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RAFT-VERSUS-HOST DISEASE OCCURRENCE AMONG CORD BLOOD TRANSPLANTATION RECIPIENTS IN RELATION TO HUMAN LEUKOCYTE ANTIGEN MATCH GRADE

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GRAFT-VERSUS-HOST DISEASE OCCURRENCE AMONG CORD BLOOD

TRANSPLANTATION RECIPIENTS IN RELATION TO HUMAN LEUKOCYTE

ANTIGEN MATCH GRADE

by

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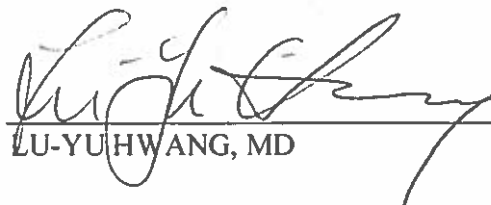SARAH CHOWDHURY
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GRAFT-VERSUS-HOST DISEASE OCCURRENCE AMONG CORD BLOOD TRANSPLANTATION RECIPIENTS IN RELATION TO HUMAN LEUKOCYTE ANTIGEN MATCH GRADE

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Umbilical cord blood (UCB) transplantation has been consistently found to have low rates of graft-versus-host disease (GVHD), high rates of engraftment, and faster turnaround times in terms of finding suitable donors. In addition, it serves as a viable option for individuals with no matched donor options especially due to its flexible human leukocyte antigen matching criteria. Despite its advantages, there has been an overall decrease in UCB transplantation. However, its advantages emphasize the need for further research and investment in resources such as cord blood banking especially since pediatric populations are most likely to benefit from continued research due to them typically being the recipients of UCB transplantation. A retrospective cohort study was conducted to determine if recipients of a mismatched UCB transplantation were at higher risk of developing acute GVHD and failure to achieve engraftment. Other risk factors including cytomegalovirus (CMV) serology status, geographical origin of UCB unit, and primary disease were examined to determine if they were associated with an increased risk of acute GVHD and failure to achieve engraftment. The results for the following factors were shown to be not significant thus not considered as an increased risk of acute GVHD: mismatched cord blood units

according to 6 loci matching criteria (RR 1.09; CI 95%, 0.28-4.24; p = 0.61), mismatched cord blood units according to 10 loci matching criteria (RR 0.86; CI 95%, 0.19-3.89; p = 0.57), malignant primary disease (RR 2.55; CI 95%, 0.65-9.94; p=0.54), international CBU origin (RR 0.63; CI 95% 0.08-4.73; p=0.54), and positive donor's maternal CMV status (RR 0.82; CI 95%, 0.20-3.35; p=0.54). In addition, the following results for potential risk factors were not significant thus not considered as an increased risk of failure to achieve engraftment: mismatched cord blood units according to 6 loci matching criteria (RR 1.28; CI 95%, 0.12-13.54; p = 0.66), mismatched cord blood units according to 10 loci matching criteria (RR 0.56; CI 95%, 0.05-5.86; p = 0.53), and malignant primary disease (RR 0.74; CI 95%, 0.07-7.86; p=0.64). The non-significant results could be contributed to the fact that the small sample size did not meet the requirement for 80% power. Despite this major limitation, the study helped further understand how match grade is related to GVHD development especially since there is a limited number of studies on this topic to begin with. This study emphasizes the need for further large-scale research to gain a more comprehensive understanding of UCB transplantation. Further research has implications to improve the transplant-related diseases in the pediatric populations around the world.

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BACKGROUND

Literature Review

       Hematopoietic stem cell (HSC) transplantation is a treatment for specific malignant diseases, such as acute myeloid leukemia (AML), and specific non-malignant diseases, such as severe combined immunodeficiency (SCID) (Galgano & Hutt, 2017). There are two types of transplantation: autologous and allogeneic. The type of transplant chosen depends on the primary disease and the purpose of the treatment. In autologous transplantation, a patient serves as their own donor and receives their own HSC. After undergoing high-dose chemotherapy as treatment for malignancy, patients often experience adverse effects including chemotherapy-induced bone marrow damage which affects the patient's ability to create new blood cells. An autologous stem cell infusion would treat this issue by helping restore the patient's HSC. Since an individual is receiving their own cells, there is no risk of graft-versus-host disease (GVHD). Allogeneic transplantation is used to describe transplantation that uses HSC derived from a donor (Hatzimichael & Tuthill, 2010). The purpose of an allogeneic transplantation is to either replace the recipient's immune system with the donor's to help eliminate malignancy in malignant disease or to replace non-functional elements of the recipient's immune system in non-malignant diseases (Galgano & Hutt, 2017). In an allogeneic transplant, the donor may be syngeneic, related, or unrelated. Syngeneic is a type of allogeneic transplantation where the donor is the identical twin of the recipient, thus the recipient and donor are genetically identical with no risk of GVHD (Hatzimichael & Tuthill, 2010). For both unrelated and related allogeneic transplants, the recipient and donor are matched according to human leukocyte antigen (HLA) compatibility

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to minimize the risk of GVHD, graft rejection, and other transplant related complications (Tiercy, 2016).

The transplantation process includes several steps: donor identification, mobilization and harvesting, conditioning, infusion, neutropenic phase, and recovery and engraftment (Galgano & Hutt, 2017). The donor identification involves analyzing the HLA compatibility between the recipient and the donor to determine if they are an ideal match. This process will be discussed in further detail later. Once a donor is identified, the HSC must be extracted from an HSC source which includes bone marrow, peripheral blood, or cord blood units (CBU). Before the actual HSC infusion can occur, the recipient must undergo conditioning (Khaddour & Mewawalla, 2019). Conditioning is also known as preparative regimen, and this process includes the recipient undergoing chemotherapy and, depending on primary disease and treatment protocol, potentially total body irradiation. The purpose of this process is to eliminate any remaining malignant cells and non-functional hematopoietic cells and suppress the immune system in order to minimize the risk of graft rejection (Hatzimichael & Tuthill, 2010). Once conditioning is completed and the HSC is collected and processed based on specific treatment protocol requirements, it is then infused into the recipient. After conditioning and infusion, the recipient undergoes a neutropenic phase where their neutrophil counts and overall blood counts are low (Galgano & Hutt, 2017). At this stage, the recipients are immunocompromised thus more at risk for infections. In addition, due to low counts of blood components, including platelets and hemoglobin, they are more susceptible to bleeding and fatigue. The final step of the transplantation process is recovery and engraftment. A successful transplantation is determined by the recipient's ability to accept the donor's HSC

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and achieve engraftment (Gonçalves, Benvegnú, & Bonfanti, 2009). A recipient has achieved engraftment once their neutrophil count has recovered and reached a threshold of >500/µL cells.

In order to determine if a donor is a suitable match for the recipient, HLA typing is completed. HLA refers to surface proteins that are located on leukocytes, or white blood cells, and most other cells (Berger, 2001). These surface proteins play a role in the regulation of immunological responses (Choo, 2007). The HLA compatibility of the donor and recipient is thus important because if poorly matched, it could elicit an immunological response that could lead to graft rejection and other adverse effects including GVHD and even death. HLA compatibility is determined through the process of HLA typing which involves collecting a blood sample from both the potential donors and recipient and identifying specific genetic markers through molecular tests (Berger, 2001). The genetic markers are found across ten loci on chromosome 6 and are typed to determine HLA compatibility across five antigens: A, B, C, DRB1, and DQB1 (Mahdi, 2019). The gold standard when it comes to the ideal donor is an HLA-identical sibling donor (Tiercy, 2016). However, only 30% of recipients are transplanted with an HLA-identical sibling. If an HLA-identical sibling is not available, then a matched unrelated donor is considered the gold standard. It is important to find an appropriate match for a recipient in order to reduce the risk of graft failure, GVHD, and death (Baxter-Lowe & Hurley, 2008). In order to find a suitable donor, two types of typing are used: high-resolution typing and low-resolution typing (Tiercy, 2016). High-resolution typing examines each locus at an allelic level, so it considers differences in alleles. Table 1 demonstrates high-resolution HLA typing which includes both the antigen and allele typed.

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For example, in Table 1, at antigen A, the recipient HLA high-resolution typing would be "A*02:01", and it includes the allele portion "01". Low-resolution typing only examines it at an antigen level. For example, it would be typed as "A*02". For bone marrow and peripheral blood-derived HSC products, high-resolution typing at all 10 loci is typically recommended, and donors are considered suitable if they match at least 9 out of 10 loci (Demiriz, Tekgunduz, & Altuntas, 2012). Table 1 demonstrates high-resolution typing of a donor and recipient. They demonstrate allelic-matching at all loci except for the allelic mismatch at A2, so the recipient and donor are considered a 9/10 match.

Table1. High Resolution Matching

Recipient HLA Typing					
	A	**B**	**C**	**DRB1**	**DQB1**
	02:01	07:06	04:02	03:02	04:01
	11:01	07:06	07:02	13:01	06:01
Donor HLA Typing					
Match Grade	**A**	**B**	**C**	**DRB1**	**DQB1**
9/10	02:01	07:06	04:02	03:02	04:01
	11:02	07:06	07:02	13:01	06:01

For cord blood products, a match of at least 4 out of 6 loci at antigen A, B, and DRB1 is recommended. In addition, low-resolution matching is examined at antigen A and B and high-resolution matching is examined at DRB1. Table 2 demonstrates how low-resolution matching occurs at antigen A and B where A2 is considered a mismatched, and high-resolution matching occurs at DRB1, where they match at an allelic level.

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Table 2. Cord Blood HLA Typing

Recipient HLA TYPING			
	A	B	DRB1
	02:XX	07:XX	03:02
	03:XX	07:XX	13:01
Donor HLA TYPING			
Match Grade	A	B	DRB1
5/6	03:XX	07:XX	03:02
	03:XX	07:XX	13:01

This demonstrates how there is more flexibility when it comes to HLA compatibility of a cord blood product and is advantageous to use for transplantation.

GVHD is a complication that can occur after allogeneic transplantation, and emphasizes the importance of HLA compatibility between the recipient and donor. It is an immune-mediated response that occurs after transplantation when the donor's immune cells react with the recipient's histocompatibility antigens and starts attacking the recipient's tissues from different organ systems, including but not limited to the skin, liver, and lungs (Kim, Kim, & Cho, 2013). GVHD can be categorized as acute GVHD (aGVHD) or chronic GVHD (cGVHD) (Schroder & DiPersio, 2011). Traditionally, the classification was based on time of development with aGVHD occurring within 100 days of transplant, and cGVHD occurring 100 days after transplant with symptoms sometimes not appearing 2-5 years after transplant. However, there has been a shift in this definition, and clinical and histological features in addition to time of development are considered for classification. Examples of clinical manifestations of acute GVHD include skin involvement such as maculopapular rash

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and gastrointestinal involvement such as diarrhea (Chao, 2019). Each organ that is affected by aGVHD is staged according to the extent of involvement. aGVHD is staged according to number of organs affected and the extent of involvement. Further details on how each organ system is staged by the International Bone Marrow Transplant Registry is demonstrated in Table 3.

Table 3. aGVHD Staging and Grade

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dl	< 500 ml/day or persistent nausea.
1	Maculopapular rash< 25% BSA	2–3 mg/dl	500–999 ml/day
2	Maculopapular rash 25 – 50% BSA	3.1–6 mg/dl	1000–1500 ml/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dl	Adult: >1500 ml/day
4	Generalized erythroderma plus bullous formation	>15 mg/dl	Severe abdominal pain with or without ileus
Grade			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2–3 or	Stage 2–4
IV	Stage 4 or	Stage 4	-

The overall extent of aGVHD is assigned an overall grade based on simple calculations of the individual staging scores (Jacobsohn & Vogelsang, 2007). Grade I-II aGVHD is typically described as mild to moderate, while grade III-IV is typically described as severe to life-threatening and thus considered high-grade aGVHD. Also, the overall aGVHD grade demonstrates correlation with mortality with recipients experiencing grade III and grade IV

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having a 30% and 5% probability of achieving long-term survival, respectively (Cahn, *et al.,*2005). In contrast, there is a >80% chance of long-term survival achievement with recipients that experience a grade I-II aGVHD. There is a similar scoring system to stage cGVHD that is based on the organs affected and extent of involvement. However, there is only two categories to determine the overall grade of cGVHD: limited and extensive (Socié & Ritz, 2014). cGVHD is categorized as limited if it only there is only skin and/or liver involvement. If there is other organ involvement including eye, lungs, salivary glands, and/or other organs, it is considered extensive cGVHD. In addition to the degree of HLA matching between donor and recipients, other risk factors for GVHD development include HSC source, the preparative regimen, age, differences in sex between donor and recipient, and donor cytomegalovirus (CMV) serology among other risk factors (Bhutani, 2015). However, the effect of CMV serostatus on transplant outcomes and risk of developing GVHD has not been consistent across studies, and further research is needed to fully understand its effects (MacMillan, *et al.,* 2009).

Although an HLA matched donor decreases the risk of GVHD development, an estimated 40% of recipients still develop aGVHD despite receiving HLA identical products (Ferrara, Levine, Reddy, & Holler, 2009). High-grade aGVHD occurs in approximately 11-18% of allogeneic transplant recipients, and the mortality rate ranges from 70-90% for the severest grade of aGVHD (Henig & Zuckerman, 2014). According to data submitted to the Center for International Blood and Marrow Transplant Research (CIBMTR), from 2015-2016, one of the main causes of transplant-related mortality is GVHD (D'Souza & Fretham, 2018). GVHD was the cause of death for 8% of recipients that died within 100 days after

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transplant and 10% for recipients that died after the 100 days mark. Overall UCB transplant recipients experience lower rates of GVHD. According to a study by Herr *et al.*, the results showed that the rates of aGVHD were approximately 12% and cGVHD were approximately 10% when examining cord blood transplants that occurred within the European Union (2010). These rates emphasize the importance of further research to understand the development of GVHD to minimize risks for future transplantation.

After identifying and confirming a donor, the HSC must be extracted from the donor. The process of extraction differs depending on the HSC source, which includes bone marrow, peripheral blood, and umbilical cord blood. For HSC extraction from a bone marrow source, the donor is under either local or general anesthesia (Khaddour & Mewawalla, 2019). The process involves inserting a hollow needle into the bone, typically the anterior or posterior iliac crest, to collect the bone marrow. Multiple aspirations are completed with the total depending on how many cells need to be collected, usually 1 to 1.5 liters with 15 mL per aspiration. For HSC extraction from peripheral blood source, the donor must take a mobilization agent, such as granulocyte colony-stimulating factors (G-CSF), prior to collection (Hatzimichael & Tuthill, 2010). The mobilization agent helps increase the number of HSC in the peripheral blood of the patient which is then collected through apheresis. Finally, HSC extraction from umbilical cord blood source takes place after birth (Risso *et al.,* 2018). A needle is inserted into the cord and at least 40 milliliters of blood is collected. The blood is then cryogenically frozen and stored at a cord blood bank.

There are different factors that are considered when choosing among these three sources. When comparing these three sources, there are differences in risk and recovery

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(Demiriz, Tekgunduz, & Altuntas, 2012). There are many factors that differentiate UCB-derived HSC products from bone marrow and peripheral blood-derived HSC products. UCB is the only source where there is no risk for the donor during the extraction process, and there is a much shorter duration of time needed to find a donor, about one month or less. Both peripheral blood and bone marrow extraction poses as a risk to the donor, and it typically takes 3 to 6 months to find a suitable donor. Recipients that receive HSC from a peripheral blood source are also at a higher risk of GVHD (Bensinger, 2012). This pattern is most likely due to the fact that there is typically a higher number of T-cells found in peripheral blood grafts compared to bone marrow grafts, thus recipients are more likely to develop an immunological response to their transplant if they received peripheral blood derived products than bone marrow derived products (Gorin *et al.,* 2002). As previously mentioned, recipients that received a UCB-sourced product typically experience lower rates of GVHD and most likely this is contributed to the fact that there are lower counts of total T-cells with the majority being naïve T-cells found in UCB-sourced products compared to peripheral blood and bone marrow (Politikos & Boussiotis, 2014). One major limitation to UCB-derived HSC products is that transplantation using these cells is typically limited to pediatric patients. The amount of HSC needed for transplantation is determined by the recipient's weight and ranges from 2 to 4 x 10^8 nucleated cells per kilogram of the recipient's weight (Karanes *et al.,* 2003). There are typically not enough cells within a single UCB unit to use for transplantation in adults unless multiple UCB units are used. Another disadvantage is that UCB recipients take a longer time to achieve engraftment compared to peripheral blood and bone marrow recipients with peripheral blood recipients having the fastest recovery time

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(Demiriz, Tekgunduz, & Altuntas, 2012). Cord blood units contain fewer HSC than other sources, and the more limited number of HSC most likely contributes to the slower engraftment time. Also, due to this delay in engraftment there is a higher risk of post-transplant infection with UCB than bone marrow or peripheral blood (Galgano & Hutt, 2017). As previously mentioned, after conditioning and infusion, recipients undergo a neutropenic phase where they are more immunocompromised, thus more susceptible to infections. Since cord blood recipients typically take a longer time to achieve engraftment, this means that they are also in this neutropenic phase longer and have an increased risk of infections. Although UCB transplants are associated with longer engraftment times, UCB transplants are also associated with high rates of engraftment at 85-100% of transplants achieving engraftment (Danby & Rocha, 2014).

As previously mentioned, UCB transplantation is an advantageous option for individuals especially those with no feasible matched donor options due to more rapid availability, lower rates of GVHD, and minimal extraction risk among other factors. As of 2017, approximately 700,000 UCB units have been donated and 40,000 UCB transplantations have occurred (Ballen, 2017). According to data published by CIBMTR, from 2013-2017, CBU transplants account for 4% (n = 4,335) of all transplants that were performed in the United States and reported to CIBMTR (D'Souza & Fretham, 2018). For comparison, peripheral blood transplants account for 86% (n = 91,636) and bone marrow transplants account for 9% (n = 9,833).

The importance investing in cord blood research, especially to better understand advantages to using it over other HSC sources, is reflected in the global burden of cancer

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especially pediatric cancer as it continues to increase with about 200,000 children and adolescent diagnosed annually (Rodriguez-Galindo *et al.,* 2013). Although there have been improvements in the diagnosis, treatment, and outcome of pediatric cancers in high-income countries (HIC), which is reflected in the over 80% 5-year survival rate, low to middle-income countries (LMIC) continue to face low rates of survival with survival rates varying across regions (Force *et al.*, 2019). For example, it is estimated that the pediatric cancer mortality rate is about 90% in Sub-Saharan Africa. This is especially concerning because more than 80% of the pediatric population at risk for developing cancer are in LMIC (Rodriguez-Galindo *et al,* 2013). The availability and accessibility of treatment is especially important due to this continued upward pattern of the global burden of pediatric cancer. Although ideally a matched donor source is used for transplantation and there are over 33 million marrow donors registered globally, only about one third of patients are able to find an HLA-matched unrelated donor (Brunstein & Weisdorf, 2009). The success rate of finding a donor varies across racial and ethnic backgrounds with recipients of racially and ethnically diverse backgrounds having a much lower success rate than Caucasian recipients. Further support of UCB banking through education, resources, and research provides more options for alternative sources especially since UCB is rapidly available and mismatched-HLA UCB products still demonstrate high rates of engraftment and low incidences of graft-versus-host disease.

In addition to potentially addressing the needs of patients facing malignant diseases such as hematological cancers, HSC transplantation also serves as the only cure for otherwise debilitating or deadly non-malignant diseases. Sickle cell disease (SCD) is an example of an

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inherited non-malignant hematological disease where currently the only cure is HSC transplantation (Bernaudin *et al.*, 2007). SCD affects millions of people globally especially in malarial endemic areas (Wastnedge *et al.*, 2018). The higher prevalence in these areas are due to the fact that individuals that are genetically heterozygotic for SCD are protected against malaria (Luzzatto, 2012). These areas are also resource poor areas with limited access to healthcare which often translates to poor disease outcomes and survival rates for SCD. In LMIC, which accounts for 90% of SCD, it is estimated that 500 children die every day due to lack of proper care and 90% of children inflicted with SCD do not reach their fifth birthday (Wastnedge *et al.*, 2018). These poor survival rates demonstrate the importance of investing resources and funding research to help create better outcomes for individuals affected by SCD especially in the area of cord blood transplantation. As previously mentioned, HSC transplantation serves as the only curative option and with the advantages of CBU over other HSC sources and a large pediatric population, CBU serves as a viable option to help combat the high global burden of disease and low survival rates.

In addition, expansion of the cord blood registry has the potential to address ethical concerns regarding the use of donors, both children and adults, to recover hematopoietic stem cells. The current process of UCB collection does not cause harm to the mother or the baby or increase their risk of adverse events (Cavusoglu *et al.,* 2017). Other sources of HSC including bone marrow and peripheral blood must be recovered directly from patients, so there are risks of injury, adverse events, and even death. The risks are extremely rare, but there is still a chance of causing adverse effects in a donor that was completely healthy prior to donation. In a study by Halter *et al.*, 383 transplant teams were given a survey to complete

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regarding transplantation outcomes (2009). The study found that out of a total of 51,024 transplantations, 37 reported severe adverse events, five donors had died, and 20 reported hematological malignancies. As the results demonstrate, the risks are very rare and could be considered statistically negligible, but there is an ethical conflict of placing an otherwise healthy individual in a situation that could be potentially harmful or fatal. Another area that is potentially ethically concerning is the use of children as donors, including both using preimplantation HLA typing to create a donor child or using a matched-sibling as a donor (Devolder, 2005). The idea of having a child solely to serve as a donor for a sibling can be a potential gray area as some people see it as creating a child to basically use their parts. Using children in general can be a potential concern because the children are typically not the ones making the decision to continue with the donation process. They do not have full autonomy in regards to donation. Using a UCB product eliminates these ethical concerns.

Public Health Significance

In 1957, the first allogeneic bone marrow transplantation was performed in New York, and involved using the identical twin of the recipient as the donor (Henig & Zuckerman, 2014). Since that successful event, the field has rapidly evolved through research and development to where allogeneic transplants no longer just involve matched siblings but also include the options of matched and mismatched unrelated donors, and umbilical cord blood (UCB) units as a donor source. The field continues to evolve and now even includes haploidentical donors. The use of haploidentical donors is an alternative option if a matched sibling donor is not available and involves using a donor that is half-matched typically the mother or father of the recipient (Zheng *et al.,* 2020). The availability of new sources of

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hematopoietic stem cell (HSC) donations means that transplantation is a more accessible treatment for individuals affected by cancer and other life-threatening disorders.

In the United States, cancer is currently the second leading cause of death overall among all ages (21.3% of total deaths), the third leading cause of death in children between 1 to 4 years of age and 10 to 14 years of age (8.4% and 13.6% of total deaths in respective age groups), and the second leading cause of death in children between 5 to 9 years of age (17.8% of total deaths within age group) (Heron, 2019). Hematological cancers are the most common type of cancer among children with the most common being pediatric leukemia, which represents about 30% of pediatric cancers (Madhusoodhan, 2016). Since HSC transplantation is an established treatment for high-risk and relapsed cancer patients, the overall rates of pediatric cancer emphasize the importance of the expansion and use of UCB units as products. UCB transplantation is typically reserved for the treatment of pediatric patients due to the limitations in cell numbers per UCB unit (Brunstein & Weisdorf, 2009). Adult patients undergoing UCB transplantation have to use two or more units of UCB in order for the number of cells to be sufficient. The expansion of the UCB registry through increased cord blood banking is especially important in the context of global cancer rates, which as previously mentioned, continues to rise.

Even though preventative care is the focus of public health and transplantation is a form of treatment for curative purposes, it has important implications in the field of public health. Most of the individuals that are eligible for transplantation are inflicted with diseases that have a genetic component, such as some hematological cancers, sickle cell disease, and severe combined immunodeficiency, and often there are no preventative measures that affect

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the development of disease, and transplantation is the only option that provides them with a better quality of life or a better chance of survival. Also, from a global perspective, diseases such as hematological cancers and SCD are concentrated within specific communities, which are often resource-poor with limited access to healthcare. This demonstrates that there needs to be more outreach in terms of educational programs, policy development, resource distribution, and projects that focus on understanding the population. In addition to making transplantation a more accessible treatment option for these populations, it is also important to understand their issues and needs to help develop outreach programs and provide resources that would improve the health of the community overall.

Although UCB units offer many advantages including more potential product sources due to less stringent HLA-matching criteria, products more rapidly available, and addresses ethical issues concerning use of donors among others, the past few years demonstrate that there is a decrease in the overall use of UCB units (Rafii *et al.,* 2016). Alternative sources of products, such as HSC from haploidentical donors, could be most likely contributing to this current trend of moving away from UCB transplantation. However, due to continued high rates of cancer both within the United States and globally especially among pediatric patients, it is important to continue investing in UCB-related treatments through research, development, and educational outreach programs relating to banking. This includes further research into understanding the advantages of using UCB source products including lower rates of GVHD and consistent levels of engraftment achievement to help encourage its use as a viable option. As GVHD is one of main causes of transplant-related mortality, it is important continue research in GVHD outcomes from using UCB source products in order to

better understand how to decrease transplant-related complications and improve survival rates.

Hypothesis and Study Objectives

The purpose of this study is to determine the difference in incidence of GVHD among pediatric cord blood transplant recipients based on human leukocyte antigen match grade. Current literature supports that unlike other stem cell products, such as peripheral blood and bone marrow, recipients do not need to be matched strictly according to high-resolution HLA requirements for cord blood transplantation in order to achieve engraftment for a successful treatment. Also, there are limited studies on how primary disease affects cord blood engraftment and GVHD, so this study will help further understand this gap in knowledge.

The purpose of the study can be further summarized through the following objectives:

- Primary Objective:
    - To determine how HLA match grade compatibility of CBU transplantation affects GVHD development and engraftment achievement status specifically if an HLA mismatched product increases the risk of GVHD development or engraftment failure
- Secondary Objectives:
    - To analyze how primary disease of transplantation recipient affects GVHD development and engraftment achievement status
    - To analyze how CBU origin affects GVHD development and engraftment achievement status

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Based on current literature, I hypothesize that there is no increased risk of failure to achieve engraftment and GVHD occurrence among mismatched UCB recipients and that primary disease has no effect on either engraftment or GVHD development.

METHODS

Study Design

A retrospective cohort study was conducted to study engraftment achievement and the incidence of GVHD among cord blood transplant recipients based on HLA compatibility and match grade.

Study Subjects and Study Setting

The study involved pediatric patients that underwent transplantation at the Bone Marrow Transplant (BMT) unit at Texas Children's Hospital (TCH) at the Texas Medical Center in Houston, Texas. Subjects were eligible for selection based on the following criteria:

<u>Inclusion Criteria:</u>

Subjects will be considered eligible for the study based on the following inclusion criteria: 1) The patient must have undergone transplantation at the BMT unit at TCH, 2) Patients must have received a cord blood product and only received product from one donor, or in other words, they only received one CBU, and 3) They must be a pediatric patient and under the age of 18, and 4) The patient must have underwent cord blood transplantation between 2008 and June 2019.

<u>Exclusion Criteria:</u>

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Subjects will not be considered eligible for the study based on the following exclusion criteria: 1) If they received multiple CBU or received mixed products, e.g. bone marrow product in addition to cord blood, 2) If they underwent transplantation at an outside hospital and is currently being followed by TCH, 3) If they are 18 years of age or older, and 4) if they underwent transplantation after June 2019, since not enough time has passed for these patients to be examined for engraftment and graft-versus-host disease.

Sample Size Calculation and/or Study Power

A sample size of 240 is required for 80% power was calculated based on the following assumptions:

- $\alpha = 0.05$

- Incidence of GVHD among matched and mismatched is 9% and 22%, respectively

Data Collection and Data Analysis

The BMT unit at TCH is part of the network of CIBMTR under the National Marrow Donor Program, which requires the unit to submit data relating to patient history, details of transplant, and outcome to CIBMTR. In addition, the BMT unit has an in-house database, StemSoft, which can be used to generate data sets for analysis based on what is reported to CIBMTR. Information regarding patient demographics, transplant history, product type (matched or mismatched), conditioning, engraftment status, acute GVHD, and chronic GVHD was obtained from StemSoft. The classification of acute GVHD and chronic GVHD at TCH is according to the traditional criteria that focuses on the time of development with GVHD occurring within 100 days of transplant considered acute GVHD, and GVHD

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occurring 100 days after transplant considered chronic GVHD (Schroder & DiPersio, 2011). Information regarding HLA typing, product country of origin, donor's maternal CMV status, and HLA match grade are not reported in the database so this information was manually ascertained through medical records and HLA reports. The information that was manually ascertained was also verified by Dr. Caridad Martinez, the assistant Director of the BMT unit at TCH, to ensure accuracy.

In order to protect patient confidentiality and ensure data security, all data that contains patient information will be compiled at the Feigin Tower at TCH. The building is limited to TCH and Baylor College of Medicine employees, and the work areas in the building are only accessible by badge by authorized individuals. All medical records that will be used in this study are electronic, so there are no risks of misplacement of physical copies. All computers at TCH are password protected and only limited to authorized users. In addition, the data prior to de-identification will be locally stored on a computer in office 1540 at the Feigin Tower that only the researcher can access. The office can be locked and is located in an area that is only accessible by badge specific individuals. Once the data is compiled, it will be de-identified for analysis.

The statistical analysis software STATA will be used for data analysis. Data analysis will include calculation of relative risk to determine the risk of occurrence of GVHD or engraftment failure after exposure to risk factors such as match grade, primary disease type, geographical origin of CBU, and donor's maternal CMV status. For all statistical analysis, a p-value of less than or equal to 0.05 will be used to determine significance. Additional tests

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of statistical analysis will include Chi-Square tests to determine associations between match-grade and acute GVHD development.

Human Subjects

During the data collection stage of the study, sensitive health information was accessed through the StemSoft database and electronic medical records in order to create data sets for analysis. There was no direct patient contact for this study. The handling of sensitive health information was completed following BMT departmental protocols. In addition, Dr. Caridad Martinez has provided a letter for her permission of use of data for the study on January 8, 2020. The data will be de-identified for analysis, and, as previously mentioned, only the primary investigator and Dr. Martinez will have access to the data prior to de-identification solely for data collection purposes. Furthermore, the study was approved by the IRB under HSC-SPH-20-0023 on January 28, 2020. IRB approval was obtained to ensure that the study adheres to strict research guidelines

<div align="center">

RESULTS

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Overall Transplant Recipient Characteristics

A total of 102 cord blood transplantations occurred at TCH between 2008 and June 2019. After the recipients' eligibility status was determined based on the previously mentioned inclusion and exclusion criteria, a total of 82 recipients were included for analysis. A summary of the transplant recipient characteristics is shown in Table 4. The median age of the recipient at transplant was 0.90 years (10.8 months) with the youngest recipient aged 0.13 years old (1.6 months) and the oldest aged 9.31 years old. As of the time of data collection, 74.39% (n=61) of recipients were alive and 25.61% (n=21) of recipients had died. Figure 1

details the cause of death of the recipients in this cohort. There was a total of 21 recipients (25.61%) that died, and their cause of death includes the following: 47.62% (n=10) from Relapse/progression/persistent disease, 9.52% (n=2) from GVHD, 9.52% (n=2) from transplant-related infections, 4.762% (n=1) from other transplant-related causes, and 28.57% (n=6) from other causes. Approximately 59.76% (n=49) of recipients underwent transplantation due to a non-malignant primary disease including anemia/hemoglobinopathy (n=4), histiocytic disorder (n=2), immune deficiency (n=37), inherited disorder of metabolism (n=4), and other non-malignant diseases (n=2). Approximately 40.24% (n=33) of recipients underwent transplantation for malignant diseases including acute leukemia (n=28) and MDS (n=5).

Overall Cord Blood Unit Characteristics

A summary of the cord blood unit characteristics is included in Table 5. The median number of years between the date of UCB collection and date of transplantation is 3.67 years (range 0.52-19.49 years). Approximately 81.71% (n=67) of CBU were from a domestic origin, meaning that they were collected and stored at a cord blood bank within the United States. In addition, 18.29% (n=15) of CBU were from an international origin thus collected and stored at a cord blood bank outside the United States. Further examination of the donor sex determined that 50.62% (n=41) were female and 40.38% (n=40) were male. Finally, the maternal CMV status of the CBU were as followed: 29.27% (n=24) negative, 47.56% (n=39) positive, and 23.17% (n=19) unknown.

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Effect of HLA Compatibility on the Development of acute GVHD and Engraftment

Table 4 shows how the 82 recipients are categorized according to both the less stringent cord blood method of matching across 6 loci and the high-resolution HLA matching across 10 loci. When examining how the recipients are grouped based on HLA matching out of 6, 1.22% (n=1) recipients had an HLA-match grade of 4/6 to the donor, 59.76% (n=49) were a 5/6 match, and 39.02% (n=32) were a 6/6 match. When examining how the recipients are grouped based on HLA matching out of 10, 21.95% (n=18) matched 10/10, 19.51% (n=16) matched 9/10, 41.46% (n=34) matched 8/10 or 7/10, 12.20% (n=10) matched 6/10 or 5/10, and 4.88% (n=4) matched 4/10 or 3/10.

A Chi-Square analysis was performed in order to determine the association between the 6 loci or 10 loci match grade and development of acute GVHD. The results for the 6 loci match-grade ($X^2 = 0.66$; p=0.96; Table 6) and 10 loci match-grade ($X^2 = 4.85$; p=0.99; Table 6) were both found to be not significant indicating that there was no association between the match grade and aGVHD development for each criterion. In addition, one important assumption of the Chi-Square analysis test is that the expected value of each cell is greater than 5. Since multiple cells during the analysis for both 6 and 10 loci match-grade does not meet this criteria, a Fisher's exact test was performed. However, the p-values for both factors for the Fisher's Exact test was greater than 0.05 thus not significant.

Examination of the aGVHD rates show that 90.12% (n=73) did not experience GVHD while 4.94% (n=4) experienced a Grade I-II of aGVHD and 4.94% (n=4) experienced a Grade III-IV of aGVHD. For cGVHD, 98.55% (n=68) experienced no cGVHD while 1.45% (n=1) experienced extensive cGVHD. Please note that the total number of recipients

that were examined for acute and chronic GVHD development do not add up to 82 recipients. This is due to the recipients passing away before they can be assessed for development of GVHD. Further analysis of the data was completed to determine if mismatched HLA based on both 6 loci and 10 loci criteria is a risk factor for aGVHD development. The results support that there is no significant increased risk of aGVHD development for recipients with mismatched donors (RR 1.09; 95% CI, 0.28-4.24; p= 0.61; Table 7) when analyzed according to the 6 loci criteria. In addition, there was no significant increased risk of aGVHD development among recipients with mismatched donors versus matched donors (RR 0.86; 95% CI, 0.199-3.89; p= 0.57; Table 7) when analyzed according to the 10 loci criteria.

In addition, 96.34% (n=79) of transplant recipients achieved engraftment while 3.66% (n=3) did not achieve engraftment. The data was analyzed to determine if mismatched HLA is a risk factor for engraftment achievement failure. Based on analysis of the relationship between HLA compatibility and engraftment achievement as shown in Table 8, there is no significant increased risk of not achieving engraftment among recipients with mismatched donors versus matched donors (RR 1.28; 95% CI, 0.12-13.54; p = 0.66) when analyzed according to the 6 loci criteria. The results for the 10 loci criteria also did not demonstrate a significant increase of risk of not achieving engraftment among recipients with mismatched donors (RR 0.56; 95% CI, 0.05-5.86; p= 0.53; Table 8).

Effect of Primary Disease on the Development of acute GVHD and Engraftment

As previously stated, 59.76% (n=49) of recipients underwent transplantation due to a non-malignant primary disease, and 40.24% (n=33) of recipients underwent transplantation for malignant diseases. The data was further analyzed to determine primary malignant

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diseases were a risk factor for developing aGVHD. Further analysis to understand the relationship between primary disease and development of aGVHD show that recipients with primary malignant diseases had 2.55 times (95% CI, 0.66-9.95; p=0.154; Table 7) the risk of developing aGVHD than recipients with primary non-malignant diseases, however the results are not significant. Since the 95% confidence interval contains the null value of 1, the results are not significant. When examining the relationship between primary disease and engraftment achievement, it was calculated that recipients with primary malignant diseases had 0.742 times (95% CI, 0.07-7.86; p=0.65; Table 8) the risk of not achieving engraftment than recipients with primary non-malignant diseases, so primary malignant disease can be interpreted as a protective factor for achieving engraftment. However, the difference in risk was found to be not significant for the same reason as previously mentioned.

Effect of CBU properties on the Development of acute GVHD and Engraftment

Since there are limited studies on the effect of geographic origin on CBU and development of GVHD, the data was analyzed to determine if a CBU of international origin had a higher risk ratio of developing GVHD than those of domestic origin. Further analysis to examine the association between geographical origin of CBU collection and development of GVHD showed that recipients transplanted with a CBU from an international origin had 0.628 times the risk (95% CI, 0.08-4.73; p=0.54; Table 7) of development of aGVHD than recipients transplanted with a CBU from a domestic origin. However, since the 95% confidence interval contains the null value of 1, the results are not significant, and there is no significant increase in risk for recipients that was transplanted with a CBU from a international origin. Finally, CMV serology status is not a well understood risk factor of

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GVHD, so the data was analyzed to determine if a positive donor's maternal CMV status was associated with an increased risk of GVHD development in the recipient. Furthermore, when examining the association between GVHD and donor's maternal CMV status, it was found that recipients that were transplanted with a CBU unit whose donor's maternal CMV status was positive had 0.821 times the risk (95% CI, 0.20-3.35; p=0.54; Table 7) of developing aGVHD than recipients that were transplanted with a CBU unit whose donor's maternal CMV status was negative, which can be interpreted as a positive CMV status being a protective factor against the development of aGVHD. However, the risk was found to be not significant based on the p-value (0.54) and the 95% CI containing the value of 1.

Effect of Recipient Sex and Mismatched Recipient-Donor Sex on Development of acute GVHD

Based on the discrepancy between the number of male (n=55) and female recipients (n=26) that underwent UCB transplantation, further analysis was completed to determine if the sex of the recipient was a potential risk factor for development of aGVHD. It was examined if males had an increased risk of aGVHD development. Based on further analysis, it was found that male recipients had 3.31 times the risk (95% CI, 0.43-25.52; p=0.20; Table 7) of developing aGVHD than female recipients. However, the risk was found to not be significant due to the Fisher's exact test p-value of 0.20 and due to the CI including 1.

In addition, it was also examined if differences in sex between the recipient and donor is a risk factor for aGVHD development. Further analysis conducted to determine if a mismatched recipient-donor sex was associated with an increased risk of aGVHD. Based on the results of the analysis, it was found that mismatched recipient-donor sex pairs had 0.59

times the risk (95% CI, 0.15-2.29; p=0.34; Table 7) of developing aGVHD than matched

recipient-donor sex pairs which indicates it is a protective factor. This result was also found

to not significant due to the p-value (0.34) and the CI containing 1.

DISCUSSION

Analysis of whether HLA match grade, primary disease, geographical origin of CBU,

and donor's maternal CMV status as potential risk factors for GVHD development were

shown to be not significant. In addition, analysis of HLA match grade and primary disease as

potential risk factors of failure to achieve engraftment were also not significant. The purpose

of this study was to analyze these risk factors to see if there is an overall consistency with

previous studies to further support that transplantation with CBU provides advantages over

other sources of HSC that would be especially beneficial to pediatric patient populations.

Overall, the results of this study were consistent with the following previous studies.

About 10% of the recipients in this cohort developed aGVHD which is similar to previous

studies that estimated around 12% of recipients develop aGVHD (Herr *et al.*, 2010).

However, the percentage of recipients that developed cGVHD for this cohort was about 1%

while it was estimated to be around 10% by Herr *et al.* (2010). Many factors could have

contributed to this discrepancy including a larger sample size for other studies, discrepancy

in GVHD treatment protocols at TCH versus other institutions, and differences in the makeup

of the population included in the study especially if other studies included a mix population

of pediatric and adult recipients. For example, in terms of differences in population make-up

as a potential factor, this study only included pediatric patients, and age is considered a

potential risk factor for GVHD development as older recipients being at increased risk

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(Bhutani, 2015). Furthermore, the severity of disease for recipients could have had an effect on the rates of GVHD. About 26% of recipients died after undergoing transplantation with about 10% of the total deaths due to GVHD complications. This is consistent with the data reported to CIBMTR that estimates 8-10% of recipients died as a result of GVHD complications (D'Souza & Fretham, 2018). Even though the findings for mismatched donor-recipient sex were not significant, the results contradicted previous studies since it was found to be a protective factor while previous studies found it to be a risk factor (Bhutani, 2015). Further research is necessary to get a comprehensive understanding of how the factor affects GVHD development. Although the intention of the study was to also analyze the risk of developing cGVHD, further analysis could not be completed due to only one recipient developing cGVHD. Analysis of the current data would not have reflected accurate findings due to the rarity of the condition. Overall, as previously discussed, this study demonstrates the consistency across studies regarding the rates of developing GVHD which further supports the benefits of CBU transplantation.

The primary objective of the study was to determine how HLA match grade compatibility of CBU transplantation affects GVHD development and engraftment achievement status. Based on statistical analysis of the data collected, there was no significant association between match-grade (both for 6 loci and 10 loci) and development of aGVHD as well as no statistically significant increased risk of aGVHD development among mismatched versus matched recipients. Analysis of engraftment status showed similar results in that there was no significant difference in risk between mismatched versus matched recipients in failing to achieve engraftment. This further supports that there is flexibility in

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terms of HLA matching in CBU without the risk of developing adverse reactions relating to

GVHD and engraftment failure. Furthermore, about 96% of recipients achieved engraftment

which is on par with previous studies that consistently showed high engraftment rates among

CBU transplant recipients where 85-100% of recipients achieved engraftment (Danby &

Rocha, 2014). Although age has been shown to be a risk factor for development of acute

GVHD, studies have consistently supported that children are 12 years old or younger at very

low risk for both aGVHD and cGHVD (Qayed *et al,* 2018). Further analysis on age as a risk

factor was not completed since the oldest recipient in this cohort is approximately 9 years old

and younger than threshold for age to be considered a risk factor.

The secondary objectives focused on other potential risk factors that could contribute

to GVHD development and engraftment failure. The type of primary disease was not found

to be a significant risk factor in the development of aGVHD or engraftment failure. Also, the

geographical origin of the CBU and donor's maternal CMV status were not found to be

significant risk factors in the development of aGVHD. There are limited prior research

studies that examined the type of primary disease and geographical origin of CBU as

potential risk factors. A positive donor's maternal CMV status is cited as a risk factor across

some studies, however, this study found no significant increase in the risk between positive

versus negative donor's maternal CMV status and development of aGVHD. Nonetheless, one

important factor that could have contributed to this outcome is the small sample size when

examining CMV status. Due to limitations in data collection, the donor's maternal CMV

status for recipients that were transplanted in the earlier years is unknown. The study

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included 82 recipients but the CMV status could not be collected for 19 recipients which could have greatly affected the outcome of the analysis.

Further research studies are recommended to gain a more comprehensive understanding of CBU transplantation. A comparison of these risk factors among bone marrow and peripheral blood transplant recipients in addition to CBU recipients would be able to provide more context for the results of analysis. In addition, severity of disease is also an important factor that could affect engraftment and GVHD development, however, due to limitations in data collection regarding this factor, further analysis could not be completed. This would be an important risk factor and potential confounding factor for this study that should be assessed in future studies.

There are notable limitations that should be considered for this study. As like most retrospective cohort studies, the data used for analysis was originally collected for other purposes so not all relevant risk factors and variables for the study objectives were necessarily collected. The data sets that were generated through the StemSoft database were mostly demographical information relating to the recipient and transplant. For example, for information relating to GVHD, it only includes the grade of GVHD but not the organs that were affected and their individual staging. Additional details relating to different aspects of transplantation would have allowed for a more comprehensive analysis of the data that would have provided more context for the study findings. Also, information relating to GVHD cannot be as easily manually ascertained through clinical notes, since the GVHD status is based on the discretion of the treating physician and not necessarily based on pathology or lab reports. This could also lead to inconsistency of interpretation of GVHD status across

physicians, however it is unlikely since there are specific requirements for each stage, grade, and type of GVHD that helps minimize discrepancies. Nonetheless, it would be beneficial to collect more comprehensive data for the database for future studies.

Another limitation for this study is that GVHD status was reported according to the traditional criteria that focused more on time of diagnosis rather than histological features. As the traditional criteria becomes more obsolete across different hospitals and clinics, and the new criteria becomes more prevalent, the findings of this study might not necessarily be as relevant, and further studies that examines the new criteria would be necessary.

In addition, another notable limitation is that there is no real time quality control for the data that is entered into the StemSoft database. The data itself especially data relating to GVHD is separately verified by primary physicians, however, there is no system in place to verify the data that is actually entered accurately into the database thus leaving room for human error especially typographical errors.

Finally, the small sample size contributed to the low-power of the study. 240 participants were required to achieve 80% power, however, the sample size of 82 fell short of this requirement. Despite the results demonstrating that specific potential risk factors do not increase the risk of aGVHD development and failure to achieve engraftment, the analysis is inconclusive due to the small sample size not providing the power necessary to determine accurate significance. The sample size could have potentially affected the results of the study including contributing to the non-significant findings. A smaller power would mean that the probability of a type II error increases, which potentially contributes to the non-significant findings in a study. Also, a larger sample size would have been more ideal in terms of

analyzing cGVHD development. Only one recipient developed cGVHD in this cohort, and in-depth analysis of cGVHD risk factors based on solely one patient could be misleading and not provide accurate results.

Despite limitations, the study further supported the advantages of CBU transplantation in terms of low-rates of GVHD development regardless of HLA match grade and high rates engraftment achievement. In addition, since there are currently limited studies on how match grade, primary disease, CBU geographical origin, and donor's maternal CMV status affects transplantation outcomes, the study provides a basic understanding that future, large-scale studies can further examine. It will be an important stepping stone to help advance future studies to help better understand CBU transplantation to further encourage its use especially in pediatric populations.

CONCLUSION

Previous research studies have shown that the use of CBU for transplantation provides advantages over other sources of HSC including less stringent HLA matching criteria, no risk to donor, lower rates of GVHD, and higher rates of engraftment among other factors (Demiriz, Tekgunduz, & Altuntas, 2012). The results of the study further support that UCB-sourced products offer flexibility in HLA matching criteria and mismatched recipients were not associated with an increased risk of developing GVHD or failure to achieve engraftment. In addition, the type of primary disease, geographical origin of CBU, and CMV status did not increase the risk of developing GVHD or failure to achieve engraftment. However, as previously mentioned, further studies large-scale studies would provide more conclusive results due to achieving the power necessary to determine significance in

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findings. As this study further supports the benefits of CBU transplantation, it emphasizes the importance of further research and investment into cord blood banking and transplantation. Further research is important to allow for a more comprehensive understanding of CBU transplantation. Due to limitations of CBU transplantation, the pediatric population would benefit the most from continued support and research. This is especially important because pediatric cancer rates continue to rise globally and transplantation offers the only curative option for many genetic diseases thus CBU transplantation serves as a viable option that could help combat the increased rates of disease.

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Table 4. Transplantation Characteristics Summary

Factors	n = 82
Recipient age at transplant (years), median (range)	0.90 (0.13-9.31)
Recipient Sex, n (%)	
Female	27 (32.93)
Male	55 (67.07)
Survival Status, n (%)	
Alive	61 (74.39)
Dead	21 (25.61)
Primary Disease, n (%)	
Acute leukemia	28 (34.15)
Anemia/hemoglobinopathy	4 (4.88)
Histiocytic disorder	2 (2.44)
Immune deficiency	37 (45.12)
Inherited disorder of metabolism	4 (4.88)
MDS	5 (6.10)
Other non-malignant diseases	2 (2.44)
Primary Disease Type, n (%)	
Malignant	33 (40.24)
Non-Malignant	49 (59.76)
Acute GVHD, n (%)	
None	73 (90.12)
Grade I-II	4 (4.94)
Grade III-IV	4 (4.94)
Chronic GVHD, n (%)	
None	68 (98.55)
Limited	0 (0)
Extensive	1 (1.45)
HLA Compatibility, n (%)	
Matching out of 6 Criteria	
4/6	1 (1.22)
5/6	49 (59.76)
6/6	32 (39.02)
Matching out of 10 Criteria	
3/10 or 4/10	4 (4.88)
5/10 or 6/10	10 (12.20)
7/10 or 8/10	34 (41.46)
9/10	16 (19.51)
10/10	18 (21.95)

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Table 5. Cord Blood Unit Characteristics Summary

Factors	n = 82
Time between collection date and transplant date (years), median (range)	3.67 (0.52-19.49)
CBU origin, n (%)	
Domestic	67 (81.71)
International	15 (18.29)
Donor CMV status, n (%)	
Negative	24 (29.27)
Positive	39 (47.56)
Unknown	19 (23.17)
Donor sex, n (%)	
Female	41 (50.62)
Male	40 (49.38)
Days to achieve engraftment, median (range)	18 (8-44)
Engraftment Achievement, n (%)	
Yes	79 (96.34)
No	3 (3.66)

Table 6. Chi-Square Analysis for HLA Match Grade and aGVHD Development

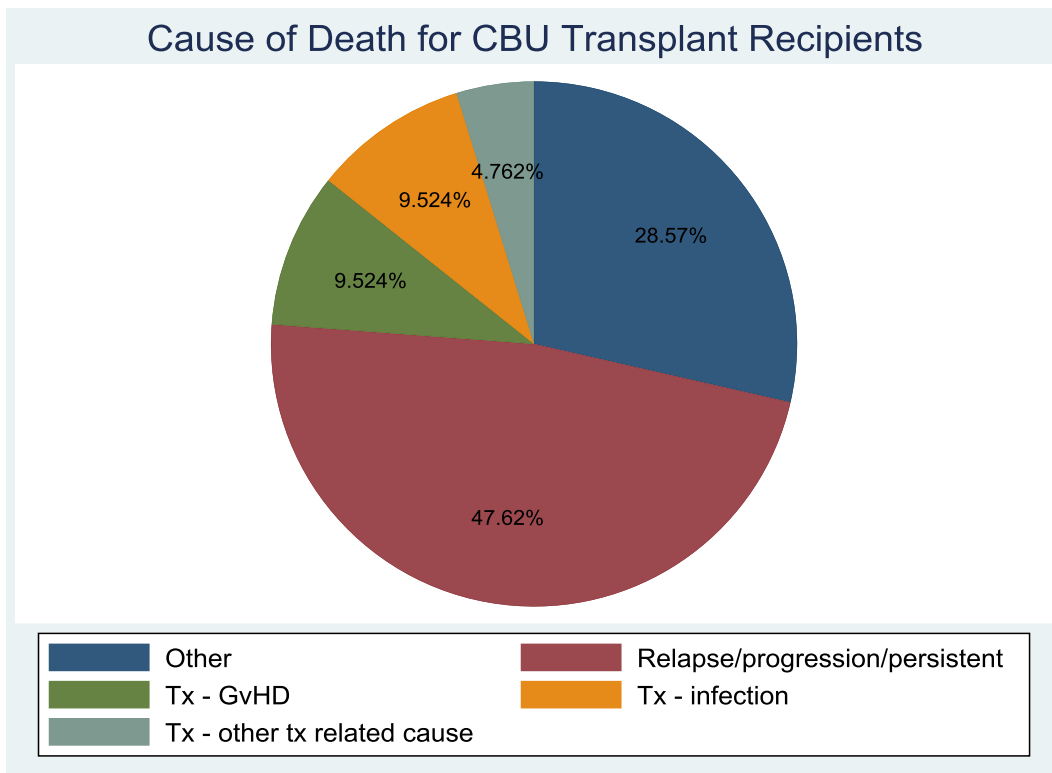| Factors	aGVHD Grade 0 (N=73)	aGVHD Grades I-II (N=4)	aGVHD Grades III-IV (N=4)	Total (N=81)	X^2	P	Fisher's Exact
6 Loci Match Grade					0.66	0.96	1.00
4/6	1	0	0	1			
5/6	43	2	3	48			
6/6	29	2	1	32			
Total	73	4	4	81			
10 Loci Match Grade					4.85	0.99	.82
3/10	1	0	0	1			
4/10	3	0	0	3			
5/10	1	0	0	1			
6/10	6	1	1	8			
7/10	12	1	1	14			
8/10	19	0	1	20			
9/10	15	1	0	16			
10/10	16	1	1	18			
Total	73	4	4	81			

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Table 7. Factors associated with aGVHD Development

Factors	Total (N=81)	aGVHD Yes (N=8)	aGVHD No (N=73)	Risk Ratio (95%CI)	P
Mismatched CBU (at 6 loci)				1.09 (0.28-4.24)	0.61
Yes	49	5	44		
No	32	3	29		
Mismatched CBU (at 10 loci)				0.86 (0.19-3.89)	0.57
Yes	63	6	57		
No	18	2	16		
Primary Disease				2.55 (0.65-9.94)	0.15
Malignant	32	5	27		
Non-Malignant	49	3	46		
Origin of CBU				0.63 (0.08-4.73)	0.54
International	15	1	14		
Domestic	66	7	59		
Donor Maternal CMV				0.82 (0.20-3.35)	0.54
Positive	39	4	35		
Negative	24	3	21		
Recipient Sex				3.31 (0.43-25.52)	0.20
Male	55	7	48		
Female	26	1	25		
Mismatched Donor-Recipient Sex				0.59 (0.15-2.29)	0.34
Yes	41	3	38		
No	39	5	35		

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Table 8. Factors Associated with Achievement of Engraftment

Factors	Total (N=82)	Engraftment Achievement No (N=3)	Engraftment Achievement Yes (N=79)	Risk Ratio (95% CI)	P
Mismatched CBU (at 6 loci)				1.28 (0.12-13.54)	0.66
Yes	50	2	48		
No	32	1	31		
Mismatched CBU (at 10 loci)				0.56 (0.05-5.86)	0.53
Yes	64	2	62		
No	18	1	17		
Primary Disease				0.74 (0.07-7.86)	0.64
Malignant	33	1	32		
Non-Malignant	49	2	47		

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Figure 1. Cause of Death of CBU Transplant Recipients

Cause of Death for CBU Transplant Recipients

- Other — 28.57%
- Relapse/progression/persistent — 47.62%
- Tx - GvHD — 9.524%
- Tx - infection — 9.524%
- Tx - other tx related cause — 4.762%

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