Impact on Outcomes of Human Leukocyte Antigen Matching by Allele-Level Typing in Adults with Acute Myeloid Leukemia Undergoing Umbilical Cord Blood Transplantation

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INTRODUCTION

One intrinsic advantage of umbilical cord blood (UCB) over other stem cell sources for allogeneic stem cell transplantation is the ability to tolerate greater degrees of human leukocyte antigen (HLA) disparity. However, the impact of donor-recipient HLA mismatch on umbilical cord blood transplantation (UCBT) outcomes is still not well established, particularly in adults.

Most registry-based studies that included a majority of pediatric population and a variety of diseases have reported an increased nonrelapse mortality (NRM) rate with HLA disparity considering HLA-A and -B by low-resolution and an increased nonrelapse mortality (NRM) rate with HLA disparity among adults. In contrast, the impact of donor-recipient HLA disparity on outcomes of unrelated donors (UDBT) has been assessed in a variety of diseases in adults with acute myeloid leukemia (AML) who received single-unit umbilical cord blood (UCB) transplant at a single institution. With extended high-resolution HLA typing, the donor-recipient compatibility ranged from 2/8 to 8/8. HLA disparity showed no negative impact on nonrelapse mortality (NRM), graft-versus-host (GVH) disease or engraftment. Considering disparities in the GVH direction, the 5-year cumulative incidence of relapse was 44% and 22% for patients receiving an UCB unit matched ≥ 6/8 and < 6/8, respectively (P = .04). In multivariable analysis, a higher HLA disparity in the GVH direction using extended high-resolution typing (Risk ratio [RR] 2.8; 95% confidence interval [CI], 1.5 to 5.1; P = .0009) and first complete remission at time of transplantation (RR 2.1; 95% CI, 1.2 to 3.8; P = .01) were the only variables significantly associated with an improved disease-free survival. In conclusion, we found that in adults with AML undergoing single-unit UCBT, an increased number of HLA disparities at allele-level typing improved disease-free survival by decreasing the relapse rate without a negative effect on NRM.

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PATIENTS AND METHODS

Eligibility Criteria

Between June 2000 and December 2012, 79 consecutive adult patients with AML underwent myeloablative UCBT from unrelated donors at our institution. Patients were eligible for enrollment if they met the following criteria: (1) high-risk AML, defined by either high-risk cytogenetics or poor prognosis gene markers, more than 1 cycle to achieve complete remission (CR), or disease status beyond first CR; and (2) no suitable related donor (HLA-identical or 1-antigen–mismatched). From 2005, patients were included in 2 subsequent prospective trials: TSCU-GETH2005 (21 patients) and TSCU-GETH/GITMO2008 (42 patients). The institutional review board approved the protocol and written informed consent was obtained from all patients according to the Declaration of Helsinki. TSCU-GETH/GITMO2008 clinical trial was registered on the EudraCT with code 2008-000927-24.

The treatment plans, including of graft selection, conditioning regimen, immune suppression, and supportive care have been reported in detail previously [4,9], and are summarized below.

Umbilical Cord Blood Unit Selection and Management

The search of UCB units was conducted by the Spanish Registry of Bone Marrow Donors (Registro Español de Donantes de Médula Ósea). Graft selection algorithm required UCB ≥ 4/6 HLA matched with the recipient (HLA class I antigens [A and B] considering the antigen level and class II antigens [DRB1] considering allele-resolution DNA typing). Minimum total nucleated cell (TNC) and CD34+ cell counts from the information provided by the different UCB banks were required. Minimum cell dose criteria changed over time. A TNC dose ≥ 1.5 × 10^7/kg recipient’s body weight was required until 2005, whereas TNC ≥ 2 × 10^7/kg and CD34+ cell dose ≥ 1 × 10^7/kg recipient’s body weight were required in 2006 and 2007. From 2008 to present, minimum cell dose criteria considered total cell dose of the UCB units without taking into account recipient’s body weight as follows: TNC >
150 × 10^6 and CD34+ cells > 70 × 10^5. Cell dose was considered the most important criteria for unit selection.

**Conditioning Regimen**

Two subsequent myeloablative conditioning regimens were used, all based on the combination of thiotaepa, busulfan, cyclophosphamide or fludarabine, and antithymocyte globulin (ATG). Until March 2005, 16 patients received thiotaepa (10 mg/kg), busulfan (12 mg/kg orally in 8 patients or 9.6 mg/kg i.v. in 8 patients), cyclophosphamide (120 mg/kg) and ATG (Lymphoglobuline, Merieux, Lyon, France; 60 mg/kg in the first 6 patients or Thymoglobulin, Genzyme Transplant, Cambridge, MA; 8 mg/kg in the remaining 10 patients). From March 2005, the remaining 63 patients received thiotaepa (10 mg/kg), i.v. busulfan as a single dose (9.6 mg/kg), fludarabine (150 mg/m²), and ATG (Thymoglobulin 8 mg/kg in the first 21 patients and 6 mg/kg in the remaining 42 patients).

**Graft-versus-host-disease Prophylaxis and Treatment**

All patients received cyclosporine 1.5 mg/kg/12 hours i.v., followed by 3 to 5 mg/kg/12 hours orally when oral intake was possible, with slow tapering starting between day +90 and +180 and discontinuation on day +180, or before if feasible. Cyclosporine was combined with long-course prednisone in the first 38 patients (5 mg/kg/day on days +7 to +14, 1 mg/kg/day on days +14 to +28, with slow tapering until discontinuation on day +180), micofunastenol-fefetil (15 mg/kg/12 hours until day +28) in the following 26 patients, or a short course of prednisone in the last 15 patients (1 mg/kg/day on days +14 to +28). Patients developing acute graft-versus-host-disease (GVHD) received high-dose methylprednisolone as initial therapy (20 mg/kg/day; halving the dose every 3 days until reaching 1 mg/kg/day, and then gradually tapered), followed by ATG in refractory cases. Chronic GVHD was treated with prednisone 1 mg/kg/day.

**HLA Typing**

For confirmation purposes, all patients and UCB units were HLA typed in our laboratory (D.P.). High-resolution typing was performed for HLA-A, -B, -C, and DRB1 in all patient and donor pairs as previously described [10]. High-resolution (allele level) and low-resolution (serologic or antigen level) HLA matching were considered in the analysis of clinical outcomes. Low-resolution disparities involved conversion of the DNA-based typing to its serologic equivalent, by collapsing the 4-digit typing result back to standard criteria [10]. The number of HLA disparities between donors and recipients was considered single mismatches.

**Definitions**

Treatment outcomes were assessed according to the revised criteria by Cheson et al. [11]. Myeloid engraftment was defined as an absolute neutrophil count (ANC) of .5 × 10^9/L or greater on 3 consecutive days. Platelet engraftment was defined as a platelet count of 20 × 10^9/L or higher, without transfusion support, for 7 consecutive days. Patients who survived more than 28 days after transplantation and who failed to achieve myeloid engraftment were considered graft failures. Time to myeloid or platelet engraftment was defined as the time required to reach the first day of engraftment. Secondary graft failure was defined as the loss of the engraftment. Acute and chronic GVHD were defined and graded according to standard criteria [12-14]. Killer cell immunoglobulin-like receptor (KIR)-ligand compatibility was defined as described by Willemsen et al. [15]. Briefly, KIR-ligand compatibility in the GVH direction was determined according to whether or not they expressed HLA-C group 1 or 2, HLA-Bw4, or HLA-A3/-A11. NRM was defined as death from any cause without evidence of relapse. Disease-free survival was defined as survival from the time of transplantation without evidence of disease relapse.

**Statistical Analysis**

The probabilities of engraftment, NRM, GVHD, and relapse were estimated by the cumulative incidence method (marginal probability) [16,17]. For cumulative incidence analyses of engraftment, GVHD, and relapse, death in CR was considered as a competing cause of failure, whereas relapse was the competing event for NRM. Unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate [18], and, for comparisons, the log-rank tests [19]. Disease-free survival was calculated from the date of UCBT. In the analysis of leukemia-free survival (LFS), relapse, or death in CR, whichever occurred first, was considered an uncensored event. The follow-up of the patients was updated on December 1, 2012. A Cox proportional hazards model [20] or the Fine and Gray method for competing events [21] were used for multivariable analysis using variables with a P value < .10 for each endpoint. The variables considered for prognostic factor analysis were age, gender, recipient weight, recipient CMV serology, disease status at transplantation, HLA compatibility considering HLA-A, -B, -C, and DRB1 by high- and low-resolution typing, ABO blood group mismatch, KIR-ligand incompatibility, conditioning regimen, GVHD prophylaxis, and TNC and CD34+ cells before freezing and infused. Continuous variables were dichotomized at the most discriminative cutoff point for each outcome. The slight modifications in the conditioning regimen and GVHD prophylaxis performed over the years did not have an impact on any outcome and. Therefore, subgroup analysis was not performed, although these variables were included in the multivariable analysis. Statistical analyses were conducted using R version 2.12.2 (The CRAN project) with packages, survival v2.37-4, rms 3.6-3, prodlim v1.3.3, and cmprsk v2.2-4 [22].

**RESULTS**

**Patient, Graft, and Transplantation Characteristics**

Patient characteristics are shown in Table 1. Briefly, median age was 37 years (range, 16 to 55) and 55 patients (72%) were in CR1 or CR2.

Graft and transplantation characteristics are shown in Table 2. Briefly, 26 (33%) males received a graft from a female donor and KIR-ligand mismatch was present in 28 (35%) patients.

**Donor-recipient HLA Matching**

Table 3 shows the number of HLA disparities between donors and recipients using the standard HLA match criteria used for unit selection, as well as the extended allele-level typing at HLA-A, -B, -C, and DRB1. The number of HLA disparities was considered in both GVH and HVG direction.

With the standard criteria, 6 patients (8%) received a fully matched 6/6 UCB unit whereas 19 patients (24%) and 54 patients (68%) received a 5/6 and 4/6 matched graft, respectively. With extended high-resolution GVHD typing, the donor-recipient compatibility ranged from 2/8 to 8/8. Ten donors had at least 1 homzygous allele resulting in a unique vector in the GVH direction, whereas 7 patients had at least 1
Table 2
Graft and Transplantation Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor-recipient sex match</td>
<td></td>
</tr>
<tr>
<td>Male-male</td>
<td>24 (30)</td>
</tr>
<tr>
<td>Male-female</td>
<td>13 (16)</td>
</tr>
<tr>
<td>Female-male</td>
<td>26 (33)</td>
</tr>
<tr>
<td>Female-female</td>
<td>16 (20)</td>
</tr>
<tr>
<td>KIR-ligand incompatibility in graft-versus-host direction</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (35)</td>
</tr>
<tr>
<td>No</td>
<td>51 (65)</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
</tr>
<tr>
<td>TT + BU + CY + ATG</td>
<td>16 (20)</td>
</tr>
<tr>
<td>TT + BU + FLU + ATG</td>
<td>63 (80)</td>
</tr>
<tr>
<td>Graft-versus-host disease prophylaxis</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine A + prednisone</td>
<td>53 (67)</td>
</tr>
<tr>
<td>Cyclosporine A + MMF</td>
<td>26 (33)</td>
</tr>
<tr>
<td>No. of nucleated cells before freezing, × 10^7/kg</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
</tr>
<tr>
<td>Range</td>
<td>1.4-6.8</td>
</tr>
<tr>
<td>No. of nucleated cells infused, × 10^7/kg</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.4</td>
</tr>
<tr>
<td>Range</td>
<td>1.0-4.9</td>
</tr>
<tr>
<td>No. of CD34⁺ cells before freezing, × 10^7/kg</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.6</td>
</tr>
<tr>
<td>Range</td>
<td>2.4-1.1</td>
</tr>
<tr>
<td>No. of CD34⁺ cells infused, × 10^7/kg</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.3</td>
</tr>
<tr>
<td>Range</td>
<td>1.6-6.1</td>
</tr>
</tbody>
</table>

TT indicates thiopeta; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; ATG, antithymocyte globulin; MMF, micofenolate-mofetil. Percentages may total 100 because of rounding. Data presented are n (%), unless otherwise indicated.

Impact of HLA Mismatch on Relapse

Overall, 21 patients relapsed at a median time of 202 days (range, 32 to 1372). The 5-year cumulative incidence of relapse of the entire cohort was 28% (95% CI, 18% to 39%). For patients who underwent transplantation in CR, cumulative incidence of relapse was 24% compared with 41% for patients who underwent transplantation while in relapse (P = .08). With extended high-resolution HLA typing, bidirectional mismatches showed no impact on the risk of relapse; however, the 5-year cumulative incidence of relapse was 44% and 22% for patients receiving an UCB unit matched ≥ 6/8 and < 6/8, respectively (P = .04) (Figure 1).

In multivariable analysis, a higher HLA disparity in the GVH direction using extended high-resolution typing (RR, 4; 95% CI, 2 to 9; P = .0004) and CR at time of transplantation (RR, 4; 95% CI, 2 to 9; P = .02) were the only variables significantly associated with a reduced risk of relapse.

Leukemia-free Survival

After a median follow-up of 66 months (range, 21 to 132) for surviving patients, 27 patients remained alive and disease free at last follow-up. The 5-year LFS for the entire cohort was 35% (95% CI, 23% to 46%). For patients who underwent transplantation in first CR, LFS was 41%, compared with 25% for patients who underwent transplantation in more advanced phases of the disease (P = .04).

Considering standard bidirectional HLA typing, the 5-year LFS was 40%, 26%, and 17% for patients who underwent transplantation with a 4/6, 5/6, and 6/6 UCB unit, respectively (P = .04). With standard HLA typing and considering mismatches in the GVH direction, the 5-year LFS was 45%, 21%, and 14% for patients who underwent transplantation with a 4/6, 5/6, and 6/6 UCB unit, respectively (P = .03).

With extended high-resolution HLA typing, bidirectional incompatibilities showed no impact on LFS, but considering mismatches in the GVH direction, the 5-year LFS was 45% and 10% for patients receiving an UCB unit matched 2 to 5/8 and 6 to 8/8, respectively (P = .003) (Figure 2).

In multivariable analysis, a higher HLA disparity in the GVH direction using extended high-resolution typing (RR, 2.8; 95% CI, 1.5 to 5.1; P = .0009) and CR1 at time of transplantation (RR, 2.1; 95% CI, 1.2 to 3.8; P = .01) were the only variables significantly associated with an improved LFS.

DISCUSSION

This study shows that a higher donor-recipient HLA disparity in myeloablative single-unit UCBT for adults with...
AML decreases the risk of relapse, suggesting an enhanced GVL effect, which translates to a benefit on long-term LFS. We could also identify donor-recipient pairs with a high number of discrepancies by extended high-resolution HLA typing without an evident deleterious effect. A separate analysis of HLA disparity according to GVH and HVG direction improved the predictive value on relapse rate. These data may provide clinically useful information for a better selection of cord blood units in adults with poor-risk AML.

The number of patients and events in this study was smaller than in registry-based studies and did not allow an analysis of the impact of specific HLA disparities. Results should, therefore, be interpreted with caution. However, this retrospective study included a series of adults with AML treated with a homogenous strategy of single-unit UCBT after busulfan-based myeloablative conditioning regimen at a single institution. Of note, most patients were included in 2 subsequent prospective trials (TSCU-GETH2005 and TSCU-GETH/GITMO2008).

With extended high-resolution typing, we identified a significant proportion of patients that received highly mismatched units. In fact, 70% of the donor-recipient pairs had 3 or more disparities at the allelic level. Compared to the standard HLA typing criteria, directional high-resolution typing was able to further discriminate disparities. For example, UCB units matched 4/6 with standard criteria had disparities that were reclassified ranging from 2/8 to 8/8 in the GVH direction at high resolution.

Our study showed no negative impact of HLA disparity on NRM, GVHD, or engraftment, whereas TNC content was the only variable associated with the risk of NRM. In contrast to pediatric patients, HLA disparity in adults has never shown a negative impact on NRM in large registry-based studies [6,23,24]. The different outcome in children compared with adults may be explained, at least in part, by the important differences in cell dose between these populations. Another important factor to consider is the importance of mismatch direction on outcomes. However, only few registry-based studies have evaluated its impact by serologic data with inconsistent results, maybe because of the heterogeneity of the population. Some studies have suggested a deleterious effect on engraftment with mismatches in the HVG direction [5,25], whereas others found an opposite effect [26]. UCBT performed with mismatches in the GVH direction had outcomes that were similar to those that were fully matched [5,25], but this effect was restricted to children in 1 of the studies [25]. No mismatch category has been previously associated with relapse.

We observed an impressive reduction in risk of relapse, suggesting an enhanced GVL effect, for patients who underwent transplantation with UCB units with a higher HLA disparity in the GVH direction. This observation was made in the context of a very high-risk population and a specific highly immunosuppressive treatment platform. Future studies are needed to determine whether this finding is true for all or only specific scenarios. In this regard, previous registry-based studies from Eurocord [27] and the Japanese Society for Hematopoietic Cell Transplantation group [6] have also found that UCBTs with a higher HLA disparity had a lower probability of relapse. However, we could not see any favorable effect of KIR-ligand incompatibility in the graft-versus-host direction as previously described [15]. A reduced relapse rate, together with the lack of a negative impact on NRM, translated into improved LFS after UCBT performed with more mismatched units in the GVH direction. To our knowledge, this has not been previously reported, maybe because of the fact that this was the first RISE-specific analysis of an adult-restricted cohort evaluating the impact directional HLA mismatches by high-resolution typing.

In conclusion, we found that in adults with AML undergoing single-unit UCBT with thiotepa, busulfan, fludarabine, and ATG, UCB units with high number of discrepancies seem acceptable for transplantation. In fact, an increased number of HLA disparities using allelic-level typing improved disease-free survival by decreasing the relapse without increasing mortality rate. Whether these findings will apply in other settings, such as double-unit UCBT or using reduced-intensity conditioning, is unknown and should be further investigated. Further studies addressing the potential alloreactivity and GVL effect of HLA disparity are warranted.

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