Original Article

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

Megan M. Morsheimer, MD, MPH[a], Jack Rychik, MD[b], Lisa Forbes, MD[c], Kathryn Dodds, CRNP[b], David J. Goldberg, MD[b], Kathleen Sullivan, MD, PhD[a], and Jennifer R. Heimall, MD[a] *Philadelphia, Pa; Houston, Tex*

What is already known about this topic? Immunologic perturbations are common among patients in heart failure. Those with single-ventricle physiology repaired with a Fontan (typically the third and final surgery in the staged reparative process for those with congenital single-ventricle physiology) frequently have lymphopenia. Previous studies of postthymectomy and post-Fontan patients did not identify a clinically significant secondary immunodeficiency.

What does this article add to our knowledge? Lymphopenia among single- ventricle survivors is common; the risk increases with time after Fontan and is presumed because of perturbations in hemodynamics more than thymic insufficiency. Delayed viral cutaneous infection clearance occurs in nearly 25%.

How does this study impact current management guidelines? Significant lymphopenia (absolute lymphocyte count of <1000 cells/μL), even in the absence of PLE, is common. Opportunistic infections typical of depressed T-cell counts are not seen, presumably because function is spared and peripheral sampling may be inaccurate.

BACKGROUND: Congenital cardiac anomalies are associated with immunologic perturbations. Surgical thymectomy, thoracic duct manipulation, and protein- losing enteropathy (PLE), a condition related to stressed Fontan hemodynamics, presumably contribute to low peripheral absolute lymphocyte counts (ALCs) and quantitative immunoglobulins. Clinical significance of lymphopenia and hypogammaglobulinemia in single-ventricle survivors requires additional study.

OBJECTIVE: Although immunologic laboratory anomalies are common in this population, we hypothesize that clinically significant immunodeficiency requiring intervention is rarely required.

METHODS: A retrospective chart review of the immunologic parameters of patients enrolled in the Single Ventricle Survivorship Program (SVSP) at the Children's Hospital of Philadelphia was performed.

RESULTS: The age range of the 178 SVSP patients was 3 to 26 years, with a median of 10.8 years. Most of the SVSP patients had some degree of lymphopenia. In the non-PLE group, the range of ALCs varied from 530 to 5322 cells/μL, with 17 patients without PLE maintaining an ALC of less than 1000 cells/μL. Among those with PLE, the median ALC and the IgG level were lower (672 cells/μL and 200 mg/dL, respectively) than in those without (1610 cells/μL and 868 mg/dL, respectively). Despite lymphopenia in the majority, few were severely clinically affected: 24% had delayed clearance of cutaneous viral infections, 63% had atopy, and 1 died of EBV-associated Hodgkin lymphoma. Immunoglobulin replacement was clinically indicated for 3 patients, 1 of whom had common variable immunodeficiency. Four patients with normal splenic function were treated with daily antibiotic prophylaxis.

CONCLUSIONS: Patients with repaired single-ventricle physiology often demonstrate T-cell lymphopenia and hypogammaglobulinemia. A significant portion of patients without PLE also have lymphopenia. The most common clinical manifestation was delayed clearance of cutaneous viral infections, but significant systemic opportunistic infections were not seen despite laboratory abnormalities and lack of antimicrobial prophylaxis. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2016;■:■-■)

Key words: *Single ventricle; Fontan; Protein-losing enteropathy (PLE); T-cell lymphopenia; Hypogammaglobulinemia; Secondary immunodeficiency; Warts*

[a]Division of Allergy & Immunology, The Children's Hospital of Philadelphia, Pa
[b]Division of Cardiology, The Children's Hospital of Philadelphia, Pa
[c]Section of Immunology, Allergy, Rheumatology, Baylor College of Medicine, Tex Children's Hospital, Houston, Tex

J. Rychik's efforts and support for the Single Ventricle Survivorship Program are provided by the Harrington Endowed Chair for Pediatric Cardiology at the Children's Hospital of Philadelphia. This project was otherwise not funded.

Conflicts of interest: L. Forbes is a member of Baxalta and Horizon; is employed by Baylor College of Medicine; and has received research support and lecture fees from Horizon. K. Sullivan has received research support from Baxter; has received consultancy fees from Immune Deficiency Foundation; and is a reviewer for UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 7, 2015; revised October 15, 2015; accepted for publication November 25, 2015.

Available online ■■

Corresponding author: Jennifer R. Heimall, MD, Division of Allergy & Immunology, Children's Hospital of Philadelphia, 3550 Market St, 3rd Fl, Philadelphia, PA 19104. E-mail: heimallj@email.chop.edu.

2213-2198

http://dx.doi.org/10.1016/j.jaip.2015.11.034

Abbreviations used
ALC- absolute lymphocyte count
NK- natural killer
PLE- protein-losing enteropathy
SVSP- Single Ventricle Survivorship Program

Children with single-ventricle heart cardiac anomalies have been noted to have frequent immunologic perturbations, including lymphopenia and hypogammaglobulinemia.[1] However, the clinical relevance of these laboratory abnormalities in terms of infection risk, immune dysfunction, and survival is not well described. Surgical palliation of single-ventricle cardiac anomalies has been optimized through a 3-step staged approach, resulting in the survival of most children; however, the resultant physiology remains abnormal. Despite maximal surgical and medical management, single-ventricle survivors live with a form of chronic heart failure and are subject to the deleterious effects of an abnormal circulation with a multitude of end-organ consequences over time, including but not limited to protein-losing enteropathy (PLE).[2,3] In adult patients with heart failure states, lymphopenia in particular has been noted to have an association with poor prognosis,[4] suggesting that the abnormal physiology of the heart failure state is intrinsic to the low lymphocyte counts seen in children with single-ventricle congenital cardiac disease following surgical palliation.

The purpose of our study was to characterize the laboratory findings and clinical correlates of immune function in a large cohort of children with single- ventricle type of congenital heart disease after the Fontan operation (typically the third and final surgery in the staged reparative process for those with congenital single-ventricle physiology).

METHODS

Research approval for a retrospective chart review was granted by the Children's Hospital of Philadelphia Committee for the Protection of Human Subjects (institutional review board no. 13-010413).

The Single Ventricle Survivorship Program (SVSP) at the Children's Hospital of Philadelphia provides multidisciplinary, long-term follow-up for children who have completed the series of surgical repairs to their underlying congenital cardiac anomaly. Although many of those enrolled have received all their surgical and cardiac care at the Children's Hospital of Philadelphia, children from across the country enroll in this unique program for long-term follow-up. To date approximately 200 patients have been evaluated in the SVSP, representing one of the largest cohorts in the country. Children are evaluated in SVSP approximately every 3 to 5 years, depending on their clinical status. For over 3 years, a clinical immunologist has provided consultation as part of the multidisciplinary evaluation.

A master list of participants was used to identify patients eligible for inclusion. All congenital single-ventricle patients status-post Fontan who established outpatient care in the SVSP from the clinic's inception through July 2013 were included. Those who were followed in general cardiology clinic alone, died before their SVSP appointment, or had not yet received a Fontan repair were ineligible.

Electronic medical records were gleaned for demographic information, diagnoses, surgical interventions, immunologic laboratory data, and medications. SVSP and immunology consultation notes, when available, were reviewed for patients' infection history. An attempt to review operative notes for details regarding the degree of surgical thymectomy was made; however, surgeons did not routinely note this detail. Chart review gleaned the following from immunology consultation notes: screening absolute lymphocyte count (ALC) and IgG for all patients; a thorough history of viral, bacterial, and fungal infections; a vaccine reaction history; evaluation for atopy and autoimmunity; screening questions for PLE and chronic diarrhea; and relevant physical examination findings such as peripheral edema and cutaneous infections.

TABLE I. SVSP cohorts' demographic characteristics

Characteristic	Value
Median age (IQR) at SVSP evaluation (y)	10.8 (7-16)
Median years post-Fontan at SVSP evaluation	7.8 (4-13.7)
Sex: male, n (%)	97 (55)
Hypoplastic left heart, n (%)	113 (63)
Heterotaxy syndrome, n (%)	23 (13)
Fenestrated Fontan, n (%)	158 (89)
PLE	31 (17)

IQR, Interquartile range.

In those with heterotaxy, functional, or anatomic asplenia, assessment of antibody-based response to vaccines (pneumococcal, *Haemophilus influenzae*, meningococcal, diphtheria, and tetanus titers) was noted. Patients with an ALC of less than 1000 cells/μL typically received immunophenotyping of T, B, and natural killer (NK) cells ($CD3^+$, $CD4^+$, $CD8^+$, $CD4/CD45RA^+$, $CD4/CD45RO^+$, $CD3^-/CD19^+$, $CD16^+$, &/or $CD56^+$). Lymphocyte mitogen stimulation to PHA was recorded when available. PLE was diagnosed by the presence of hypoalbuminemia and hypoproteinemia, and in some cases was confirmed with stool alpha-1 antitrypsin testing.[5]

Data gleaned from the EPIC-based electronic medical record were stored in an Excel worksheet and ultimately de-identified for analysis. STATA10 (StataCorp LP, College Station, Tex) was used to perform all statistical queries. Standard descriptive statistical analysis were performed for parametric and nonparametric continuous variables. The Wilcoxon rank-sum test was used to compare the distribution of continuous, nonparametric values. The Fisher exact test was used for low-frequency events, and a 2-sample test of proportion was used to compare categorical frequencies. Multiple logistical regression was performed to explore risk factors for significant lymphopenia; risk factors included sex, heterotaxy, Fontan fenestration, hypoplastic ventricle laterality, number of years post-Fontan, and history of PLE. A 2-tailed *P* value of less than .05 was considered statistically significant.

RESULTS

The median age of the 178 SVSP patients was 10.8 years, with ages ranging from 3 to 26 years (Table I). On average, patients established care at the SVSP 7.6 years after their Fontan procedure. Males and females were equally represented. Nearly two-third of the patients seen had a history of hypoplastic left heart syndrome, and a minority (13%) had heterotaxy syndrome. Most of the Fontan procedures performed were fenestrated. A significant proportion of the cohort was found to have PLE (17.4%).

Screening ALC values for the overall cohort were typically in the lower end of normal values[6] for healthy children with a