible difference in risk based on the interval since childbearing. Donor type (Matched sibling, mismatched related/unrelated) was also examined as an effect modifier. A variable was considered a confounder if the adjusted OR differed from the unadjusted OR by >10%.

**Results:**

Results – Patient characteristics

There were 2,305 patients at Fred Hutchinson Cancer Research Center from 1991-2011 who met the inclusion criteria for this study. In general, very few data were missing with the exception of donor parity and gravidity, both of which ranged from 11-17% missing, and was roughly similar in both case groups, and slightly higher than the no cGVHD controls.

Patients and, their respective donors, in the liver and non-liver cGVHD case groups were older than those in the no cGVHD control group. Also, relative to no cGVHD controls, liver and non-liver cGVHD cases were more likely to have received peripheral blood stem cells (PBSCs)

than bone marrow, and also more likely to have had acute GVHD grade II-IV. Donor and recipient sex frequencies were similar between the no-cGVHD and non-liver cGVHD groups, but the liver cGVHD group was more likely to have a female donor and a male recipient. Liver cGVHD cases had a higher proportion of related donors and non-liver cGVHD patients had a higher proportion of unrelated donors. Liver cGVHD cases were more likely than all other groups to have had a myeloablative conditioning regimen. ABO compatibility, CMV serostatus, and GVHD prophylaxis were similar across all groups (Table 1).

Table 1. Selected characteristics of donors and recipients of allogeneic HCTs, by cGVHD status, Fred Hutchinson Cancer Research Center 1991-2011.*

| | No cGVHD (N=1102) | Non-liver cGVHD (N=1050) | Liver cGVHD (N=153) |
|---|---|---|---|
| Characteristics | N (%) | N (%) | N (%) |
| **Donor age (years)** | | | |
| Mean (range) | 34.2 (0.4-76.6) | 38.9 (1.4, 79.7) | 38.0 (5.1, 69.6) |
| Unknown | 78 (7.0) | 172 (16.3) | 19 (12.4) |
| **Recipient age (years)** | | | |
| Median (range) | 35.9 (0.3-75.2) | 42.0 (0.4-74.0) | 40.2 (0.6-65.2) |
| **Donor sex** | | | |
| Male | 659 (59.8) | 552 (52.6) | 84 (45.1) |
| Female | 442 (40.1) | 498 (47.4) | 69 (54.9) |
| Unknown | 1 (<0.1) | 0 (0) | 0 (0) |
| **Recipient sex** | | | |
| Male | 583 (52.9) | 628 (59.8) | 96 (62.7) |
| Female | 519 (47.1) | 422 (40.2) | 57 (37.3) |
| **Donor parity** | | | |
| Nulliparous | 841 (76.3) | 687 (65.4) | 104 (67.8) |
| Parous | 130 (11.8) | 192 (18.3) | 26 (17.0) |
| Unknown | 131 (11.9) | 171 (16.3) | 23 (15.0) |
| **Donor gravidity** | | | |
| Nulligravid | 817 (74.1) | 664 (63.2) | 99 (64.7) |
| Previously gravid | 157 (14.3) | 218 (20.8) | 28 (18.3) |
| Unknown | 128 (11.6) | 168 (16.0) | 26 (17.0) |
| **Recipient parity** | | | |
| Nulliparous | 813 (73.8) | 784 (74.7) | 109 (71.2) |
| Parous | 279 (25.3) | 258 (24.6) | 42 (27.5) |
| Unknown | 10 (0.9) | 8 (0.8) | 2 (1.3) |
| **Recipient gravidity** | | | |
| Nulligravid | 778 (70.6) | 763 (72.7) | 102 (66.7) |
| Previously gravid | 249 (22.6) | 252 (24.0) | 41 (26.8) |
| Unknown | 75 (6.8) | 35 (3.3) | 10 (6.5) |
| **Donor/recipient parity** | | | |
| Nulliparous/nulliparous | 641 (58.2) | 522 (49.7) | 77 (50.3) |
| Nulliparous/parous | 193 (17.5) | 158 (15.0) | 26 (17.0) |
| Parous/nulliparous | 82 (7.4) | 146 (13.9) | 18 (11.8) |
| Parous/parous | 46 (4.2) | 45 (4.3) | 8 (5.2) |

| | | | |
|---|---|---|---|
| Unknown | 140 (12.7) | 179 (17.0) | 24 (15.7) |
| **Donor Recipient gravidity** | | | |
| Nulligravid/nulligravid | 604 (54.8) | 495 (41.4) | 68 (44.4) |
| Nulligravid/previously gravid | 161 (14.6) | 146 (13.9) | 25 (16.3) |
| Previously gravid/nulligravid | 99 (9.0) | 151 (14.4) | 19 (12.4) |
| Previously gravid/previously gravid | 50 (4.5) | 60 (5.7) | 8 (5.2) |
| Unknown | 188 (17.1) | 198 (18.9) | 33 (21.6) |
| **Year of transplant** | | | |
| 1991-1995 | 278 (25.2) | 250 (23.8) | 41 (26.8) |
| 1996-2000 | 249 (22.6) | 355 (33.8) | 56 (36.6) |
| 2001-2005 | 252 (22.9) | 256 (24.4) | 46 (30.1) |
| 2006-2011 | 323 (29.3) | 189 (18.0) | 10 (6.54) |
| **Diagnosis** | | | |
| Aplastic anemia | 85 (7.7) | 20 (1.9) | 1 (0.7) |
| Acute lymphocytic leukemia | 156 (14.2) | 139 (13.2) | 10 (6.5) |
| Acute myeloid leukemia | 277 (25.1) | 261 (24.9) | 44 (28.8) |
| Chronic myeloid leukemia | 289 (26.2) | 314 (30.0) | 46 (30) |
| Myelodysplastic syndrome | 149 (13.5) | 159 (15.1) | 34 (22) |
| Other hematologic malignancy** | 146 (13.3) | 157 (15.0) | 18 (11.8) |
| **Donor Type** | | | |
| HLA-identical Sibling | 576 (52.3) | 379 (36.1) | 89 (58.2) |
| Mismatched sibling or other relative | 67 (6.1) | 77 (7.3) | 13 (8.5) |
| Unrelated donor | 459 (41.7) | 594 (56.6) | 51 (33.3) |
| **Stem cell source** | | | |
| Bone marrow | 729 (66.2) | 582 (52.8) | 79 (51.6) |
| Peripheral blood | 372 (33.7) | 465 (44.3) | 74 (48.4) |
| Bone marrow and peripheral blood | 1 (<0.1) | 3 (2.9) | 0 (0) |
| **ABO compatibility** | | | |
| Matched | 633 (57.4) | 571 (54.4) | 82 (53.6) |
| Major incompatilibility | 246 (22.3) | 264 (25.2) | 40 (26.1) |
| Minor imcompatibility | 222 (20.2) | 214 (20.4) | 31 (20.3) |
| Unknown | 1 (<0.1) | 1 (<0.1) | 0 (0) |
| **CMV serostatus (recipient/donor)** | | | |
| Negative/negative | 384 (34.8) | 370 (35.2) | 55 (36.0) |
| Negative/positive | 147 (13.3) | 139 (13.2) | 20 (13.1) |
| Positive/negative | 262 (23.9) | 266 (25.3) | 30 (19.6) |
| Positive/positive | 302 (23.8) | 273 (26.0) | 41 (26.8) |
| Unknown | 7 (<0.1) | 2 (<0.1) | 7 (4.5) |
| **Conditioning regimen[&]** | | | |
| Myeloablative | 942 (85.5) | 874 (83.2) | 139 (90.8) |
| Reduced intensity | 160 (14.5) | 176 (16.8) | 14 (9.2) |
| Unknown | | | |
| **GVHD prophylaxis[$]** | | | |

| | | | |
|---|---|---|---|
| Calcineurin inhibitor + methotrexate +/- other | 224 (20.3) | 247 (23.5) | 25 (16.3) |
| Other | 818 (74.2) | 784 (74.6) | 127 (83.0) |
| Unknown | 60 (5.4) | 29 (2.8) | 1 (0.7) |
| **Acute GVHD grade** | | | |
| 0-I | 279 (25.3) | 105 (10.0) | 27 (17.6) |
| II-IV | 820 (74.4) | 943 (89.8) | 124 (82.0) |
| Unknown | 3 (0.3) | 2 (0.2) | 2 (1.3) |

Total percentages may not add to 100% because of rounding and an 'unknown' category listed only if there is missing data

** Other hematologic malignancies include chronic lymphoblastic leukemia, multiple myeloma, non-Hodgkins lymphoma, Hodgkin's lymphoma, and

& Reduced intensity conditioning includes any regimens with total body irradiation between 200 and 300 cGy +/- fludarabine +/- other chemotherapy

Donor parity and donor gravidity were highly correlated (r = 0.937). The same was true for recipient parity and gravidity (r = 0.958). The parous and previously gravid recipient groups had very similar characteristics. ABO compatibility, conditioning regimen, cGVHD prophylaxis and acute GVHD did not differ across parous/previously gravid donor/recipient groups. However, parous/previously gravid donors were more likely than recipients to have been transplanted earlier (1991-1995) and have bone marrow as the stem cell source than parous/previously gravid recipients (Table 2).

Table 2. Selected characteristic of donor and recipients of allogeneic HTCs, by donor and recipient parity and gravidity, Fred Hutchinson Cancer Research Center 1991-2011*

| | Parous donor (N=348) | Previously Gravid donor (N=403) | Parous recipient (N=578) | Previously Gravid recipient (N=542) |
|---|---|---|---|---|
| Characteristics | N (%) | N (%) | N (%) | N (%) |
| **Donor age (years** | | | | |
| Median (range) | 43.3 (21.3-78.0) | 41.7 (20.3-77.6) | 40.6 (5.7-78.0) | 40.5 (5.7-78.0) |
| Unknown | 35 (10.0) | 43 (10.7) | 58 (10.0) | 62 (11.4) |
| **Recipient age (years)** | | | | |
| Median (range) | 41.4 (0.8-71.4) | 40.0 (0.5-75.2) | 46.1 (18.3-75.2) | 45.5 (18.3-75.2) |
| **Donor sex** | | | | |
| Male | 0 (0) | 0 (0) | 306 (52.8) | 284 (52.4) |
| Female | 348 (100) | 403 (100) | 273 (47.2) | 258 (47.6) |
| **Recipient sex** | | | | |
| Male | 191 (55.0) | 216 (53.6) | 0 (0) | 0 (0) |
| Female | 157 (45.0) | 187 (46.4) | 579 (100) | 542 (100) |
| **Year of transplant** | | | | |
| 1991-1995 | 125 (35.8) | 131 (32.5) | 126 (21.8) | 112 (20.7) |
| 1996-2000 | 142 (41.0) | 132 (32.8) | 167 (28.8) | 164 (30.3) |
| 2001-2005 | 40 (11.5) | 71 (17.6) | 137 (23.7) | 132 (24.4) |
| 2006-2011 | 41 (11.7) | 69 (17.1) | 149 (25.7) | 134 (24.7) |
| **Diagnosis** | | | | |
| Aplastic anemia | 13 (3.7) | 79 (19.6) | 20 (3.5) | 18 (3.3) |

| | | | | |
|---|---|---|---|---|
| Acute lymphocytic leukemia | 38 (11.2) | 48 (11.9) | 34 (5.9) | 34 (6.) |
| Acute myeloid leukemia | 74 (21.2) | 81 (20.1) | 183 (31.6) | 167 (30.8) |
| Chronic myeloid leukemia | 121 (34.7) | 138 (34.2) | 174 (30.1) | 164 (30.3) |
| Myelodysplastic syndrome | 46 (13.2) | 57 (14.1) | 100 (17.3) | 93 (17.2) |
| Other hematologic malignancy** | 56 (16.1) | 63 (15.6) | 68 (11.7) | 67 (12.4) |
| **Donor Type** | | | | |
| HLA-identical Sibling | 204 (58.7) | 175 (43.4) | 291 (50.3) | 276 (50.9) |
| Mismatched sibling or other relative | 38 (10.9) | 35 (8.7) | 21 (3.6) | 18 (3.3) |
| Unrelated donor | 106 (30.4) | 193 (47.9) | 267 (46.1) | 248 (45.8) |
| **Stem cell source** | | | | |
| Bone marrow | 252 (72.5) | 280 (69.5) | 301 (52.0) | 280 (51.7) |
| Peripheral blood | 94 (26.9) | 121 (30.0) | 278 (48.0) | 262 (48.3) |
| Bone marrow and peripheral blood | 2 (0.6) | 2 (0.5) | 0 (0) | 0 (0) |
| **ABO compatibility** | | | | |
| Matched | 193 (55.6) | 206 (51.1) | 336 (58.0) | 307 (56.6) |
| Major incompatilibility | 80 (22.9) | 94 (23.3) | 119 (20.6) | 124 (22.9) |
| Minor imcompatibility | 74 (21.2) | 102 (25.3) | 124 (21.4) | 111 (20.5) |
| Unknown | 1 (0.3) | 1 (0.3) | 0 (0) | 0 (0) |
| **CMV serostatus (recipient/donor)** | | | | |
| Negative/negative | 91 (26.1) | 111 (27.5) | 143 (24.7) | 141 (26.1) |
| Negative/positive | 74 (21.3) | 86 (21.3) | 64 (11.1) | 62 (11.4) |
| Positive/negative | 61 (17.5) | 74 (18.4) | 167 (28.8) | 156 (28.8) |
| Positive/positive | 121 (34.8) | 129 (32.0) | 202 (34.9) | 180 (33.2) |
| Unknown | 1 (0.3) | 3 (0.7) | 3 (0.5) | 3 (0.6) |
| **Conditioning regimen[&]** | | | | |
| Myeloablative | 309 (88.8) | 350 (86.9) | 480 (83.0) | 452 (83.4) |
| Reduced intensity | 39 (11.2) | 53 (13.2) | 99 (17.1) | 90 (16.6) |
| **GVHD prophylaxis[$]** | | | | |
| Calcineurin inhibitor + methotrexate +/- other | 278 (79.7) | 308 (76.4) | 440 (76.0) | 412 (76.0) |
| Other | 61 (17.5) | 79 (19.6) | 131 (22.6) | 123 (22.7) |
| Unknown | 9 (2.6) | 16 (4.0) | 8 (1.4) | 7 (1.3) |
| **Acute GVHD grade** | | | | |
| 0-I | 60 (17.2) | 65 (16.1) | 124 (21.4) | 113 (20.9) |
| II-IV | 287 (82.5) | 336 (83.3) | 452 (78.1) | 427 (78.8) |
| Unknown | 2 (0.3) | 2 (0.5) | 3 (0.5) | 2 (0.4) |

* Total percentages may not add to 100% because of rounding and an 'unknown' category listed only if there is missing data

** Other hematologic malignancies include chronic lymphoblastic leukemia, multiple myeloma, non-Hodgkins lymphoma, Hodgkin's lymphoma, and

& Reduced intensity conditioning includes any regimens with total body irradiation between 200 and 300 cGy +/- fludarabine +/- other chemotherapy

Results- Parity and Gravidity

Prior donor delivery and prior donor pregnancy both increased the risk of non-liver cGVHD with an OR for a parous donor of 1.81 (95%CI 1.38, 2.39) and an OR for a previously gravid donor of 1.52 (95%CI: 1.18, 1.96). Slightly weaker, non-statistically significant increases in risk were found for liver cGVHD. The ORs for risk of non-liver cGVHD and liver cGVHD based on donor parity and gravidity were not statistically different (Table 3). The ORs for risk of non-liver cGVHD and liver cGVHD based on donor parity and gravidity were not statistically different (Table 3). The OR estimates for all outcomes were affected by donor age which was adjusted for in all analyses. Similarly, recipient age was found to confound the association between recipient parity/gravidity and both cGVHD outcomes and was adjusted for. Also, donor type confounded the relationship between donor parity and both cGVHD outcomes we adjusted for it.

Recipient parity and gravidity were associated with a decrease in risk of non-liver cGVHD (parity OR: 0.74 (95%CI: 0.60, 0.91), gravidity OR: 0.78 (0.63, 0.98)) but were not associated with risk of liver cGVHD (Table 4). Risk estimates for the association between recipient parity/gravidity liver and non-liver cGVHD were not appreciably different when donor age (≤45 years, >45 years) was evaluated as an effect modifier, based on the p-values for the interaction term for donor age and recipient parity (Table 5).

When donor type was assessed as an affect effect modifier of the association between recipient parity and liver cGVHD the ORs for matched siblings and mismatched related donors/ unrelated donors, respectively, were 0.79 (95%CI: 0.47, 1.33) and 0.67 (95%CI:0.50, 0.92). The p-value for the interaction term was non significant and p= 0.361. When the same analyses were done for non-liver cGVHD the ORs were 1.13 (95%CI:0.55, 2.33) and 0.82 (95%CI:0.59, 1.13) and p=0.645 for the interaction term.

The association between recipient gravidity and cGVHD was not modified by donor type. The ORs for the association of recipient gravidity and liver cGVHD for matched donors was 0.82 (95%CI: 0.48, 1.39) and 1.55 (95%: 0.74, 3.22) for mismatched/unrelated donors. The p-value for the interaction term was not significant with p=0.162. The association between recipient gravidity and non-liver cGVHD was also not modified by donor type and the ORs for this association with a matched donor was 0.71 (95%CI:0.52, 0.97) and for a mismatched donor it was 0.97 (95%CI: 0.69, 1.37) and the p-value for the interaction term was 0.321. All of these analyses were adjusted for donor and recipient age