Risk Factors and Clinical Significance of Lymphopenia in Survivors of the Fontan Procedure for Single-Ventricle Congenital Cardiac Disease


PURPOSE OF THE STUDY. To determine the clinical significance of immunologic laboratory anomalies (lymphopenia and hypogammaglobulinemia) commonly seen in survivors of the Fontan procedure for single-ventricle congenital heart disease.

STUDY POPULATION. The study included 178 patients ages 3 to 26 years with congenital single-ventricle cardiac anomaly (status: postcompletion of surgical repair) with Fontan who had established outpatient care in the Single Ventricle Survivorship Program (SVSP) at the Children’s Hospital of Philadelphia.

METHODS. This was a retrospective chart review of the immunologic parameters of patients enrolled in the SVSP. Data on demographics, diagnoses, surgical interventions, immunologic laboratory data, and medications were gleaned from the electronic medical records. SVSP and immunology consult notes were reviewed for infectious history, absolute lymphocyte count (ALC), and IgG levels. A $P$ value of <.05 was considered statistically significant.

RESULTS. Most SVSP patients had some degree of lymphopenia. Those with protein-losing enteropathy (PLE) had lower median ALC and IgG levels (672 cells/µL and 200 mg/dL, respectively) than those without (1610 cells/µL and 868 mg/dL, respectively). Approximately 12% of those in the non-PLE group had significant asymptomatic lymphopenia (ALC <1000 cells/µL). In a logistic regression analysis, PLE and increased number of years after Fontan were found to be the only significant risk factors for lymphopenia. Despite lymphopenia in the majority, few patients were significantly clinically affected; 24% had a delayed clearance of cutaneous viral infections, 63% had atopy, and 1 died of EBV-associated Hodgkin lymphoma. Severe opportunistic infections typical of cellular immune defects were not observed, even among those with significant lymphopenia.

CONCLUSIONS. Patients with repaired single-ventricle physiology often demonstrate T-cell lymphopenia and hypogammaglobulinemia. The most common clinical manifestation was a delayed clearance of cutaneous viral infections. Significant systemic opportunistic infections were not seen despite laboratory abnormalities and a lack of antimicrobial prophylaxis or immunoglobulin replacement.

CRISPR/Cas9 β-globin Gene Targeting in Human Haematopoietic Stem Cells


PURPOSE OF THE STUDY. Sickle cell disease (SCD) is caused by a single nucleotide mutation (A to T) in the β-globin (*HBB*) gene leading to a Glu6Val amino acid change. The purpose of this study is to show that this gene can be effectively edited in human hematopoietic stem and progenitor cells (HSPCs) using the CRISPR/Cas9 system and to develop a method to enable efficient engraftment of edited human cells in mice.

STUDY POPULATION. HSPCs from mobilized peripheral blood of healthy donors and SCD patients were used for gene editing ex vivo. Immune-deficient NSG mice were used as recipients of edited HSPCs.

METHODS. Cas9 protein can generate double-strand breaks in DNA that can then be repaired by nonhomologous end joining or homologous recombination (HR) if a donor strand is provided. HSPCs were electroporated with Cas9 protein combined with sgRNA (Cas9 RNP) targeting the *HBB* gene. Cells were then infected with recombinant adeno-associated viral vectors of serotype 6 (rAAV6) carrying a donor sequence with a single nucleotide mutation (A to T) in the *HBB* gene and a green fluorescent protein (GFP) sequence for truncated nerve growth factor receptor (tNGFR) expression cassette. tNGFR is expressed at the surface of transfected cells and allows for enrichment of transduced cells by utilizing magnetic bead-based
When using an antisickling method of 50% of disease-causing variant alleles to wild type, expression of corrected cells were able to differentiate into erythroid cells in vitro. Donor, 11% of tNGFR-positive cells were achieved. These targeted sickle cell patients (HbAS3), and those differentiated from tNGFRhigh expressed 20% of corrected (HbAS3) out of total transplanted with GFPhigh cells had a median of 90% engraftment of gene-edited cells. SCD patients may benefit from gene therapy. This study not only sets the ground for gene therapy but also for other congenital hematologic and immune diseases.

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SECONDARY IMMUNODEFICIENCY (HIV)

Maternal HIV Infection Influences the Microbiome of HIV-Uninfected Infants

PURPOSE OF THE STUDY. Multiple studies have demonstrated that perinatally HIV-exposed but HIV-uninfected (HEU) infants experience greater morbidity and mortality than HIV-unexposed, uninfected infants (HUU). Newborn acquisition of the maternal microbiome provides metabolic and immunologic health benefits. The purpose of this study was to examine the effects of HIV infection on the maternal microbiome and breast milk oligosaccharides and the subsequent impact these may have on the microbiomes of their HEU infants.

STUDY POPULATION. A total of 50 Haitian mother-infant pairs were studied. All infants were breastfed, and no infants were on antibiotics at the time of the study. A total of 25 HIV-positive mothers and their infants were studied and compared with 25 HIV-uninfected mothers and their infants. Notably, all HIV-positive mothers were on combination antiretroviral therapy, and most had low HIV viral loads and normal CD4+ T-cell counts.

METHODS. Demographic and clinical data were obtained from patient records. Selected body sites were sampled for bacterial microbiological analysis from each subject. 16S ribosomal DNA was analyzed by using a previously described method. Breast milk oligosaccharides were characterized with high-pressure liquid chromatography.

RESULTS. Surprisingly, the microbiomes of the 2 groups of mothers did not differ appreciably. However, the microbiomes of the HEU infants had significant differences compared with the microbiomes from the HUU infants. Uninfected infants born to HIV-infected mothers showed lower microbial diversity and a different taxonomic composition. For example, HEU infants had an increased proportion of Pseudomonadaceae and less mature bacterial communities. HUU infants had an increase in Prevotellaceae and a Pseudomonadaceae profiles.

CONCLUSIONS. The Gut microbiome in HIV-exposed, uninfected infants differs from that of HIV-unexposed and uninfected infants, and human milk oligosaccharides were associated with specific bacterial species. It was speculated that this dysbiosis may contribute to the increased risk of illness in HEU infants.

REVIEWER COMMENTS. It is not unexpected that the presence of a chronic infection would alter individual microbiomes.
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